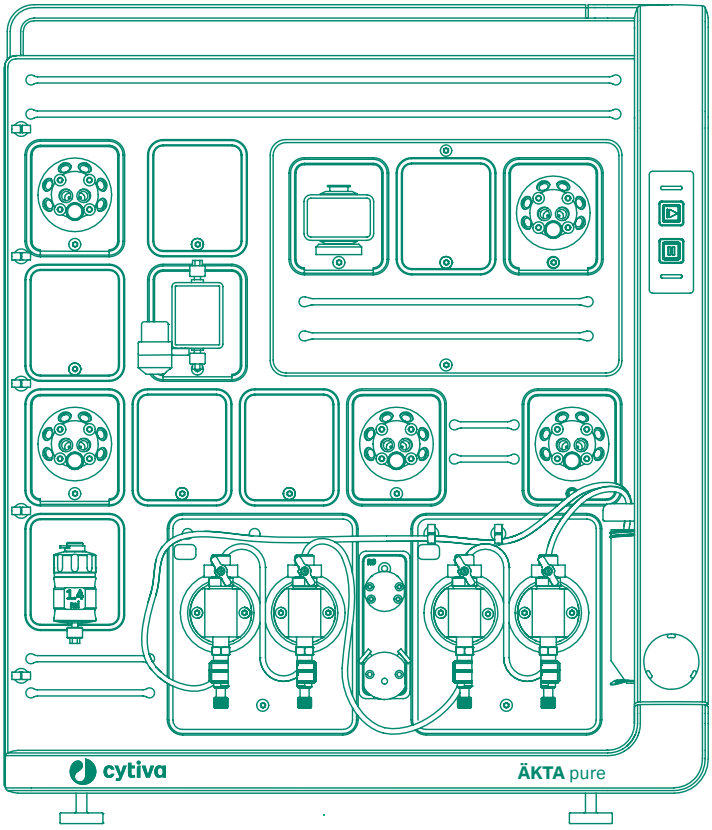
ÄKTA pure™

User Manual



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Table of Contents

1 Introduction 8
1.1 Important user information 9
1.2 ÄKTA pure overview 11
1.3 ÄKTA pure user documentation 13

2 The ÄKTA pure instrument 16
2.1 Overview illustrations 17
2.2 Liquid flow path 32
2.3 Instrument control panel 34
2.4 Instrument modules 39
2.4.1 System pumps 40
2.4.2 Mixer 44
2.4.3 Overview of the valves 46
2.4.4 Inlet valves 47
2.4.5 Mixer valve 54
2.4.6 Injection valves V9-Inj and V9H-Inj 57
2.4.7 Injection valve V9M-J 61
2.4.8 Loop valve 63
2.4.9 Column valves 66
2.4.10 Versatile valve 74
2.4.11 pH valve 75
2.4.12 Outlet valves 80
2.4.13 Pressure monitors 84
2.4.14 UV monitors 87
2.4.15 Conductivity monitors 92
2.4.16 Flow restrictor 94
2.5 Installation of internal modules 96
2.6 Accessories 100
2.6.1 Holders 101
2.6.2 Tubing kits 108
2.6.3 Micro kit 110
2.6.4 Other accessories 116

3 ÄKTA pure external modules 118
3.1 External air sensors 119
3.1.1 Description 120
3.1.2 Tubing connections 121
3.2 Fraction collector F9-C 122
3.2.1 Function 123
3.2.2 Fraction collector F9-C illustrations 125
3.2.3 Cassettes, Cassette tray and racks 129
3.2.4 Fraction collector tubes and bottles 132
3.2.5 Deep well plates 133
3.3 Fraction collector F9-R 135
3.3.1 Function 136
3.3.2 Illustrations 137
3.3.3 Connect the Fraction collector F9-R 139
3.3.4 Tubes and tube racks 140
3.4 Fraction collector F9-T 143

3.4.1 Function ... 144
3.4.2 Illustrations and parts ... 145
3.4.3 Connecting the Fraction collector F9-T ... 148
3.4.4 Plates and tubes ... 149
3.5 Sample pump S9 and S9H ... 152
3.6 I/O-box E9 ... 157
3.6.1 Overview of the I/O-box ... 158
3.6.2 Analog connector and signals ... 160
3.6.3 Digital connector and signals ... 162
3.6.4 Connect external equipment to the I/O-box ... 164
3.7 Connection of external modules ... 167

4 System configuration ... 170
4.1 Configuration overview ... 171
4.2 Configure modules ... 178
4.2.1 Configuration of injection valves ... 179
4.2.2 Configuration of inlet valves ... 180
4.2.3 Configuration of mixer valves ... 182
4.2.4 Configuration of loop valves ... 183
4.2.5 Configuration of column valves ... 184
4.2.6 Configuration of versatile valves ... 186
4.2.7 Configuration of pH valves ... 187
4.2.8 Configuration of outlet valves ... 188
4.2.9 Configuration of UV monitors ... 189
4.2.10 Configuration of conductivity monitor ... 191
4.2.11 Configuration of external air sensors ... 192
4.2.12 Configuration of fraction collectors ... 194
4.2.13 Configuration of I/O-box ... 199
4.3 Configure ÄKTA pure micro ... 201
4.4 General system settings ... 203

5 Operation ... 206
5.1 Before preparing the system ... 207
5.2 Prepare the flow path ... 208
5.2.1 Overview ... 209
5.2.2 Select mixer chamber ... 211
5.2.3 Select UV flow cells ... 212
5.2.4 Prepare tubing ... 213
5.2.5 Waste ... 214
5.3 Start UNICORN and connect to system ... 217
5.4 Prime inlets and purge pump heads ... 220
5.4.1 System pumps ... 221
5.4.2 Sample pump ... 228
5.5 Connect a column ... 233
5.6 Pressure alarms ... 238
5.7 Sample application ... 242
5.7.1 Overview ... 243
5.7.2 Sample application using direct injection onto the column ... 245
5.7.3 Sample application using Superloop ... 249
5.7.4 Sample application using a sample loop ... 256
5.8 Fractionation ... 260
5.8.1 Fractionation overview ... 261
5.8.2 Delay volume and pre-fractionation volume ... 263

5.8.3 Prepare Fraction collector F9-C ... 266
5.8.4 Prepare Fraction collector F9-R ... 272
5.8.5 Prepare Fraction collector F9-T ... 278
5.8.6 Fractionation settings ... 282
5.9 Create a method and perform a run ... 284
5.9.1 Create a method ... 285
5.9.2 Prepare and perform a run ... 291
5.9.3 Monitor a run ... 293
5.9.4 After run procedures ... 295
5.10 ÄKTA pure micro or Micro kit operation ... 299
5.10.1 Columns ... 300
5.10.2 Injection techniques ... 302
5.10.3 Gradients ... 303
5.10.4 Fraction collection ... 304

6 Performance tests ... 305
6.1 General performance test actions ... 306
6.2 Air sensor A and Inlet valve A tests ... 310
6.3 Air sensor B and Inlet valve B tests ... 312
6.4 Air sensor S and Sample inlet valve tests ... 314
6.5 Column valve C tests ... 316
6.6 Fraction collector F9-C test ... 318
6.7 Fraction collector F9-R test ... 320
6.8 Fraction collector F9-T test ... 322
6.9 Sample pump tests ... 325
6.10 System test UV U9-M ... 327
6.11 System test UV U9-T or U9-L ... 332
6.12 System test Micro kit ... 336

7 Maintenance ... 339
7.1 Maintenance Manager ... 340
7.2 Maintenance program ... 345
7.3 Calibration procedures ... 348
7.3.1 Calibrate the pH monitor ... 349
7.3.2 Calibrate the pressure monitors ... 351
7.3.3 Calibrate the conductivity monitor ... 355
7.3.4 Calibrate the UV monitors ... 360
7.4 Cleaning procedures ... 364
7.4.1 Clean the instrument externally ... 365
7.4.2 Perform System CIP ... 366
7.4.3 Perform Column CIP ... 377
7.4.4 Clean Fraction collector F9-C ... 381
7.4.5 Clean the Fraction collector F9-C sensors ... 383
7.4.6 Clean Fraction collector F9-R ... 385
7.4.7 Clean Fraction collector F9-T ... 386
7.4.8 Clean the Fraction collector F9-T drop sync sensor ... 388
7.4.9 Clean the pH electrode ... 389
7.4.10 Clean the UV flow cell ... 392
7.4.11 Clean the conductivity flow cell ... 395
7.4.12 Clean the pump head check valves ... 397
7.4.13 Wipe off excess oil from the pump head ... 400
7.5 Replacement procedures ... 401

7.5.1 Replace tubing and connectors ... 402
7.5.2 Replace internal tubing in Fraction collector F9-C ... 404
7.5.3 Replace the pH electrode ... 407
7.5.4 Replace the mixer ... 408
7.5.5 Replace the O-ring inside the mixer ... 409
7.5.6 Replace the UV monitor U9-M flow cell ... 411
7.5.7 Replace the UV monitor U9-T and U9-L flow cell ... 414
7.5.8 Replace flow restrictor ... 416
7.5.9 Replace the online filter ... 417
7.5.10 Replace the inlet filters ... 418
7.5.11 Replace the pump head check valves ... 419
7.5.12 Replace pump piston seals of Pump P9 or P9H ... 422
7.5.13 Replace pump piston seals of Pump P9-S ... 431
7.5.14 Replace pump pistons ... 439
7.5.15 Replace pump rinsing system tubing ... 440
7.6 Other maintenance procedures ... 443
7.6.1 Change pump rinsing solution ... 444
7.6.2 Check the flow restrictor ... 449
7.6.3 Storage of the pH electrode ... 451

8 Troubleshooting ... 453
8.1 Introduction to troubleshooting ... 454
8.2 Troubleshooting: General Checklist ... 456
8.3 Troubleshooting: Monitors ... 458
8.3.1 UV monitor U9-M and UV detector U9-D ... 459
8.3.2 UV monitor U9-T and U9-L ... 463
8.3.3 Conductivity monitor ... 467
8.3.4 pH monitor and pH valve ... 470
8.3.5 Pressure monitors ... 472
8.4 Troubleshooting: Valves ... 474
8.5 Troubleshooting: Fraction collectors ... 475
8.5.1 Fraction collector F9-C ... 476
8.5.2 Fraction collector F9-R ... 484
8.5.3 Fraction collector F9-T ... 486
8.6 Troubleshooting: Pumps ... 490
8.6.1 Pumps ... 491
8.6.2 Examples of pump pressure curves ... 494
8.6.3 Remove persistent air bubbles ... 497
8.7 Troubleshooting: Other components ... 498
8.7.1 General hardware: All modules ... 499
8.7.2 Mixer ... 500
8.7.3 Module Panel ... 502
8.7.4 Superloop ... 503
8.7.5 Cabinet ... 504
8.7.6 Power and ICU ... 505
8.7.7 External air sensors ... 507
8.7.8 I/O-box E9 ... 508
8.7.9 Instrument control panel ... 510
8.8 Troubleshooting: Instrument communication ... 511
8.9 Troubleshooting: Method development ... 513
8.10 Error codes ... 514

9 Reference information 523
9.1 System specifications 524
9.2 Module specifications 526
9.3 Tubing and connectors 536
9.4 Recommended tubing kits for prepacked columns 546
9.5 Chemical resistance guide 549
9.5.1 General information about biocompatibility and chemical resistance 550
9.5.2 Chemical resistance specifications 551
9.6 Wetted materials 555
9.7 Predefined methods and phases 557
9.7.1 Predefined purification methods 558
9.7.2 Predefined maintenance methods 564
9.7.3 Predefined phases 565
9.8 System settings 567
9.8.1 Introduction 568
9.8.2 System settings - UV 569
9.8.3 System settings - Conductivity 572
9.8.4 System settings - Versatile valve 574
9.8.5 System settings - pH 575
9.8.6 System settings - Pressure alarms 576
9.8.7 System settings - Air sensor 578
9.8.8 System settings - I/O-box 580
9.8.9 System settings - Fraction collection 581
9.8.10 System settings - Tubing and Delay volumes 585
9.8.11 System settings - Wash settings 587
9.8.12 System settings - Watch parameters 589
9.8.13 System settings - Advanced 591
9.8.14 System settings - Data collection 592
9.9 Manual instructions 593
9.9.1 General instruction 594
9.9.2 Manual instructions - Pumps 595
9.9.3 Manual instructions - Flow path 599
9.9.4 Manual instructions - Monitors 602
9.9.5 Manual instructions - Alarms 605
9.9.6 Manual instructions - Fraction collector F9-R 609
9.9.7 Manual instructions - Fraction collector F9-C 610
9.9.8 Manual instructions - Fraction collector F9-T 612
9.9.9 Manual instructions - Fraction collector Outlet 614
9.9.10 Manual instructions - Peak fractionation parameters 615
9.9.11 Manual instructions - I/O-box 616
9.9.12 Manual instructions - Wash settings 617
9.9.13 Manual instructions - Watch parameters 619
9.9.14 Manual instructions - Advanced 621
9.9.15 Manual instructions - Other 624
9.10 Available Run data 625
9.11 Available Curves 630
9.12 Injection volumes and peak broadening 634
9.13 Delay volumes 635
9.14 Component volumes 640
9.15 Pressure control 643
9.16 Node IDs 645

10 Ordering information 649

Index 658

1 Introduction

Purpose of the User Manual

The User Manual provides you with instructions and information to run the ÄKTA pure™ system. It also includes relevant guidance for practical handling and maintenance of instrument components.

In this chapter

Section		See page
1.1	Important user information	9
1.2	ÄKTA pure overview	11
1.3	ÄKTA pure user documentation	13

1.1 Important user information

Read this before operating ÄKTA pure



All users must read the entire *ÄKTA pure Operating Instructions* before installing, operating, or maintaining the instrument. Always keep the *ÄKTA pure Operating Instructions* at hand when operating ÄKTA pure.

Do not operate ÄKTA pure in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

ÄKTA pure is intended for purification of bio-molecules, in particular proteins, for research purposes by trained laboratory staff members in research laboratories.

ÄKTA pure shall not be used in any clinical procedures, or for diagnostic purposes.

System definition

In this manual, the combination of the ÄKTA pure instrument and the UNICORN™ software is referred to as the system.

The ÄKTA pure instrument without the software is referred to as the instrument.

Prerequisites

In order to operate ÄKTA pure in the way it is intended:

- The user must know how to use a computer with Microsoft Windows.
- The user must understand the concepts of liquid chromatography.
- The user must read and understand the Safety instructions chapter in the *Operating Instructions*.
- The ÄKTA pure instrument must be installed in accordance with the site requirements and instructions in the *Operating Instructions*.

Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

Tip: *The text can include clickable hyperlinks to reference information.*

Definitions

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the system. See definitions below.



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the system or other equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the system.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

1.2 ÄKTA pure overview

Introduction

This section gives an overview of the ÄKTA pure instrument and the UNICORN software. For detailed information about UNICORN, see the UNICORN manuals listed in *UNICORN user documentation, on page 14*. For detailed information about the instrument, see *Chapter 2 The ÄKTA pure instrument, on page 16*.

Main features

The main features of ÄKTA pure are listed below.

- ÄKTA pure is a flexible system that allows the user to configure both hardware and software to meet many purification needs.
- The instrument can be easily extended with additional valves, detectors and fraction collectors.
- There are a large number of different hardware modules to choose from. The user can adjust, for example, the number of columns, inlets, outlets and detectors and choose between different ways to apply and collect samples.
- Purification and maintenance methods are easily created using predefined methods and method phases. A method phase refers to a specific step/part in the method, such as column wash or elution. Method phases are described in ***Phase Properties*** and are displayed graphically in a method outline. This makes methods and phases easy to understand and edit.
- ÄKTA pure is controlled by the UNICORN software: a complete package for control, supervision and evaluation of chromatography instruments and purification runs.
- UNICORN has different software license options, such as Design of Experiments (DoE) and Column logbook, to further add user support.

UNICORN modules overview

UNICORN consists of four modules: ***Administration***, ***Method Editor***, ***System Control*** and ***Evaluation***. The main functions of each module are described in the following table.

Module	Main functions
Administration	Perform user and system setup, system log and database administration.
Method Editor	Create and edit methods using one or a combination of: • Predefined methods with built-in application support • Drag-and-drop function to build methods with relevant phases • Line-by-line text editing The interface provides easy viewing and editing of run properties.
System Control	Start, monitor and control manual and method runs. The current flow path is illustrated in the ***Process Picture***, which allows manual interactions with the system and provides feedback on run parameters.
Evaluation	Open results, evaluate runs and create reports. The default ***Evaluation*** module includes a user interface optimized for workflows like quick evaluation, compare results and work with peaks and fractions. To perform operations like Design of Experiments, users can easily switch to ***Evaluation Classic***.

When working with the modules ***Administration***, ***Method Editor***, ***System Control*** and ***Evaluation Classic*** it is possible to access descriptions of the active window by pressing the **F1** key. This can be especially helpful when editing methods. The module ***Evaluation*** contains a guide with video clips.

1.3 ÄKTA pure user documentation

Introduction

This section describes the user documentation that is delivered with ÄKTA pure.

User documentation

The user documentation is listed in the table below.

Translations of the Operating Instructions are provided on the User Documentation DVD together with the User Manual, Product Documentation, and Unpacking Instructions. Printed copies of the User Manual are available on request from Cytiva.

Document	Main contents
ÄKTA pure Operating Instructions, 29022997	Instructions needed to install, operate and maintain the system in a correct and safe way. System overview, site requirements, and instructions for moving the system within the same building. Instructions for basic maintenance.
ÄKTA pure User Manual, 29119969 (this document)	Additional information in order to get the optimal performance from the system. Functional description of modules. Instructions for maintenance and troubleshooting activities.
ÄKTA avant, ÄKTA pure, and ÄKTA go Site Preparation Guide, 29117084	Information needed to prepare the site for installation and use of the system.
ÄKTA pure Unpacking Instructions, 29020657	Instructions for handling the delivery package and unpacking the system.
ÄKTA pure 25 Product Documentation, 29020658 or *ÄKTA pure 150 Product Documentation, 29050426*[1]	System specification and declaration of material conformity.
ÄKTA avant, ÄKTA pure, ÄKTA go, and ÄKTA pcc Privacy and Security Manual, 29488174	Describes the privacy and security considerations of the use of the system. The manual describes the expected intended use of the system, the privacy and security capabilities included, and how these capabilities are configured.

Document	Main contents
Fraction collector F9-C Operating Instructions, 29656879	Instructions needed to install, operate and maintain Fraction collectors F9-C in a safe way.
Fraction collector F9-R Operating Instructions, 29656880	Instructions needed to install, operate and maintain Fraction collectors F9-R in a safe way.
Fraction collector F9-T Operating Instructions, 29478336	Instructions needed to install, operate and maintain Fraction collectors F9-T in a safe way.
Sample pump S9 and S9H Operating Instructions, 29025607	Instructions needed to install, operate and maintain Sample pump S9 and S9H in a safe way.

[1] The instrument is delivered with the relevant document.

UNICORN user documentation

The user documentation listed in the following table is available from the ***Help*** menu in UNICORN or from the ***UNICORN Online Help and Documentation*** software accessed by pressing the **F1** key in any UNICORN module.

Documentation	Main contents
UNICORN Help	Descriptions of UNICORN dialog boxes (available from the ***Help*** menu).
Administration and Technical Manual[1]	Overview and detailed description of network setup and complete software installation. Administration of UNICORN and the UNICORN database.
UNICORN System Control Manual[1]	Overview and detailed description of the system control features in UNICORN. Includes general operation, system settings and instructions on how to perform a run.
UNICORN Method Manual[1]	Overview and detailed descriptions of the method creation features in UNICORN. Workflow descriptions for common operations.
Getting started with Evaluation ***Note:*** *Available in UNICORN 7.0 and later.*	Video clips showing common workflows in the Evaluation module. Overview of features of the Evaluation module.

Documentation	Main contents
UNICORN Evaluation Manual[1]	Overview and detailed descriptions of the Evaluation Classic module in UNICORN. Description of the evaluation algorithms used in UNICORN.

[1] Current UNICORN version is added to the title of the manual.

Additional literature

For practical tips on chromatography, refer to *ÄKTA Laboratory-scale Chromatography Systems Handbook, CY13989* on cytiva.com/handbooks.

2 The ÄKTA pure instrument

About this chapter

This chapter provides an overview of the ÄKTA pure instrument. It also describes the internal instrument components and how these are installed in the instrument.

In this chapter

Section		See page
2.1	Overview illustrations	17
2.2	Liquid flow path	32
2.3	Instrument control panel	34
2.4	Instrument modules	39
2.5	Installation of internal modules	96
2.6	Accessories	100

2.1 Overview illustrations

Introduction

This section provides an overview of the instrument and its available modules.

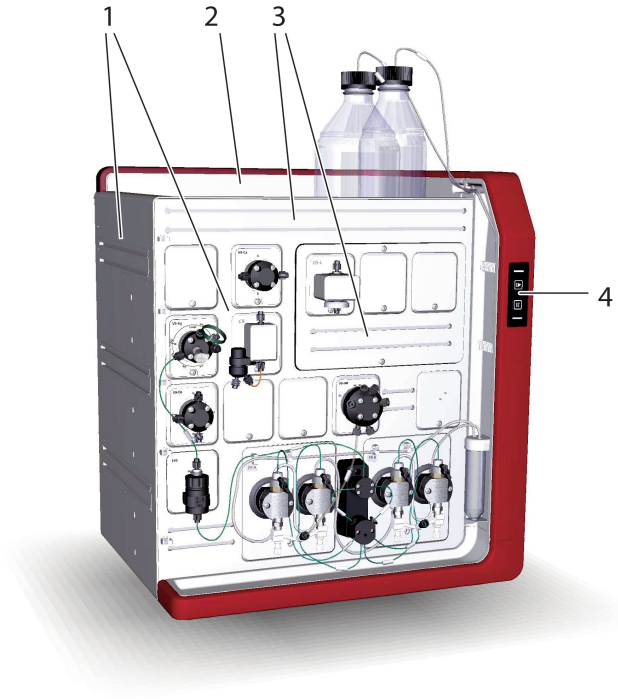
Core module configurations

ÄKTA pure is available with two core module configurations, one for flow rates up to 25 mL/min and one for flow rates up to 150 mL/min. In this manual they are referred to as ÄKTA pure 25 (25 mL/min), and ÄKTA pure 150 (150 mL/min).

ÄKTA pure micro is a special version of ÄKTA pure 25, intended for micro-scale applications. The recommended flow rate range is up to 2 mL/min.

Illustrations of the main parts of the instrument

The illustrations below show the location of the main parts of the instrument.

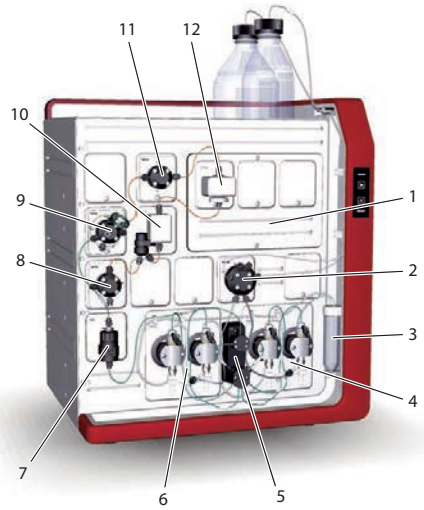




Part	Description
1	Wet sides
2	Buffer tray
3	Holder rails
4	Instrument control panel
5	Power switch
6	Ventilation panel

Example of a typical configuration of the wet side

A typical configuration of the ÄKTA pure instrument is illustrated below.



Part	Description
1	Multi-module panel
2	Inlet valve
3	Pump rinsing liquid tube
4	System pump B
5	Pressure monitor
6	System pump A
7	Mixer
8	Outlet valve
9	Injection valve
10	Conductivity monitor
11	Column valve
12	UV monitor

Available modules

The modular design allows the user to customize ÄKTA pure in multiple ways. The system is always delivered with the core modules of the selected configuration, but optional modules may be added to the flow path.

The table below lists the available modules for ÄKTA pure 25 and ÄKTA pure 150. Core modules are indicated with an asterisk (*).

Note: *The valves for ÄKTA pure 25 and ÄKTA pure 150 are compatible with both systems but for the best performance the specific valve type must be used. The narrow channels in the valves for ÄKTA pure 25 will give too high back pressure if used above 50 mL/min. The larger volumes in the "H" valves for ÄKTA pure 150 may decrease resolution and increase peak broadening if used in ÄKTA pure 25.*

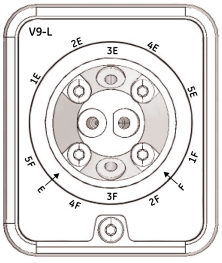
Note: *The modules* **V9M-Inj**, **C9M** *and* **V9M-Os** *are intended for micro-scale applications. If used with flow rates above 2 mL/min there will be excessive back pressure.*

Module	Label in	
	ÄKTA pure 25	**ÄKTA pure 150**
System pump A*	**P9 A**	**P9H A**
System pump B*	**P9 B**	**P9H B**
Pressure monitor*	**R9**	**R9**
Mixer*	**M9**	**M9**
Injection valve*	**V9-Inj** or **V9M-J**	**V9H-Inj**
Inlet valve A	**V9-IA**	**V9H-IA**
Inlet valve B	**V9-IB**	**V9H-IB**
Inlet valve AB	**V9-IAB**	**V9H-IAB**
Inlet valve IX	**V9-IX**	**V9H-IX**
Sample inlet valve	**V9-IS**	**V9H-IS**
Mixer valve	**V9-M**	**V9H-M**
Loop valve	**V9-L**	**V9H-L**
Column valves	**V9-C**	**V9H-C**
	V9-Cm	N/A
	V9-Cs	**V9H-Cs**
pH valve	**V9-pH**	**V9H-pH**
Outlet valves	**V9-O**	**V9H-O**

Module	Label in	
	ÄKTA pure 25	ÄKTA pure 150
	V9-Os or **V9M-Os**	**V9H-Os**
Versatile valve	**V9-V**	**V9H-V**
UV monitors	**U9-L**	**U9-L**
	U9-T	**U9-T**
	U9-M	**U9-M**
Conductivity monitor	**C9** or **C9M**	**C9**
External air sensor	**L9-1.5**	**L9-1.5**
	L9-1.2	**L9-1.2**
Fraction collectors	**F9-C**	**F9-C**
	F9-R	**F9-R**
	F9-T	N/A
I/O-box	**E9**	**E9**
Sample pump	**S9**	**S9H**

Illustration convention

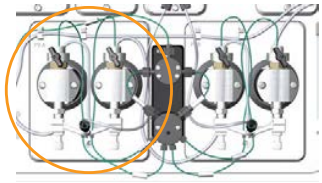
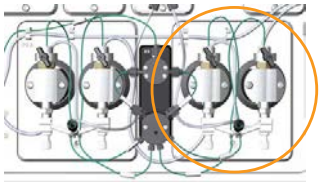
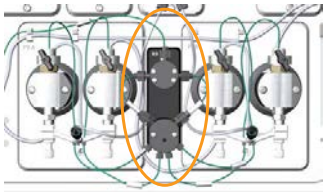
In the valve illustrations below, the following convention is used to point out the location of the ports on the valve head. Loop valve **V9-L** is used as an example.

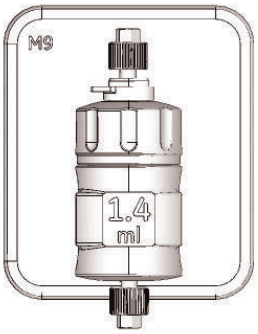
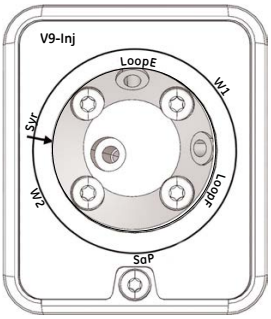


- Ports located on the valve head rim are indicated outside the black ring (e.g., 1E, 2E, etc.).
- Ports located on the pivot part of the valve head are indicated on the inside of the black ring (e.g., 3E and 3F).
- Ports located on the valve head front are indicated by an arrow (e.g., E and F).

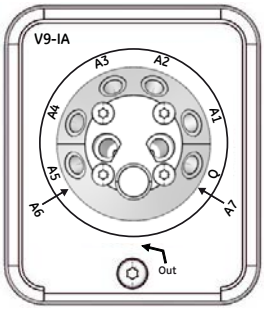
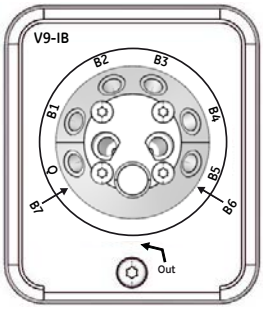
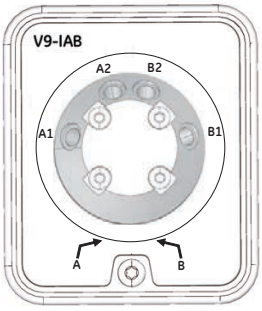
  Some valves indicate ports located on the valve head front by an dot instead of an arrow (for example, see the Column valve **V9-Cm** in *Optional modules, on page 24*).

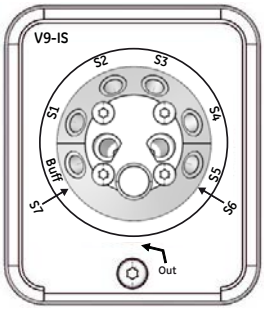
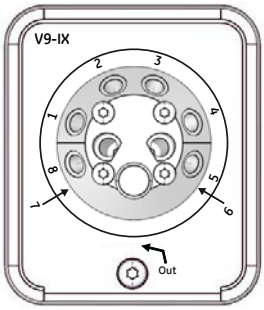
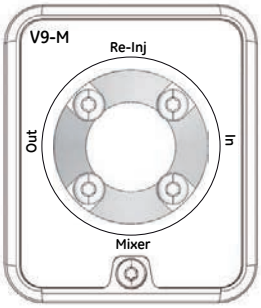
Core modules

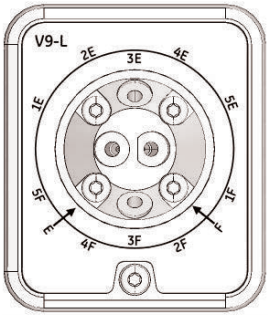
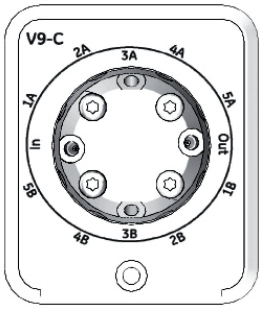
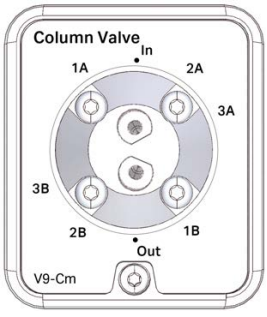
Core module	Description
System pump **P9 A** or **P9H A** 	A high precision pump, which delivers buffer or sample in purification runs. For further information, refer to *Section 2.4.1 System pumps, on page 40*.
System pump **P9 B** or **P9H B** 	A high precision pump, which delivers buffer in purification runs. For further information, refer to *Section 2.4.1 System pumps, on page 40*.
Pressure monitor **R9** 	Reads the system pressure after System pump A and System pump B. Contains a pump flow restrictor in order to stop siphoning effects. For further information, refer to *Section 2.4.13 Pressure monitors, on page 84*.

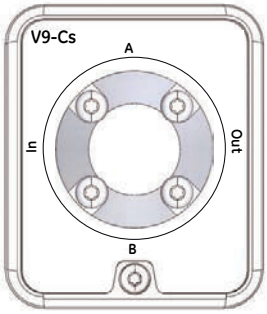
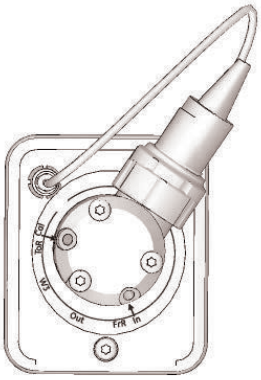
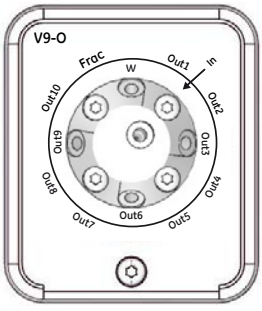
Core module	Description
Mixer **M9** 	Mixes the buffers delivered from the system pumps to a homogeneous buffer composition. Three Mixer chambers are available for ÄKTA pure 25: • 0.6 mL • 1.4 mL (mounted at delivery) • 5 mL Three Mixer chambers are available for ÄKTA pure 150: • 1.4 mL (mounted at delivery) • 5 mL (included in delivery) • 15 mL **CAUTION** **Explosion risk.** Do not use Mixer **M9** with the Mixer chamber 15 mL on an ÄKTA pure 25 system configuration. The maximum pressure for Mixer chamber 15 mL is 5 MPa. ***Note:*** *ÄKTA pure micro is delivered with an online filter clamp on the mixer module. The online filter clamp must only be used with the red tubing kit.* For further information, refer to *Section 2.4.2 Mixer, on page 44*.
Injection valve **V9-Inj**, **V9H-Inj** or **V9M-J** 	Directs sample onto the column. For further information, refer to *Section 2.4.6 Injection valves V9-Inj and V9H-Inj, on page 57* and *Section 2.4.7 Injection valve V9M-J, on page 61*.

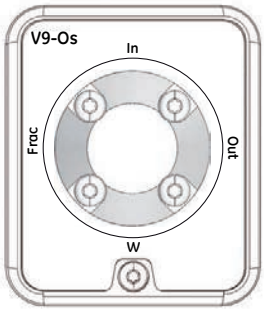
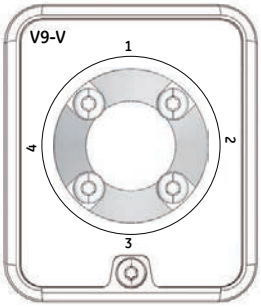
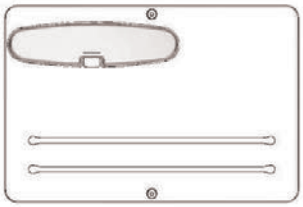
Optional modules

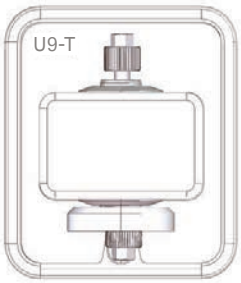
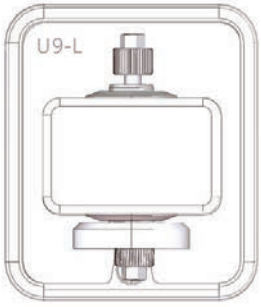
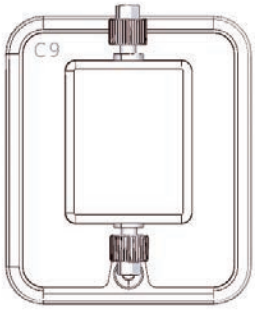
Module	Description
Inlet valve **V9-IA** or **V9H-IA** 	Inlet valve for System pump A with seven inlet ports and integrated air sensor. For further information, refer to *Section 2.4.4 Inlet valves, on page 47*.
Inlet valve **V9-IB** or **V9H-IB** 	Inlet valve for System pump B with seven inlet ports and integrated air sensor. For further information, refer to *Section 2.4.4 Inlet valves, on page 47*.
Inlet valve **V9-IAB** or **V9H-IAB** 	Inlet valve with two A inlet ports and two B inlet ports. No integrated air sensor. For further information, refer to *Section 2.4.4 Inlet valves, on page 47*.

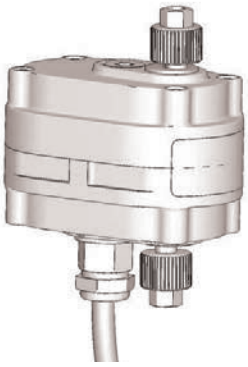
Module	Description
Sample inlet valve **V9-IS** or **V9H-IS** 	Inlet valve with eight inlet ports (seven sample inlets and one buffer inlet) and integrated air sensor. The Sample inlet valve requires an external Sample pump module. For further information, refer to *Section 2.4.4 Inlet valves, on page 47*.
Inlet valve **V9-IX** or **V9H-IX** 	Inlet valve with eight inlet ports. No integrated air sensor. For further information, refer to *Section 2.4.4 Inlet valves, on page 47*.
Mixer valve **V9-M** or **V9H-M** 	Directs the flow to the Injection valve, bypassing the Mixer, or to the Injection valve via the Mixer. For further information, refer to *Section 2.4.5 Mixer valve, on page 54*.

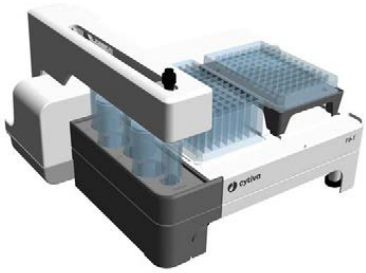
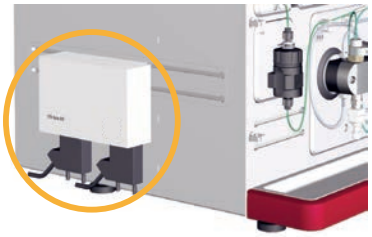
Module	Description
Loop valve **V9-L** or **V9H-L** 	Enables the use of up to five loops connected to the instrument. For further information, refer to *Section 2.4.8 Loop valve, on page 63*.
Column valve **V9-C** or **V9H-C** and **V9-C2** or **V9H-C2** 	**V9-C** or **V9H-C** can connect up to five columns to the instrument. Up to ten columns can be connected by installing the optional column valves **V9-C2** or **V9H-C2** . The column valves direct the flow to one column at a time and feature two integrated pressure sensors. The valves allow the user to choose flow direction through the column, or to bypass the column. For further information, refer to *Section 2.4.9 Column valves, on page 66*.
Column valve **V9-Cm** 	Connects up to three columns to the instrument. The column valves direct the flow to one column at a time. The valves allow the user to choose flow direction through the column, or to bypass the column. For further information, refer to *Section 2.4.9 Column valves, on page 66*.

Module	Description
Column valve **V9-Cs** or **V9H-Cs** 	Connects a single column to the instrument. Allows the user to chose flow direction through the column, or to bypass the column. For further information, refer to *Section 2.4.9 Column valves, on page 66*.
pH valve **V9-pH** or **V9H-pH** 	Enables the pH electrode to be included in the flow path or bypassed during a run. The pH electrode may be calibrated when installed in the pH valve. For further information, refer to *Section 2.4.11 pH valve, on page 75*.
Outlet valve **V9-O** or **V9H-O** 	Directs the flow to the Fraction collector, Fraction collector 2 (out 10), any of the ten outlet ports, or waste. For further information, refer to *Section 2.4.12 Outlet valves, on page 80*.

Module	Description
Outlet valve **V9-Os**, **V9M-Os** or **V9H-Os** 	Directs the flow to the Fraction collector, Fraction collector 2, the outlet port, or waste. For further information, refer to *Section 2.4.12 Outlet valves, on page 80*.
Versatile valve **V9-V** or **V9H-V** 	A 4-port, 4-position valve, which can be used when adding extra features to the flow path. For further information, refer to *Section 2.4.10 Versatile valve, on page 74*.
UV monitor **U9-M** 	Measures the UV/Vis absorbance at up to three wavelengths simultaneously in the range 190 to 700 nm. For further information, refer to *Section 2.4.14 UV monitors, on page 87*.

Module	Description
UV monitor **U9-T** 	Measures the UV absorbance at the fixed wavelengths of 280 nm, or 260 nm and 280 nm. For further information, refer to *Section 2.4.14 UV monitors, on page 87*.
UV monitor **U9-L** 	Measures the UV absorbance at a fixed wavelength of 280 nm. For further information, refer to *Section 2.4.14 UV monitors, on page 87*.
Conductivity monitor **C9** or **C9M** 	Measures the conductivity of buffers and eluted proteins. For further information, refer to *Section 2.4.15 Conductivity monitors, on page 92*.

Module	Description
External air sensor **L9-1.5** or **L9-1.2** 	Detects air in the flow path. For further information, refer to *Section 3.1 External air sensors, on page 119*.
Fraction collector F9-C 	Flexible fraction collector that can collect up to 576 fractions. Up to two fraction collectors can be connected to the system. Fraction collector F9-C can only be installed as the primary fraction collector. For further information, refer to *Section 3.2 Fraction collector F9-C, on page 122*.
Fraction collector F9-R 	Round fraction collector that can collect up to 175 fractions. Up to two fraction collectors can be connected at the same time. Fraction collector F9-R can be used both as primary and secondary fraction collector. For further information, refer to *Section 3.3 Fraction collector F9-R, on page 135*.

Module	Description
Fraction collector F9-T 	Flexible fraction collector that can collect up to 192 fractions. Up to two fraction collectors can be connected to the system. Fraction collector F9-T can only be installed as the primary fraction collector. For further information, refer to *Section 3.4 Fraction collector F9-T, on page 143*.
I/O-box **E9** 	Receives analog or digital signals from, or transfers analog or digital signals to, external equipment that has been connected to the system. For further information, refer to *Section 3.6 I/O-box E9, on page 157*.
Sample pump **S9** or **S9H** 	A high precision pump with an integrated pressure monitor. The sample pump delivers buffer or sample in purification runs. For further information, refer to *Section 3.5 Sample pump S9 and S9H, on page 152*.

2.2 Liquid flow path

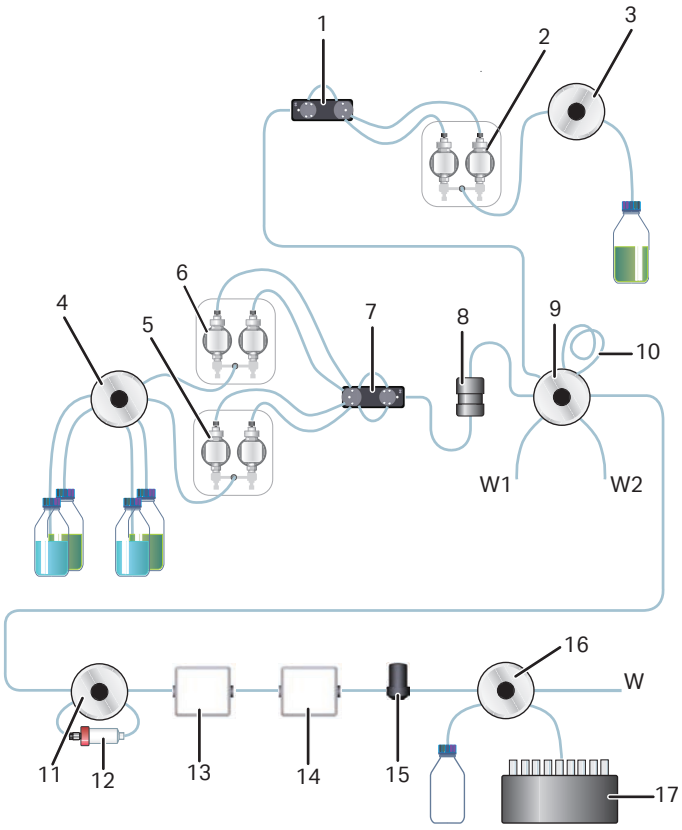
Introduction

ÄKTA pure is a liquid chromatography system with a flexible flow path.

This section provides an overview of the liquid flow path, and its possibilities.

Example of a typical liquid flow path

The illustration below shows the flow path for a typical instrument configuration. The individual instrument modules are presented in the table below. The configuration of the system is defined by the user.



Part	Description
1	Pressure monitor
2	Sample pump
3	Sample inlet valve
4	Inlet valve
5	System pump B
6	System pump A
7	Pressure monitor
8	Mixer
9	Injection valve
10	Sample loop or Superloop™
11	Column valve
12	Column
13	UV monitor
14	Conductivity monitor
15	Flow restrictor
16	Outlet valve
17	Fraction collector
W, W1, W2	Waste

2.3 Instrument control panel

Introduction

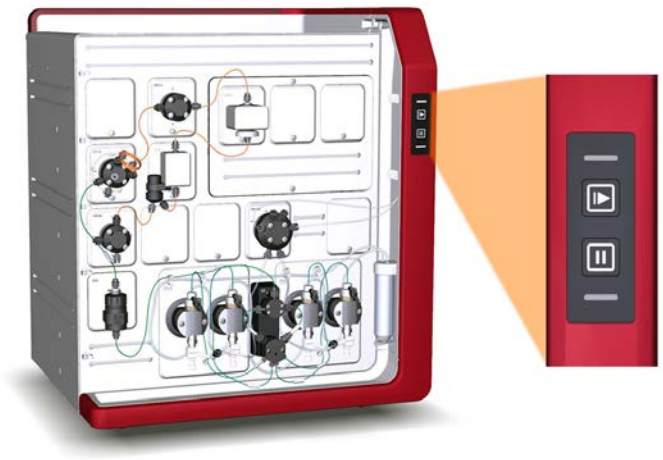
This section describes the design and main function of the Instrument control panel **B9**.

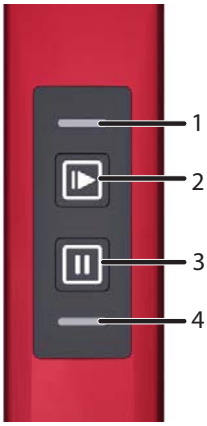
Function of the Instrument control panel

The Instrument control panel shows the current state of the system. The **Pause** and **Continue** buttons can be used to control an ongoing run. It is possible to lock and unlock the Instrument control buttons from UNICORN.

Location and illustration

The illustration below shows the location and detailed view of the Instrument control panel.





Part	Description
1	**Power/Communication** indicator (white)
2	**Continue** button with a green light indicator
3	**Pause** button with an orange light indicator
4	**Alarm and error** indicator (red)

Buttons

The Instrument control panel includes the following buttons:

Part	Description
	Resumes instrument operation from the following states: • ***Pause*** • ***Wash*** • ***Hold***
	Pauses the run and stops all pumps.

Status indications

The light indicators on the Instrument control panel indicate the current status of ÄKTA pure.

The table below describes the different states that can be displayed.

Display	State	Description
All light indicators are off. 	***Off***	The instrument is off.
The **Power/Communication** indicator flashes slowly. 	***Power-on***	The instrument has no communication with the Instrument server.
The **Power/Communication** indicator flashes quickly. 	***Connecting***	The system is starting up.
The **Power/Communication** indicator displays a constant light. 	***Ready***	The instrument is ready to use.

Display	State	Description
Both the **Power/Communication** indicator and **Continue** button display a constant light. 	***Run***	A run is ongoing.
The **Power/Communication** indicator displays a constant light and the **Continue** button flashes slowly. 	***Wash***	A wash instruction or a pump synchronization is ongoing.
	Hold	A run has been put on hold.
Both the **Power/Communication** indicator and **Pause** button display a constant light. 	***Pause***	A run has been paused.

Display	State	Description
The **Power/Communication** indicator displays a constant light and the **Alarm and error** indicator flashes. 	***Alarms and errors***	The system has been paused due to an alarm. To resume the run, acknowledge the alarm and continue the run in UNICORN.
The **Power/Communication** indicator displays a pulsating light. 	***Power-save***	The system is in power-saving mode.
All indicators are lit in a wave pattern. 	***Re-programming***	A module is being re-programmed to be compatible with the current instrument configuration.

2.4 Instrument modules

Introduction

This section describes the design and main functions of the instrument modules.

In this section

Section		See page
2.4.1	System pumps	40
2.4.2	Mixer	44
2.4.3	Overview of the valves	46
2.4.4	Inlet valves	47
2.4.5	Mixer valve	54
2.4.6	Injection valves V9-Inj and V9H-Inj	57
2.4.7	Injection valve V9M-J	61
2.4.8	Loop valve	63
2.4.9	Column valves	66
2.4.10	Versatile valve	74
2.4.11	pH valve	75
2.4.12	Outlet valves	80
2.4.13	Pressure monitors	84
2.4.14	UV monitors	87
2.4.15	Conductivity monitors	92
2.4.16	Flow restrictor	94

2.4.1 System pumps

Introduction

This section describes the design and main functions of the system pumps, and also the pump piston rinsing systems. The system can also be equipped with an external, optional sample pump, see *Section 3.5 Sample pump S9 and S9H, on page 152*.

Function of the system pumps

The ÄKTA pure instrument is fitted with two high precision system pumps, System pump A and System pump B. The system pumps can be used individually, or in combination to generate isocratic or gradient elution in purification methods.

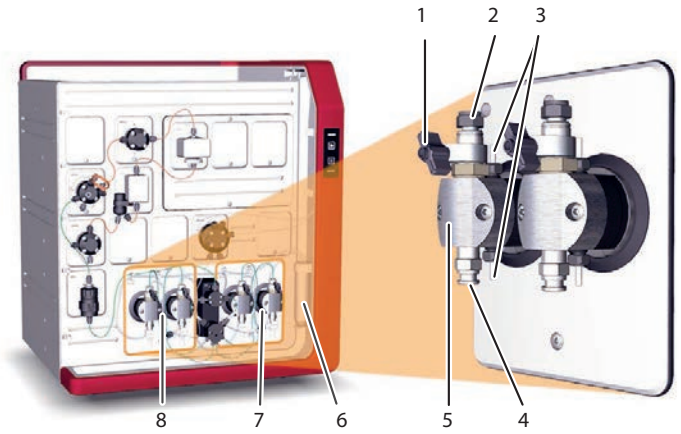
Each pump module consists of two pump heads that work alternately to give a continuous, low pulsation, liquid delivery. To make sure delivery of correct liquid volume, the pumps must be free from air. Each pump head is equipped with a purge valve that is used for this purpose. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.

The table below contains the operating limits and labels of the system pumps of ÄKTA pure 25 and ÄKTA pure 150, respectively.

Configuration	Label	Pump type	Flow rate	Max. pressure
ÄKTA pure 25	**P9 A** and **P9 B**	P9	0.001 – 25 mL/min ***Note:*** *When running the **Column packing flow** instruction, the maximum flow rate is 50 mL/min.*	20 MPa
ÄKTA pure 150	**P9H A** and **P9H B**	P9H	0.01 – 150 mL/min ***Note:*** *When running the **Column packing flow** instruction, the maximum flow rate is 300 mL/min.*	5 MPa

Location and illustration

The illustration below shows the location of System pump A and System pump B, together with a detailed view of a system pump.



Part	Description
1	Purge valve: Used to remove air from the pump
2	Outlet port with check valve
3	Connections to pump piston rinsing system: Tubing is connected between the pumps and the Pump piston rinsing system tube (6)
4	Inlet port with check valve
5	Pump head: Encapsulates the inner parts of the pump
6	Pump piston rinsing system tube
7	System pump B
8	System pump A

The pump piston rinsing system

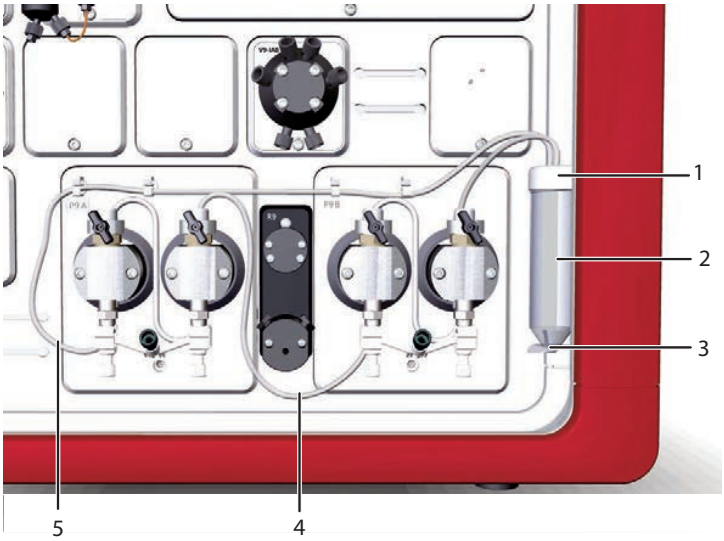
A seal prevents leakage between the pump chamber and the drive mechanism. The seal is continuously lubricated by the presence of liquid. The pump piston rinsing system continuously flushes the low pressure chamber behind the piston with a low flow of 20% ethanol. This prevents salts from aqueous buffers to contaminate the pistons, and prolongs the working life of the seals.

The pump piston rinsing system tubing is connected to the rearmost holes on the pump heads.

For instructions on how to fill the rinsing system, see *Prime the system pump piston rinsing system, on page 445*.

Illustration of the system pump piston rinsing system

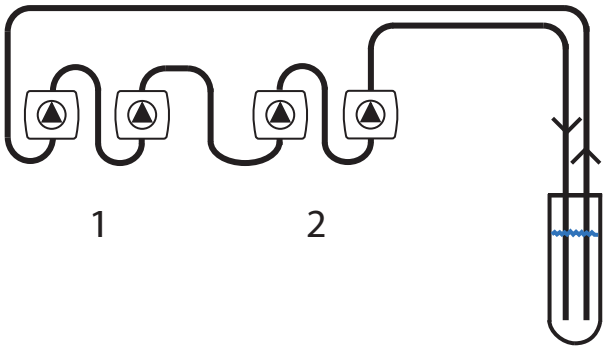
The illustration below show the parts and tubing of the system pump piston rinsing system.



Part	Description
1	Rinsing system tube holder, top
2	Rinsing system tube
3	Rinsing system tube holder, bottom
4	Outlet tubing
5	Inlet tubing

System pump rinsing systems flow path

The illustration below shows the tubing configuration of the pump piston rinsing system of the system pumps.



Part	Description
1	System pump A
2	System pump B

2.4.2 Mixer

Function of the Mixer M9

Mixer **M9** is located after System pump A and System pump B, and before the Injection valve.

The Mixer **M9** is a dynamic mixer for high-performance gradients. It is used to make sure that the buffers from the System pumps are mixed to give a homogenous buffer composition.

The mixer chamber has a built-in filter that prevents particles from reaching the column.

An online filter can be placed on the Mixer **M9** module by using the Online filter clamp. The online filter must only be used with ÄKTA pure micro and the red tubing kit.

The mixer chamber contains an identification chip that transmits the internal volume to UNICORN. This value is stored in the result file.



CAUTION

Explosion risk. Do not use Mixer **M9** with the Mixer chamber 15 mL on an ÄKTA pure 25 system configuration. The maximum pressure for Mixer chamber 15 mL is 5 MPa.

Note: *Replace the sealing ring of the mixer with a highly resistant O-ring if the system is going to be exposed to organic solvents or high concentrations of organic acids for longer periods of time. Refer to* *Section 7.5.5 Replace the O-ring inside the mixer, on page 409* *for how to change the ring.*

Location and illustration

The illustration below shows the location, together with a detailed view of Mixer **M9**.



Part	Description
1	Outlet
2	Mixer chamber (0.6, 1.4, 5 or 15 mL)
3	Inlet

2.4.3 Overview of the valves

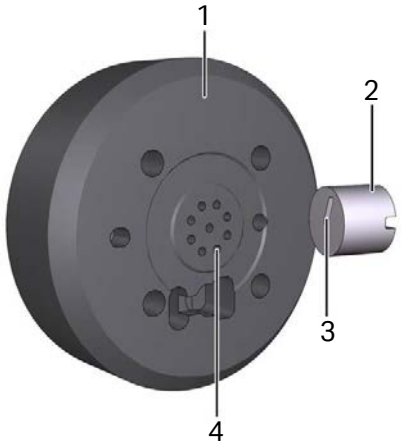
General design and function of rotary valves

The valves of the ÄKTA pure instrument allow flexibility in the liquid flow path.

All valves used in the ÄKTA pure instrument are rotary valves. The motorized rotary valve consists of a valve connection block with a number of defined bores with channels to the inlet and outlet ports of the valve. The rotary disc, mounted on the motor, has a number of defined channels. The pattern of channels of the rotary disc together with the pattern and location of the ports of the valve connection block, define the flow path and function of each type of valve. When the rotary disc turns, the flow path in the valve changes.

Illustration of valve components

The illustration below shows the components of a disassembled valve. Inlet valve A is used as an example.



Part	Description
1	Valve connection block (stator)
2	Rotary disc (rotor)
3	Defined channel(s) in the Rotary disc
4	Defined bores in the Valve connection block

Note: *Inlet and outlet ports are not visible in the picture. They are located on the opposite side of the Valve connection block.*

2.4.4 Inlet valves

Function of the inlet valves

The inlet valves are used to select which buffers or samples to use in a run. The inlet valves available for ÄKTA pure and their functions are described in the table below.

Inlet valve	Label		Description
	ÄKTA pure 25	ÄKTA pure 150	
Inlet valve A	**V9-IA**	**V9H-IA**	Enables automatic change between different buffers and wash solutions (seven inlet ports) . Can be used together with Inlet valve B to generate gradients by mixing buffer from System pump A and buffer from System pump B.
Inlet valve B	**V9-IB**	**V9H-IB**	Enables automatic change between different buffers and wash solutions (seven inlet ports). Can be used together with Inlet valve A to generate gradients by mixing buffer from System pump A and buffer from System pump B.
Inlet valve AB	**V9-IAB**	**V9H-IAB**	Enables automatic change between different buffers and wash solutions (two A and two B inlet ports). Can be used to generate gradients by mixing buffer from System pump A and buffer from System pump B.
Sample inlet valve	**V9-IS**	**V9H-IS**	Enables automatic loading of up to seven samples when used together with a sample pump.
Inlet valve X	**V9-IX**	**V9H-IX**	Increases the total number of inlets to the system. Can be used in two different configurations X1 and X2. To be able to select X2, X1 needs to be enabled.

The air sensors integrated in Inlet valve A, Inlet valve B, and Sample inlet valve detect the presence of air and prevent the air from entering the pump.

Inlet valve AB and Inlet valve IX lack built-in air sensors, but can be used together with external air sensors.

Combination of Inlet valves

The modular design of ÄKTA pure allows the use of several combinations of Inlet valves.

The possible combinations of Inlet valve A, Inlet valve B and Inlet valve AB are:

- One Inlet valve A
- One Inlet valve B
- Inlet valve A and Inlet valve B
- Inlet valve AB together with Inlet valve A or Inlet valve B
- One Inlet valve AB
- No installed Inlet valves

The Sample inlet valve can be used together with any of the combinations listed above.

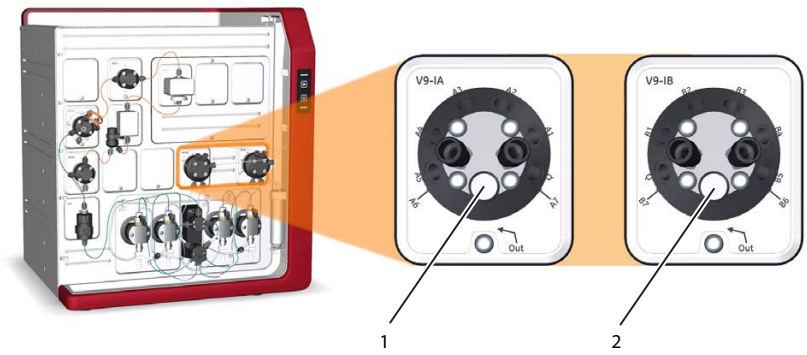
Location of inlet valves

The locations of the inlet valves are described in the following table:

Inlet valve	Location
Inlet valve A	Before System pump A
Inlet valve B	Before System pump B
Inlet valve AB	Before both System pump A and System pump B
Sample inlet valve	Before the Sample pump
Inlet valve IX	For example, before another inlet valve

Illustration of Inlet valve A and Inlet valve B

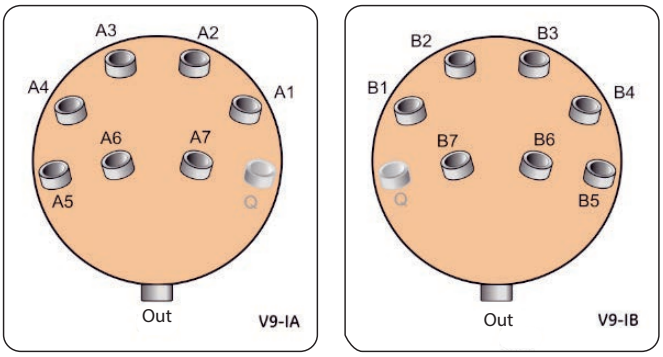
The illustration below shows a detailed view of Inlet valve A and Inlet valve B.



Part	Description
1	Integrated air sensor of Inlet valve A (located under the plug)
2	Integrated air sensor of Inlet valve B (located under the plug)

Ports of Inlet valve A and Inlet valve B

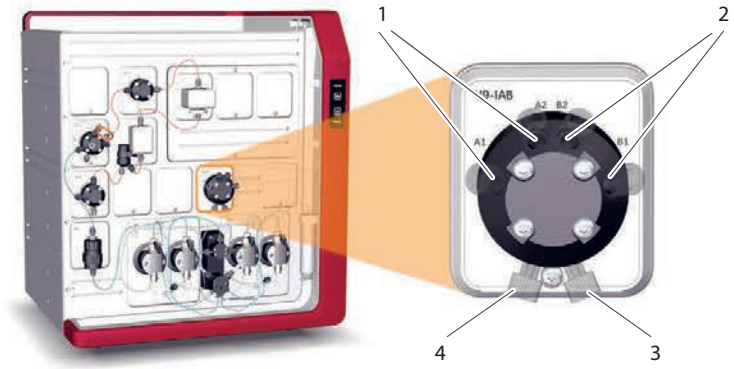
The illustration below shows the ports of Inlet valve A and Inlet valve B.



Port	Description
A1-**A7**	Buffer inlets of Inlet valve A
B1-**B7**	Buffer inlets of Inlet valve B
Q	Not used for ÄKTA pure
Out	To the respective System pump

Illustration of Inlet valve AB

The illustration below shows a detailed view of Inlet valve AB.

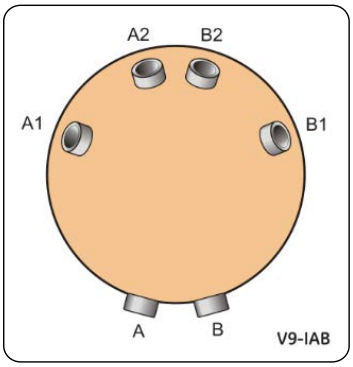


Part	Description
1	A inlet ports
2	B inlet ports
3	Outlet port to System pump B
4	Outlet port to System pump A

Note: *Inlet valve AB does not have any integrated air sensor.*

Ports of Inlet valve AB

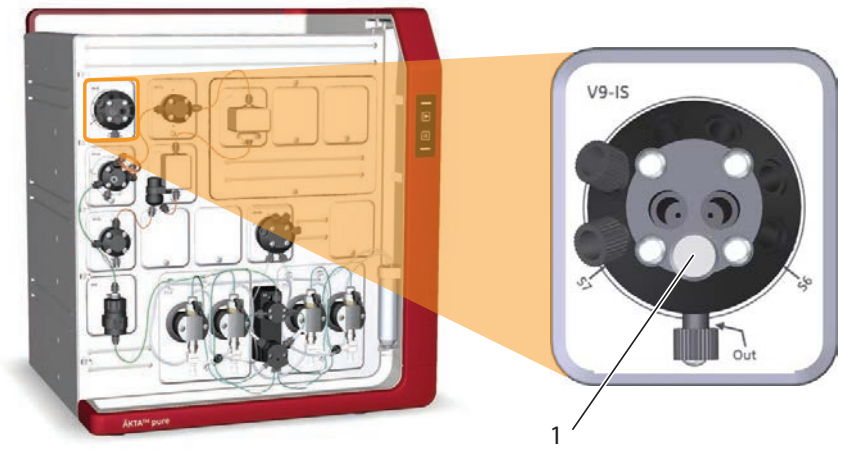
The illustration below shows the ports of Inlet valve AB.



Port	Description
A1-A2	Inlet ports **A1** and **A2** of Inlet valve AB are used when buffers or samples must be delivered to System pump A
B1-B2	Inlet ports **B1** and **B2** of Inlet valve AB are used when buffers or samples must be delivered to System pump B
A	Outlet port **A** of Inlet valve AB leads to System pump A
B	Outlet port **B** of Inlet valve AB leads to System pump B

Illustration of Sample inlet valve

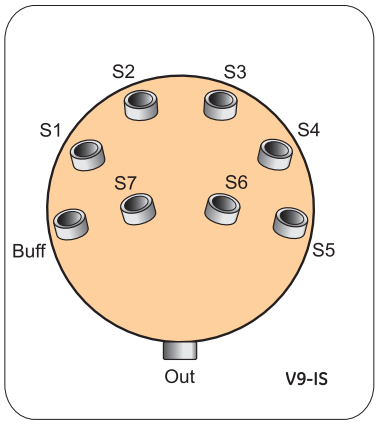
The illustration below shows a detailed view of the Sample inlet valve.



Part	Description
1	Integrated air sensor (located under the plug)

Ports of Sample inlet valve

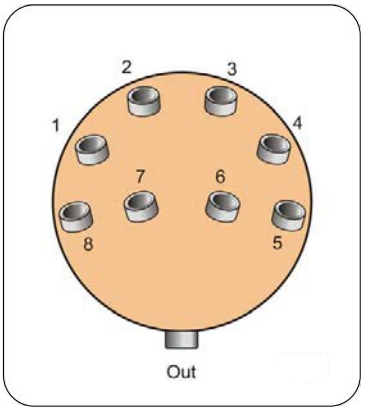
The illustration below shows the ports of Sample inlet valve.



Port	Description
S1-S7	Sample inlets
Buff	Buffer inlet
Out	To Sample pump

Ports of Inlet valve IX

The illustration below shows the ports of Inlet valve IX.



Port	Description
1-8	Inlets
Out	For example, to another inlet valve

Note: *Inlet valve IX does not have an integrated air sensor.*

Connect tubing

The table below shows the tubing and connectors that is delivered together with the optional inlet valves.

The connectors used in all occasions are:

- for ÄKTA pure 25: 5/16" with Ferrule (yellow), 1/8"
- for ÄKTA pure 150: 5/16" with Ferrule (blue), 3/16"

Tubing label	Connection	Tubing		Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150	
A1-**A2** and **B1**-**B2**	Inlets to Inlet valve AB	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500
A1-**A7**	Inlets to Inlet valve A	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500
B1-**B7**	Inlets to Inlet valve B	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500
InA	From Inlet valve A or Inlet valve AB to System pump A	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	300
InB	From Inlet valve B or Inlet valve AB to System pump B	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	300
S1-**S7**	Sample inlets to Sample inlet valve	FEP o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1000
Buff	Buffer inlet to Sample inlet valve	FEP o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1000
InS	Sample inlet valve to Sample Pump	FEP o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	580

Note: *Narrow inlet tubing is available for* **S1-S7**. *Refer to Section 9.3 Tubing and connectors, on page 536 for more information.*

2.4.5 Mixer valve

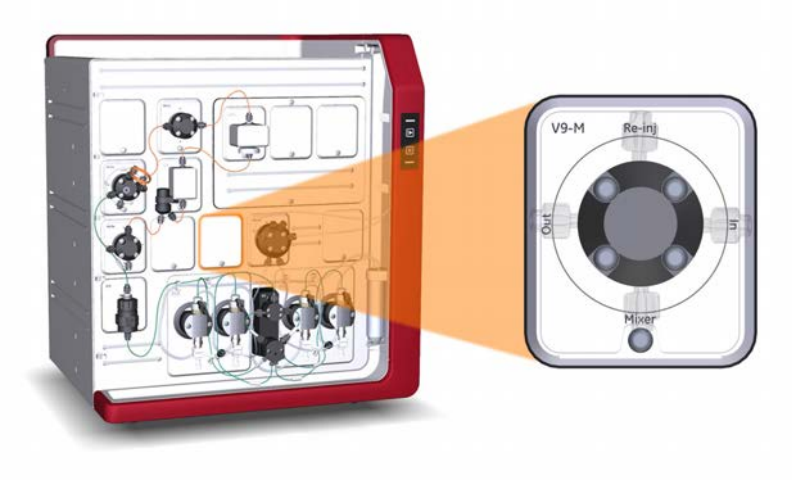
Function of mixer valve

Mixer valve (**V9-M** or **V9H-M**) allows the user to bypass the mixer. It is intended to be used when the System pump is used for sample application or when a sample is re-injected.

Note: *Mixer valve (**V9-M** or **V9H-M**) cannot be used together with Sample pump **S9** or Sample pump **S9H**.*

Location and illustration of mixer valve

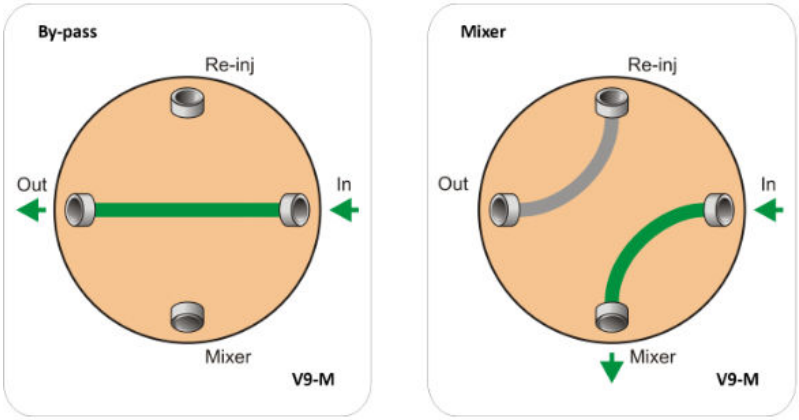
The illustration below shows the recommended location, together with a detailed view of the mixer valve.



Ports and flow paths of the mixer valve

The Mixer valve (**V9-M**, **V9H-M**) has two available flow paths; ***By-pass***, and ***Mixer*** (default). If the mixer valve is installed in the recommended location before the mixer, ***By-pass*** allows the flow to bypass the mixer, and ***Mixer*** directs the flow from the system pumps to the mixer.

The illustration below shows the different flow paths through the Mixer valve **V9-M**.



Port	Description
In	Port in which the flow enters the valve. Must be connected to the System pressure monitor outlet.
Out	Port from which the flow leaves the mixer valve and bypasses the mixer. Connect to the injection valve **SaP** port.
Re-inj	Port for advanced configurations including re-injection.
Mixer	Port from which the flow leaves the valve and is directed to the mixer.

Connect tubing

The table below shows recommended tubing and connectors.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
3-1	Pressure monitor R9 to Mixer valve V9-M/V9H-M port **In**	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.00 mm	Fingertight connector, 1/16"	160
3-2	Mixer valve V9-M/V9H-M port **Mixer** to Mixer M9	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.00 mm	Fingertight connector, 1/16"	330
3-3	Mixer valve V9-M/V9H-M port **Out** to Injection valve V9-Inj/ V9H-Inj port **SaP**	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.00 mm	Fingertight connector, 1/16"	260

2.4.6 Injection valves V9-Inj and V9H-Inj

Function the injection valve

The injection valve is used to direct sample onto the column. The valve enables a number of different sample application techniques.

The injection valve is labeled **V9-Inj** or **V9H-Inj**.

A sample loop or a Superloop can be connected to the injection valve and filled either automatically, using a Sample Pump or System pump A, or manually, using a syringe. The sample can also be injected directly onto the column using a Sample pump, or System pump A together with the mixer valve.

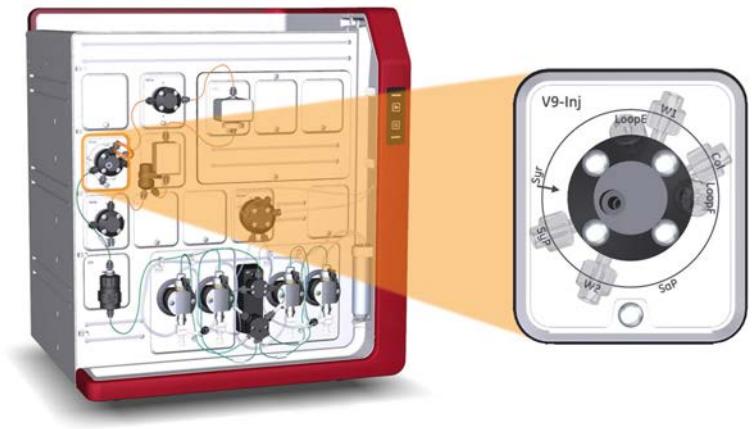
For instructions on how to connect and use loops, see *Section 5.7 Sample application, on page 242*.

Note: *Make sure that the inlet ports **SaP** and **Syr** are plugged with a stop plug when not in use.*

Note: *In order to avoid sample carry-over when switching techniques for loading samples, wash the injection valve with buffer between the loading of two different samples. See Cleaning the injection valve, on page 259.*

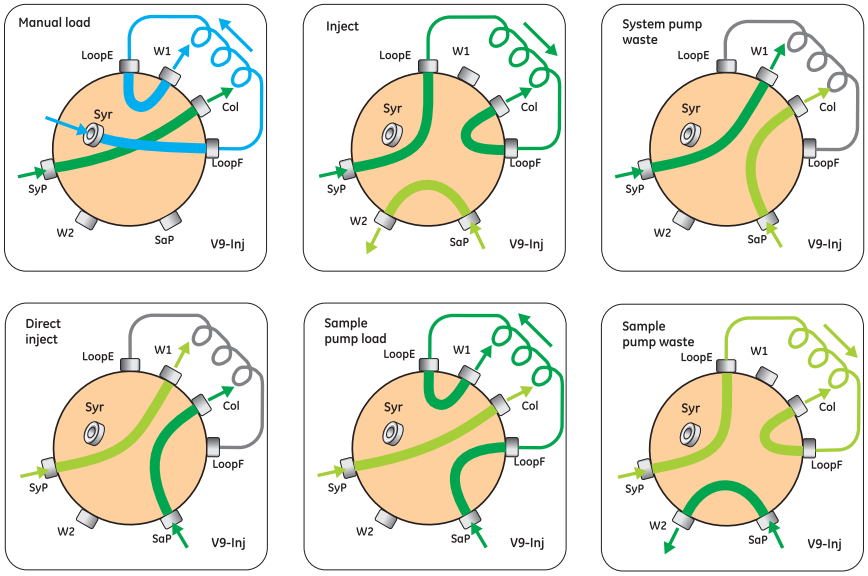
Location and illustration of Injection valves V9-Inj and V9H-Inj

The illustration below shows the location, together with a detailed view of the Injection valve.



Ports and flow paths of Injection valves V9-Inj and V9H-Inj

The injection valve can be set to different positions that give different flow paths through the valve, as shown in the illustration and tables below.


Manual load
Inject
System pump waste
Direct inject
Sample pump load
Sample pump waste
LoopE
W1
Col
Syr
LoopF
SyP
W2
SaP
V9-Inj
Primary flow path
Alternative flow path
Flow path for manual load
Closed flow path

Port	Description
SaP	Inlet from • Sample pump, or • System pump via the mixer valve **Out** port.
SyP	Inlet from the System pumps via the mixer
Syr	Syringe connection
Col	Outlet to one of the column valves or to the column.
LoopF	Port for connection of a loop. Used to fill the loop.
LoopE	Port for connection of a loop. Used to empty the loop into the flow path.
W1	Loop and System pump waste
W2	Sample flow waste

Flow path	Description
Manual load - Default position of the valve	The system flow is directed onto the column or column valve. Sample can be manually injected into the loop. Excess sample leaves the valve through waste port **W1**.
Inject	The system flow is directed through the loop and onto the column or column valve. If a mixer valve or the sample pump is used, the flow entering the **SaP** port is directed to waste port **W2**.
System pump waste	The system flow is directed to waste port **W1**. If a Mixer valve or the Sample pump is used, the flow entering the **SaP** port is directed to the column or the Column valve.
Direct inject	The flow entering the **SaP** port is directed to the column or the Column valve. This position is used with: • the Sample pump, or • a Mixer valve and System pump A, bypassing the mixer. Flow entering the **SyP** port is directed to waste port **W1**.
Sample pump load	The flow entering the **SaP** port is directed to the loop. This position is used with: • the Sample pump, or • a Mixer valve and System pump A, bypassing the mixer. Excess sample leaves the valve through waste port **W1**. The flow entering the **SyP** port is directed to the column or the Column valve.
Sample pump waste	The flow entering the **SaP** port is directed to waste port **W2**. This position is used with: • the Sample pump, or • a Mixer valve and System pump A. The flow entering the **SyP** port is directed to the column via the loop.

2.4.7 Injection valve V9M-J

Function

The micro injection valve **V9M-J** is used for manual micro injection or sample injection using an autosampler. It has minimal channel contribution to the loop volume.

Note: *Make sure that the inlet port **Syr** is plugged with a stop plug when not in use.*

Note: *In order to avoid sample carry-over when switching techniques for loading samples, wash the injection valve with buffer between the loading of two different samples. See* *Cleaning the injection valve, on page 259**.*

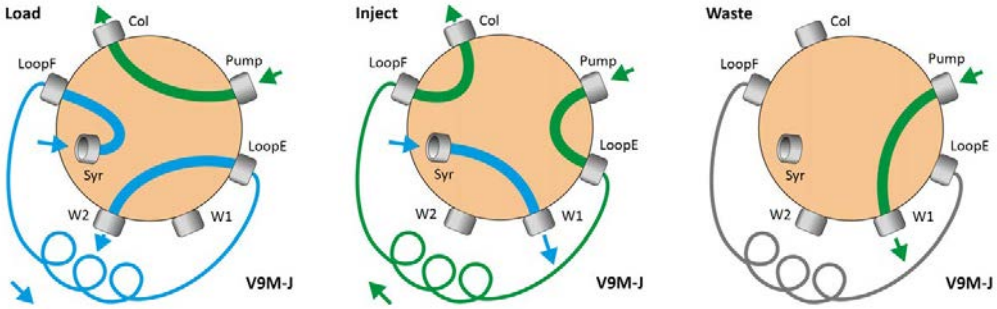
Location and illustration of Injection valve V9M-J

The illustration below shows the location, together with a detailed view of micro injection valve **V9M-J**.



Ports and flow paths of Injection valve V9M-J

The Injection valve can be set to different positions that give different flow paths through the valve, as shown in the illustration and tables below.



Primary flow path

Flow path for manual load

Closed flow path

Port	Description
Pump	Inlet from the System pumps
Syr	Syringe connection
Col	Outlet to the column
LoopF	Port for connection of a loop. Used to fill the loop.
LoopE	Port for connection of a loop. Used to empty the loop into the flow path.
W1	System pump waste
W2	Loop waste

Flow path	Description
Load – Default position of the valve	The flow is directed onto the column or column valve. Sample can be loaded manually into loop through the syringe port **Syr**. Excess sample leaves through the waste port **W2**.
Inject	The flow is directed through the loop and onto the column or column valve. The syringe port can be washed manually in this position.
Waste	The flow is directed to waste through the **W1** port. This flow path is used for performing a pump wash.

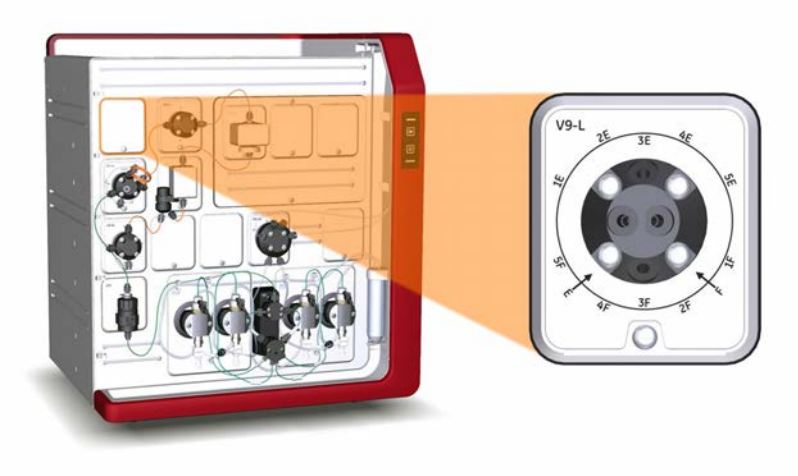
2.4.8 Loop valve

Function of the loop valve

The Loop valve allows the user to connect several loops simultaneously to the instrument. It can for example be used for storing intermediate fractions in multi-step purifications, for storing samples to be used in scouting runs, or for storing eluents needed in low volumes. The valve also has a built-in bypass function that enables bypassing all loops. The loop valve is labeled **V9-L** or **V9H-L**.

Location and illustration of loop valve

The illustration below shows the recommended location, together with a detailed view of Loop valve **V9-L**.

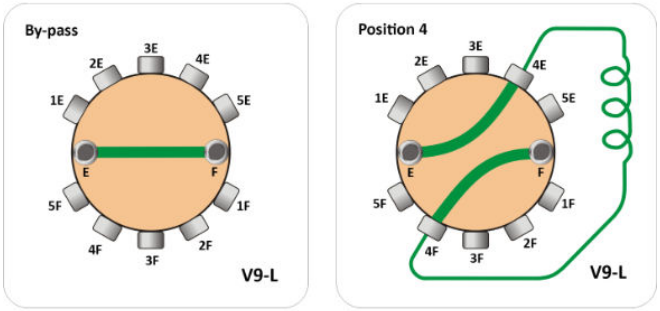


Note: *It is possible to place the loop valve in other positions in the flow path than the one described above. However, in that case, the volume in predefined phases, methods, and* ***Loop wash*** *will be incorrect, as well as the system configuration shown in the process picture.*

Ports and flow paths of the loop valve

The illustration and tables below describe the ports and different flow paths through the loop valve.

In the image below that is labeled Position 4, the sample loop is connected to port **4E** and **4F**. In this position, the sample loop can be emptied.



Primary flow path

Port	Description
F	Port connected to the **LoopF** port of the Injection valve.
1F and **1E**	Ports for connection to loop 1.
2F and **2E**	Ports for connection to loop 2.
3F and **3E**	Ports for connection to loop 3.
4F and **4E**	Ports for connection to loop 4.
5F and **5E**	Ports for connection to loop 5.
E	Port connected to the **LoopE** port of the Injection valve.

Note: *Ports labeled with the letter F are used for filling the loop and ports labeled with the letter E are used for emptying the loop.*

Flow path	Description
Position ***1-5***	The flow direction depends on the Injection valve position.
By-pass	The flow bypasses the loop(s). ***By-pass*** is the default flow path.

Connect tubing

The table below shows recommended tubing and connectors.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
L1	Injection valve position **LoopF** to Loop valve position **F**	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	Fingertight connector, 1/16"	160
L2	Injection valve position **LoopE** to Loop valve position **E**	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	Fingertight connector, 1/16"	160

Connect a loop valve

Instead of using a loop connected to the injection valve, a loop valve can be connected.

Follow the steps below to connect a loop valve.

Step	Action
1	Connect port **E** on the loop valve to port **LoopE** on the Injection valve.
2	Connect port **F** on the loop valve to port **LoopF** on the Injection valve.
3	Connect one or many loops to the loop valve. See *Section 5.7 Sample application, on page 242*. ***Note:*** *Always use the the first positions of the valve for the connected loops (e.g., if three loops will be used, use port* **1F**-**3F** *and the corresponding ports* **1E**-**3E***) to avoid cross-contamination.*

2.4.9 Column valves

Function of the column valves

The column valves are used to connect columns to the system, and to direct the flow onto the column. The column valves available for ÄKTA pure and their functions are described in the table below.

Label in		Description
ÄKTA pure 25	**ÄKTA pure 150**	
V9-C	**V9H-C**	Connects columns to the system and allows the user to choose column, flow direction through the column, or to bypass the columns. Up to five columns can be connected to the valve.
V9-C2	**V9H-C2**	Optional column valve allows up to ten columns to be connected when combined with **V9-C** or **V9H-C**. The user can choose column, flow direction through the column, or to bypass the columns.
V9-Cm	N/A	Connects columns to the system and allows the user to choose column, flow direction through the column, or to bypass the columns. Up to three columns can be connected to the valve.
V9-Cs	**V9H-Cs**	Connects one column to the system and allows the user to choose flow direction through the column, or to bypass the column.

The inlet and outlet ports of Column valves **V9-C**, **V9H-C** and **V9-C2**, **V9H-C2** have built in pressure sensors that measure the actual pressure over the column. For further information on the pressure sensors, see *Function of pressure monitors integrated in Column valves V9-C or V9H-C, on page 85*.

Column valves **V9-Cm**, **V9-Cs** and **V9H-Cs** have no pressure sensors. See *Section 5.5 Connect a column, on page 233* for information on how to set the pressure alarm to protect the column when using **V9-Cm**, **V9-Cs** or **V9H-Cs**.

Location of system with one column valve

The following illustration shows the recommended location of the column valve, when one column valve is installed.



Part	Description
1	Column valve **V9-Cm**, **V9-Cs** or **V9H-Cs** (no integrated pressure sensors)
2	Column valve **V9-C** or **V9H-C** (integrated pressure sensors)

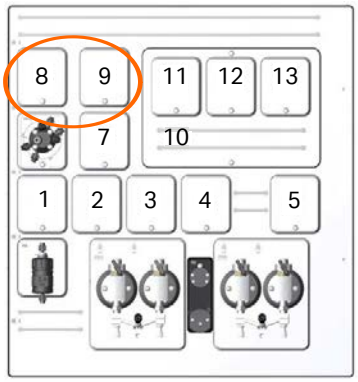
Location of system with two column valves

The standard configuration for ÄKTA pure is with one column valve.

Column valves **V9-C** or **V9H-C** have 5 column positions. To increase the number of column positions to 10, **V9-C** or **V9H-C** must be combined with a second column valve (either **V9-C2** or **V9H-C2**).

Use positions 8 and 9 to install two column valves with the shortest possible flow path. Install the primary Column valve (**V9-C** or **V9H-C**) in position 8. Install the secondary Column valve (**V9-C2** or **V9H-C2**) in position 9.

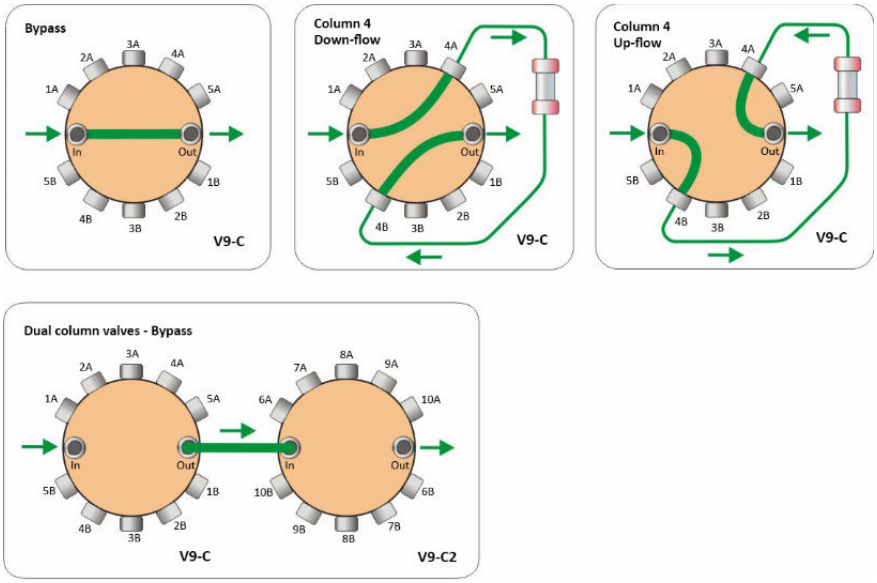
Note: *Do not install the secondary column valve before the primary column valve in the flow path. If you do, the pre-column, post-column and delta-column pressure sensors will show incorrect data.*



Ports and flow paths of the Column valves V9-C and V9H-C

The illustration and tables below describe the different ports of and flow paths through Column valves **V9-C**, **V9H-C**, **V9-C2** and **V9H-C2**. In the example below the column is connected to column position 4 and the valve is labeled **V9-C**.

Note: *The valves need to be placed in the exact order as shown in the below image. If not, then the pre-pressure, post-pressure and delta-pressure sensors will show incorrect readings.*



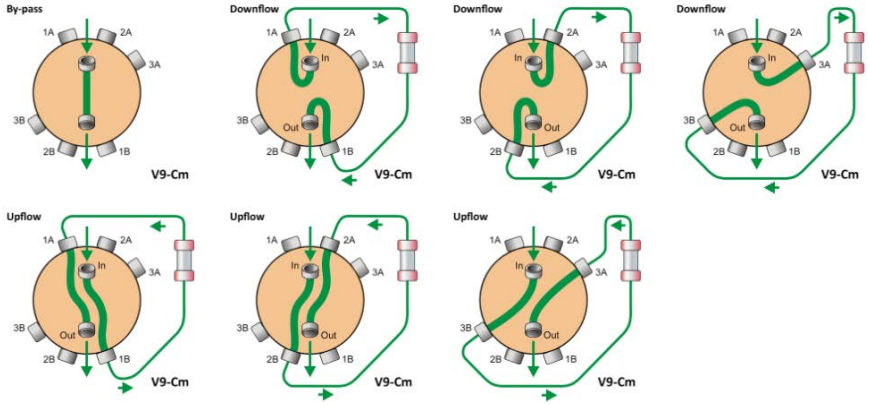
Primary flow path

Port	Description
In	Inlet from Injection valve via a built-in pressure monitor.
1A-5A	Ports for connection to the top of columns.
1B-5B	Ports for connection to the bottom of columns.
6A-10A	Ports for connection to the top of columns on the secondary Column valve.
6B-10B	Ports for connection to the bottom of columns on the secondary Column valve.
Out	Outlet to UV monitor via a built-in pressure monitor.

Flow path	Description
By-pass	The flow bypasses the column(s). ***By-pass*** is the default flow path.
Down flow	The flow direction is from the top of the column to the bottom of the column. Down flow is the default flow direction.
Up flow	The flow direction is from the bottom of the column to the top of the column.

Ports and flow paths of the Column valve V9-Cm

The illustration and tables below describe the different ports of and flow paths through Column valve **V9-Cm**.



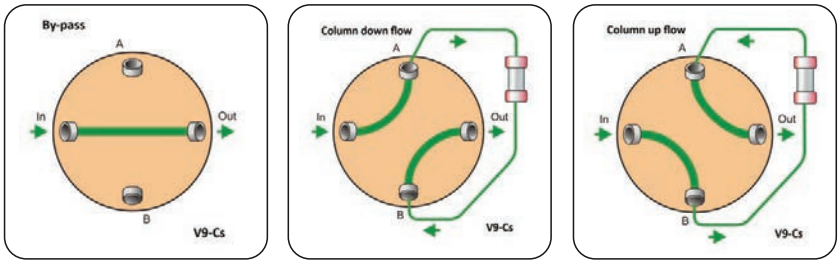
Primary flow path

Port	Description
In	Inlet from Injection valve.
1A-3A	Ports for connection to the top of columns.
1B-3B	Ports for connection to the bottom of columns.
Out	Outlet to UV monitor.

Flow path	Description
By-pass	The flow bypasses the column(s). ***By-pass*** is the default flow path.
Down flow	The flow direction is from the top of the column to the bottom of the column. Down flow is the default flow direction.
Up flow	The flow direction is from the bottom of the column to the top of the column.

Ports and flow paths of Column valves V9-Cs and V9H-Cs

The illustration and tables below describe the different ports and flow paths of Column valve **V9-Cs**.



Primary flow path

Port	Description
In	Inlet from Injection valve.
1A	Port for connection to the top of a column.
1B	Port for connection to the bottom of a column.
Out	Outlet to UV monitor.

Flow path	Description
By-pass	The flow bypasses the column. ***By-pass*** is the default flow path.
Down flow	The flow direction is from the top of the column to the bottom of the column. Down flow is the default flow direction.
Up flow	The flow direction is from the bottom of the column to the top of the column.

Connect tubing

The table below shows recommended tubing and connectors for a system with one column valve.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)	
		ÄKTA pure 25	ÄKTA pure 150		V9-Cm	All other column valves
5	Injection valve port **Col** to Column valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	180	160
6	Column valve port **Out** to UV monitor	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	180	160

The table below shows recommended tubing and connectors for a system with two column valves.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		V9-C, V9H-C
5	Injection valve port **Col** to primary Column valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	170
5C2	Primary Column valve port **Out** to secondary Column valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	120
6	Secondary Column valve port **Out** to UV monitor	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	160

Connect a column valve

Follow the steps below to connect tubing to the column valves.

Step	Action
1	Remove the Union F/F between tubing **5** and tubing **6**.
2	Connect tubing **5** from the Injection valve to the column valve. ***Note:*** *The built-in pressure sensors for Column valves* **V9-C**, **V9-C2**,**V9H-C** *and* **V9H-C2** *have to be re-calibrated after installation. See* *Calibrating the pressure sensors, on page 351**.*
3	If applicable, connect tubing **5C2** from the primary column valve to the secondary column valve. ***Note:*** *When using two column valves, combine* **V9-C** *with* **V9-C2**, *or* **V9H-C** *with* **V9H-C2**. *The other column valves cannot be used to set up a system with two column valves.* ***Note:*** *When using two column valves, only the pre-column pressure sensor on the first column valve and the post-column pressure sensor on the second column valve are used.*
4	Connect tubing **6** from the column valve to the UV monitor.

2.4.10 Versatile valve

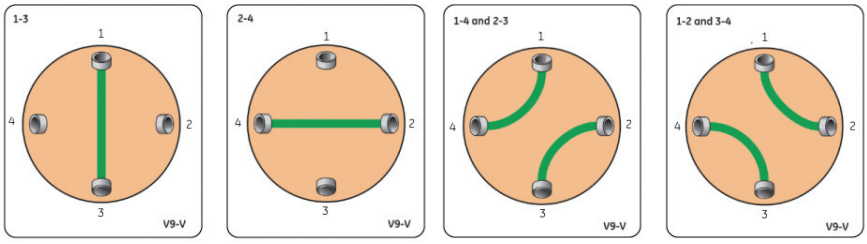
Function of the versatile valve

The versatile valve is a 4-port, 4-position valve, which can be used to add extra features to the flow path. For example, the valve can be used to connect external equipment to the flow path during parts of a run. The versatile valve can be installed in any position. The versatile valve is labeled **V9-V** or **V9H-V**.

It is possible to install up to four versatile valves simultaneously in ÄKTA pure. The configuration is defined by the module's Node ID. The default position of the versatile valve can be set in ***System Settings***.

Ports and flow paths of the versatile valve

The illustration and table below describe the different ports of and flow paths through the versatile valve. The valve has four ports (**1**-**4**).



Primary flow path

The versatile valve has four available sets of flow paths; two where a single flow channel is used and two where the flow can be directed through two different channels simultaneously.

Flow path	Description
1-3	A single flow channel where the flow is directed between port **1** and port **3**.
2-4	A single flow channel where the flow is directed between port **2** and port **4**.
1-4 and 2-3	Two simultaneously used flow channels where the flow is directed between port **1** and port **4** and between port **2** and port **3**.
1-2 and 3-4	Two simultaneously used flow channels where the flow is directed between port **1** and port **2** and between port **3** and port **4**.

2.4.11 pH valve

Function of the pH valve

The pH valve is used to direct the flow to a pH electrode when inline monitoring of pH is desired during a run. The pH valve is labeled **V9-pH** or **V9H-pH**.

The pH valve has an integrated flow cell in which the pH electrode can be installed.

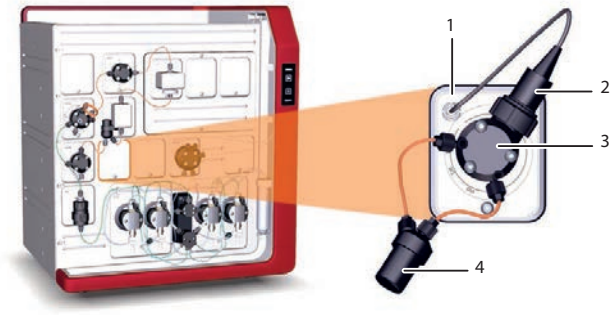
A flow restrictor must be connected to the pH valve. The Flow restrictor is used to generate a back pressure high enough to prevent the formation of air bubbles in the UV flow cell.

The valve directs the flow to the pH electrode and to the Flow restrictor, or bypasses one or both.

Note: *The flow restrictor is normally included in the flow path after the conductivity monitor. When installing the pH valve on ÄKTA pure, the flow restrictor must be moved from its normal position on the conductivity monitor to the pH valve.*

Location and illustration of the pH valve

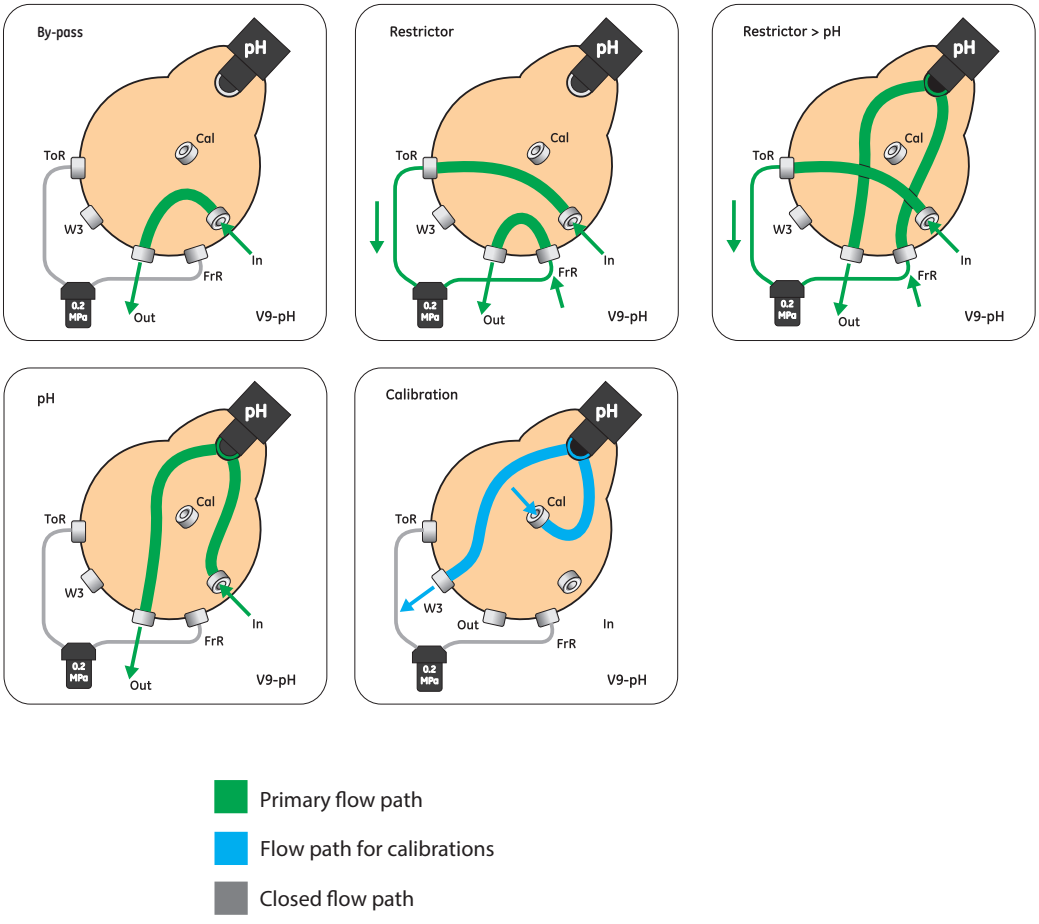
The illustration below shows the recommended location, together with a detailed view of the pH valve.



Part	Description
1	pH valve
2	pH electrode
3	pH flow cell
4	Flow restrictor

Ports and flow paths of the pH valve

The illustration and table below describe the different ports of and flow paths through the pH valve.



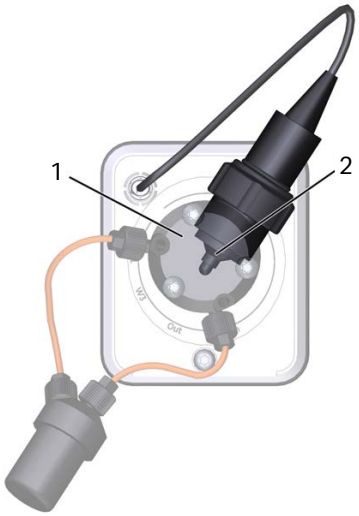
Port	Description
In	From conductivity monitor
ToR	To flow restrictor
FrR	From flow restrictor
Out	To outlet valve
Cal	Calibration port
W3	To waste

Flow path	Description
By-pass	Both pH electrode and Flow restrictor are bypassed.
Restrictor	Flow restrictor is in use and pH electrode is bypassed.
Restrictor and pH	Both pH electrode and Flow restrictor are in use.
pH	pH electrode is in use and Flow restrictor is bypassed.
Calibration	Flow path used when calibrating the pH monitor and when filling the pH flow cell with storage solution. The **Cal** port is used to inject solution into the flow cell using a syringe. Excess solution leaves the valve through port **W3**.

pH monitor

The pH monitor continuously measures the pH of the buffer and eluted proteins when the pH electrode is inline. A pH electrode can be installed in the pH flow cell. For instruction on how to install the pH electrode, see *Section 7.5.3 Replace the pH electrode, on page 407*. The pH electrode must not be exposed to more than 0.5 MPa during a normal run, but can withstand pressure spikes of 0.8 MPa. It is therefore important to place it after the column.

The illustration below shows the location of the pH flow cell and a pH electrode installed in the pH valve.



Part	Description
1	pH flow cell
2	pH electrode

Connect tubing

The table below shows recommended tubing.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
8pH	Conductivity monitor to pH valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	180
1R	pH valve port **ToR** to Flow restrictor port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	80
2R	Flow restrictor port **Out** to pH valve port **FrR**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	80
9pH	pH port **Out** to Outlet valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	160
W3	pH valve port **W3** to Waste	ETFE i.d. 1 mm	ETFE i.d. 1 mm	Fingertight connector, 1/16"	1500

2.4.12 Outlet valves

Function of the outlet valves

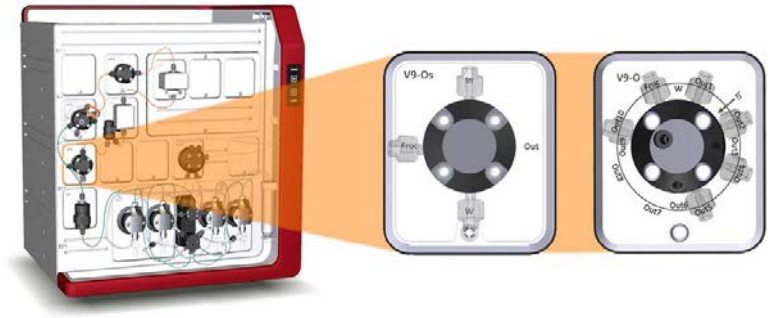
The outlet valve is used to direct the flow to the fraction collector, to an outlet port, or to waste. The table below shows the labeling of the outlet valves for ÄKTA pure 25, ÄKTA pure 150, and ÄKTA pure micro.

Label in		Description
ÄKTA pure 25	**ÄKTA pure 150**	
V9-O	**V9H-O**	10 outlet ports
V9-Os	**V9H-Os**	1 outlet port
V9M-Os (Micro)	N/A	1 outlet port

Note: *The outlet valve* **V9M-Os** *for ÄKTA pure micro is selected as outlet valve* ***V9-Os*** *in UNICORN.*

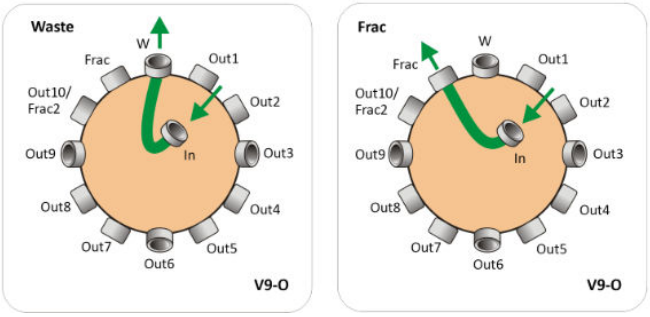
Location and illustration of outlet valves

The illustration below shows the recommended location, together with a detailed view of the outlet valves.



Ports of Outlet valves V9-O and V9H-O

The illustration below shows the ports of Outlet valve **V9-O** and **V9H-O**.

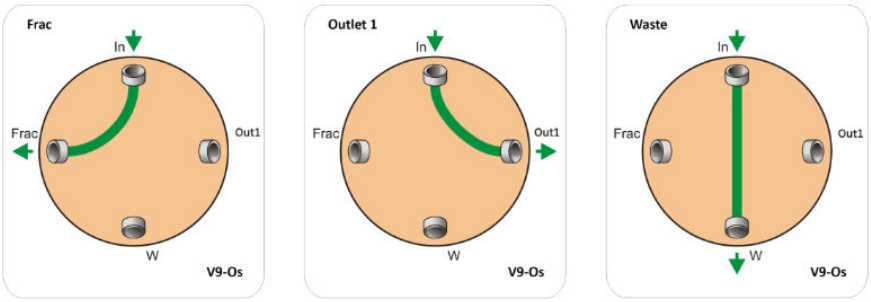


Primary flow path

Port	Description
In	Inlet port
Out1 - **Out10**	Outlet ports 1 - 10
Frac	Port to fraction collector ***Note:*** *If a secondary Fraction collector* **F9-R** *is used it must be connected to port* **Out10**.
W	Waste port

Ports of Outlet valves V9-Os, V9H-Os and V9M-Os

The illustration below shows the ports of Outlet valve **V9-Os**, **V9H-Os** and **V9M-Os**.



Primary flow path

Port	Description
In	Inlet port
Out1	Outlet port
Frac	Port to fraction collector ***Note:*** *If a secondary* **Fraction collector F9-R** *is used it must be connected to port* **Out1**.
W	Waste port

Connect tubing

The table below shows recommended tubing and connectors.

For tubing for the micro outlet valve **V9M-Os**, see *Install the tubing kit, on page 112*.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
9	Flow restrictor port **Out** to Outlet valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	135

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
9pH (Only used if pH valve is installed)	pH valve port **Out** to Outlet valve port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	160
Out1 - **Out10** (Tubing **Out2** - **Out10** are only used with **V9-O**/**V9H-O**)	Outlets of the Outlet valves	ETFE, o.d. 1/8", i.d. 1 mm	ETFE, o.d. 1/8", i.d. 1 mm	Fingertight connector, 1/16"	1500

Note: *If ÄKTA pure previously has been configured without an outlet valve, remove the Union F/F between tubing* **9** *and tubing* **W** *before installation of the outlet valve. Then connect tubing* **9** *to the* **In** *port on the outlet valve and the waste tubing* **W** *to the* **W** *port on the outlet valve.*

2.4.13 Pressure monitors

Introduction

This section describes the location and function of the pressure monitors.

Up to four pressure monitors are included in ÄKTA pure.

Function of the system pump and the sample pump pressure monitors

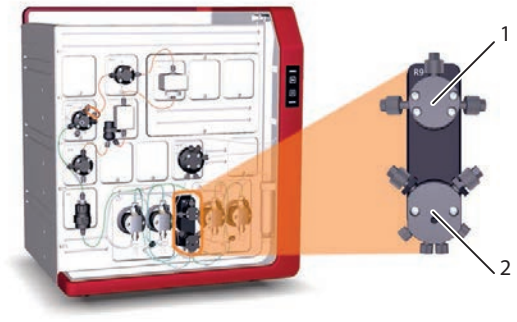
One pressure monitor is always connected to the system pumps (**P9 A**, **P9H A**, **P9 B** or **P9H B**), and one pressure monitor is part of the sample pump (**S9** or **S9H**). The monitors of the pumps are both labelled **R9**.

The system pump pressure monitor measures the pressure after the system pumps, the system pressure. The pressure monitor of the sample pump measures the pressure after the sample pump, the sample pressure. To provide a reading of the highest pressure in the system there are connections to all pump cylinders.

The pressure monitor also contains a pump flow restrictor in order to stop siphoning effects. Siphoning effects can occur if tubing in the system is disconnected while the inlet tubing is fully put into the buffer that is placed on a higher level than the pump.

Location and illustration of System pressure monitor

The illustration below shows the location, together with a detailed view of System pressure monitor.



Part	Description
1	Pressure monitor
2	Pump flow restrictors

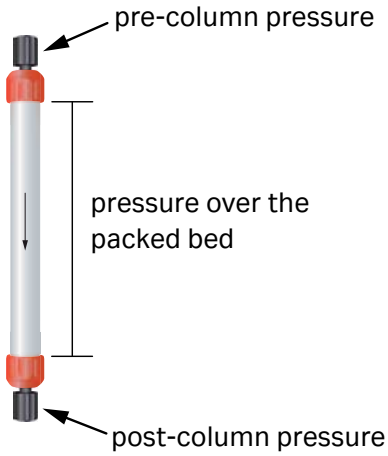
Function of pressure monitors integrated in Column valves V9-C or V9H-C

Pressure monitors are integrated into the Column valves: **V9-C**, **V9H-C**, **V9-C2** , and **V9H-C2**. Column valves **V9-Cm**, **V9-Cs** and **V9H-Cs** do not have pressure monitors.

Pressure monitors are integrated in the inlet and outlet ports of the column valve to measure pre-column pressure, and post-column pressure. The delta-column pressure, or pressure drop, is the pressure over the packed resin bed.

Pressure alarms can be set for both the pre-column pressure and the delta-column pressure. Pressure control of flow can use either the pre-column pressure or the delta-column pressure.

The table below shows the pressure monitor settings when the instrument is configured with one or two column valves.



Module	Label	Settings	
		Node ID	Pressure monitor
First column valve	**V9-C** or **V9H-C**	5	N/A
Pre-column pressure monitor in Column valve **V9-C** and **V9H-C**	N/A	N/A	2
Post-column pressure monitor in Column valve **V9-C** and **V9H-C**	N/A	N/A	3
Second column valve	**V9-C2** or **V9H-C2**	6	N/A
Pre-column pressure monitor in Column valve **V9-C2** and **V9H-C2**	N/A	N/A	4
Post-column pressure monitor in Column valve **V9-C2** and **V9H-C2**	N/A	N/A	5

Change settings

To change the settings of a pressure monitor, follow the steps below.

Step	Action
1	Set the pre-column and post-column pressure monitors by turning the arrows on the switches on the left side and right side of the module with a screwdriver.
2	Set the Node ID for the column valve by turning the arrows in the two rotating switches at the back of the module. See *Set the Node ID, on page 647*.

2.4.14 UV monitors

Introduction

This section describes the design and function of UV monitors **U9-M**, **U9-T**, and **U9-L**.

The modules include a monitor unit, and a detector with a UV flow cell.

Function of UV monitor U9-M

UV monitor **U9-M** measures the UV absorbance at a wavelength range of 190 to 700 nm. The ratio between the UV absorbance of the secondary wavelength (***UV 2***) and the primary wavelength (***UV 1***) can be displayed in the ***Chromatogram*** pane in UNICORN.

A flip-mode enables measuring of UV/Vis absorbance at three wavelengths simultaneously during a run.

The measurement of the second and third wavelength can be switched on and off in:

- ***Method Editor →Phase Properties →Method settings***
- ***System Control →Manual Instructions***
- ***System Control →System →Settings***

If not needed during a run, the UV lamp can be shut off with a manual instruction in UNICORN. The lamp starts automatically for the next run.

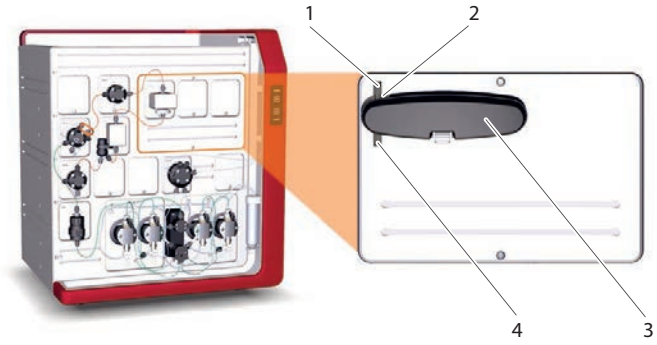
Note: *Installation of UV monitor* **U9-M** *must be performed by Cytiva Service personnel.*

Note: *The resolution is decreased when more than one wavelength is used simultaneously due to lower sampling frequency per wavelength. Do not use more wavelengths than necessary.*

Location and illustration of UV monitor U9-M

The illustration below shows the location of UV monitor **U9-M**, together with a detailed view of the monitor unit and detector. The monitor unit is labelled **U9-M** and the UV detector **U9-D**.

Note: *When UV monitor* **U9-M** *is used, the entire Multi-module panel shown in the illustration is replaced by* **U9-M**.



Part	Description
1	Inlet
2	UV flow cell. Three different path lengths are available: 0.5 mm, 2 mm (default) and 10 mm
3	UV detector
4	Outlet

Function of UV monitor U9-T

The UV monitor **U9-T** measures the UV absorbance at the fixed wavelengths of 280 nm, or 260 nm and 280 nm. The ratio between the UV absorbance at 260 nm and 280 nm can be displayed in the ***Chromatogram*** pane in UNICORN.

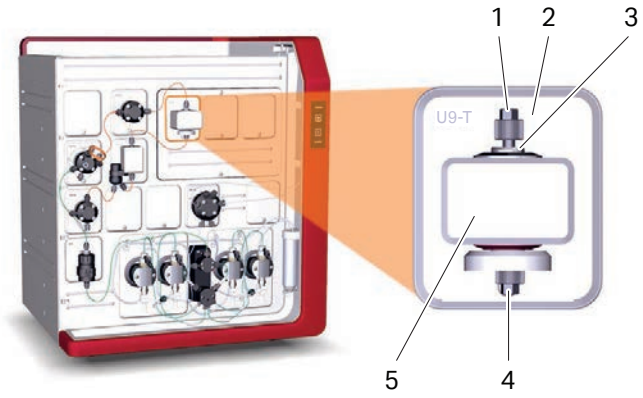
The measurement of wavelength at 260 nm can be switched on and off in:

- ***Method Editor*** →***Phase Properties*** →***Method settings***
- ***System Control*** →***Manual Instructions***
- ***System Control*** →***System*** →***Settings***

If not needed during a run, the UV lamp can be shut off with a manual instruction in UNICORN. The lamp starts automatically for the next run.

Location and illustration of UV monitor U9-T

The illustration below shows the recommended location of UV monitor **U9-T**, together with a detailed view of the monitor and detector. It is recommended to install the Multi-module panel when using the UV monitor **U9-T**, see *Example of a typical configuration of the wet side, on page 19*. The UV monitor can also be installed in an Extension box, see *Extension box, on page 117*.



Part	Description
1	Inlet
2	UV monitor **U9-T**
3	UV flow cell. Three different path lengths are available: 0.4 mm, 2 mm (default) and 5 mm
4	Outlet
5	UV detector

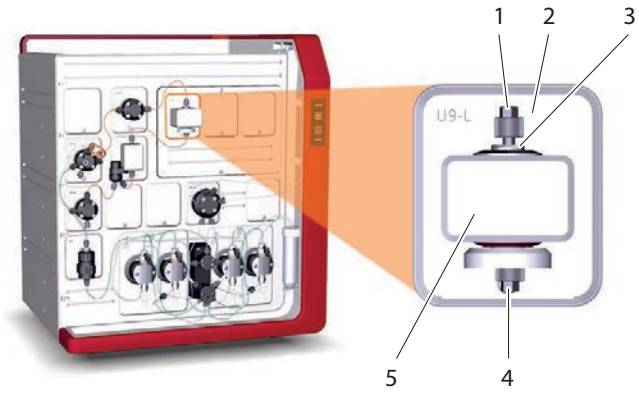
Function of UV monitor U9-L

The UV monitor **U9-L** measures the UV absorbance at the fixed wavelength of 280 nm.

It is not possible to vary the wavelength. Therefore it is not shown in the ***Phase Properties*** pane of the ***Method Settings Phase*** in ***Method Editor***. The UV lamp can be shut off with a manual instruction in UNICORN if not needed during a run. The lamp starts automatically for next run.

Location and illustration of UV monitor U9-L

The illustration below shows the recommended location of UV monitor **U9-L**, together with a detailed view of the monitor and detector. It is recommended to install the Multi-module panel when using the UV monitor **U9-L**, see *Example of a typical configuration of the wet side, on page 19*. The UV monitor can also be installed in an Extension box, see *Extension box, on page 117*.



Part	Description
1	Inlet
2	UV monitor **U9-L**
3	UV flow cell. Three different path lengths are available: 0.4 mm, 2 mm (default) and 5 mm
4	Outlet
5	UV detector

Using two UV monitors

It is possible to use two UV monitors in ÄKTA pure. See *Section 4.2.9 Configuration of UV monitors, on page 189* for more information.

Connect tubing

The table below shows the tubing and connectors to connect one UV monitor, and when using a column valve.

If the column is connected directly to the UV monitor, if a secondary UV monitor is used, or if the UV monitor is connected to Column valve **V9-Cm**, the tubing needs to be cut manually. Move the tubing label to the new tubing. A male/male connector can be used to connect some columns directly onto the UV monitor.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
6	Column valve port **Out** to UV monitor	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	160[1]
7	UV monitor to Conduc-tivity monitor	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	170[2]

[1] To perform a run with the Column valve **V9-Cm**, a longer tubing **6** is needed. Replace the 160 mm tubing to the UV monitor with tubing that is 180 mm long.

[2] To perform a run in the reverse flow direction (***Up flow***) through UV monitor **U9-T** or **U9-L**, a longer tubing **7** is needed. Replace the 170 mm tubing from the UV monitor with tubing that is 210 mm long and adjust the delay volume accordingly. For example, changing from 170 mm to 210 mm for 0.5 mm i.d. tubing increases the delay volume with 8 µL. See *Set the delay volume, on page 203*.

2.4.15 Conductivity monitors

Function of the conductivity monitors

The conductivity monitors continuously measure the conductivity of buffers and eluted proteins. The monitor for ÄKTA pure 25 and ÄKTA pure 150 is labelled **C9**. The monitor for the micro kit is labelled **C9M**.

Note: *The Conductivity monitor* ***C9M*** *for ÄKTA pure micro is selected as Conductivity monitor* ***C9*** *in UNICORN.*

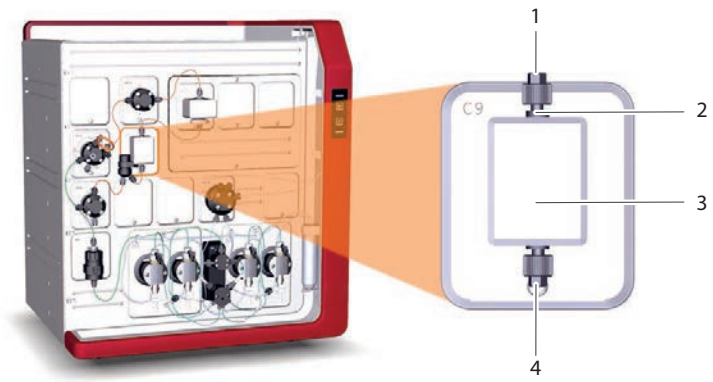
The conductivity flow cell has two electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the eluent.

The conductivity is automatically calculated by multiplying the measured conductance by the cell constant of the flow cell. The cell constant is factory-calibrated on delivery but can be re-calibrated if needed, see *Section 7.3.3 Calibrate the conductivity monitor, on page 355*.

As variation in temperature influences conductivity readings, the conductivity flow cell is fitted with a temperature sensor that measures the temperature of the eluent. A temperature compensation factor is used to report the conductivity in relation to a set reference temperature.

Location and illustration of the conductivity monitor

The illustration below shows the recommended location of Conductivity monitor **C9**, together with a detailed view of the conductivity monitor.



Part	Description
1	Inlet
2	Conductivity flow cell
3	Conductivity monitor
4	Outlet

Using two conductivity monitors

It is possible to use two conductivity monitors in ÄKTA pure. See *Section 4.2.10 Configuration of conductivity monitor, on page 191* for more information.

Connect tubing

The table below shows recommended tubing and connectors.

Tubing label	Connection	Tubing		Connector	Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150		
7	UV monitor to Conductivity monitor	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	170
8	Conductivity monitor to Flow restrictor port **In**	PEEK, o.d. 1/16", i.d. 0.50 mm (orange)	PEEK, o.d. 1/16", i.d. 0.75 mm (green)	Fingertight connector, 1/16"	95

2.4.16 Flow restrictor

Function of Flow restrictor FR-902

The flow restrictor is included in the flow path to generate a steady back pressure of approximately 0.2 MPa, to prevent formation of air bubbles in the UV flow cell.

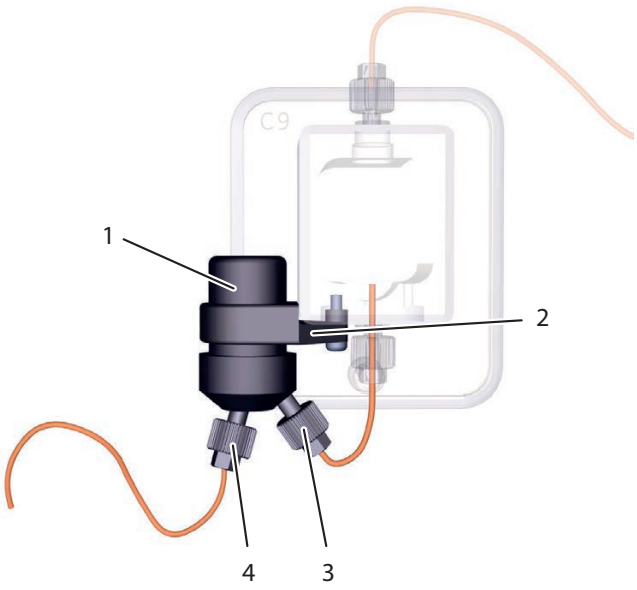
Note: *Do not remove the flow restrictor to lower the pressure in the system. There is a risk that air bubbles cause large disturbances in the UV flow cell. Use the automatic pressure control function to avoid pressure alarms, see* *Recommended pressure control parameters, on page 644**.*

Note: *Decrease the flow rate when performing a run in the cold room to prevent overpressure.*

Location and illustration of Flow restrictor FR-902

The flow restrictor is normally included in the flow path after the conductivity monitor. The conductivity monitor is equipped with a special holder for the flow restrictor.

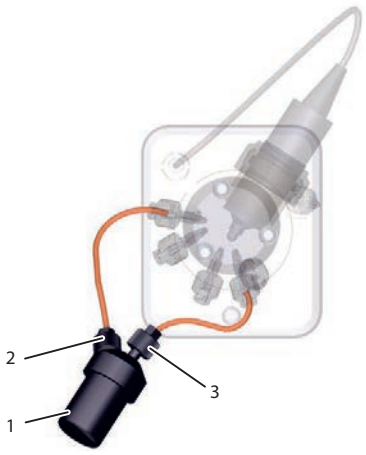
The illustration below shows Flow restrictor **FR-902** fitted on the Conductivity monitor **C9**.



Part	Description
1	Flow restrictor
2	Holder
3	Inlet
4	Outlet

If ÄKTA pure is fitted with pH valve **V9-pH** or **V9H-pH**, the flow restrictor must be moved from the conductivity monitor to the pH valve.

The illustration below shows Flow restrictor **FR-902** fitted on the pH valve.



Part	Description
1	Flow restrictor
2	Flow restrictor inlet connection from pH valve **ToR** port
3	Flow restrictor outlet connection to pH valve **FrR** port

2.5 Installation of internal modules

Introduction

Optional modules and valves are easy to install in the instrument. The existing module or Module Panel is removed with a Torx T20 screwdriver and the cable is disconnected. The cable is then connected to the optional module, which is subsequently inserted into the instrument. The newly installed module is then added to the ***System properties*** in UNICORN.

Node ID

All of the available optional modules are preconfigured to give the desired function. However, the function of a module can be changed by changing the Node ID of the module. The Node ID is a unit number designation that is used by the instrument to distinguish between several units of the same type.

In a troubleshooting situation it may be useful to check the Node ID of the module. See *Section 9.16 Node IDs, on page 645* for a list of the Node ID of modules.

Note: *The function of a module is defined by its Node ID, not by its physical position.*

The Node ID is set by positioning the arrows of the one or two rotating switches at the back of the valve. Use a screwdriver to set the arrows of the switches to the desired number.



The illustration shows an example of a valve module with two rotating switches.

- The first rotating switch, labeled **A**, sets the tens.
- The second switch, labeled **B**, sets the units.

Hardware installation of a module

The instruction below describes how to install a module in the instrument.

Note: *The illustrations show the principle how to install an optional module. The position of the module on the instrument and the used type of module will depend on the module being installed.*

CAUTION

Disconnect power. Always switch off power to the ÄKTA pure instrument before replacing any of its components, unless stated otherwise in the user documentation.

Step	Action
1	Disconnect power from the instrument by switching off the instrument power switch.
2	Loosen the connectors and remove the tubing from the existing module. ***Note:*** *This step does not apply for a Module Panel.*
3	Loosen the module with a Torx T20 screwdriver. 
4	Remove the module. 

Step	Action
5	Disconnect the cable and secure it in the slit.



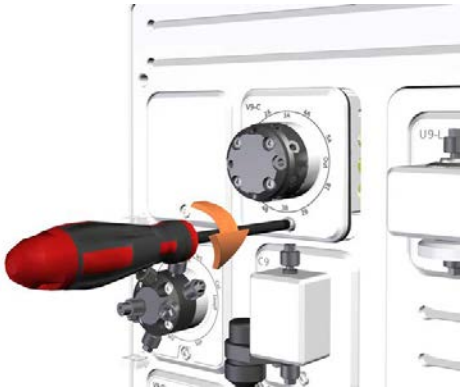
6	Connect the cable to the module to be installed.



7	Insert the module.



Step	Action
8	Fasten it with a Torx T20 screwdriver.



Note: *A warning message is displayed at start up if a module has been installed in the instrument but not added to the current system configuration in UNICORN. To update the system configuration, see* *Edit system properties, on page 175**.*

Install internal modules in the Extension box

The Extension box can be used to install extra modules on the ÄKTA pure instrument. See *Extension box, on page 117* for more information.

2.6 Accessories

Introduction

This section describes the holders, tubing kits and other available accessories.

The holders are used to attach and organize columns, tubing and bottles to the ÄKTA pure instrument. The holders are attached to the instrument using the holder rails on the left side and the front of the instrument.

The tubing kits can be used to optimize the volume of the flow path.

Other available accessories are for instance: the module panel, and the extension box.

In this section

Section		See page
2.6.1	Holders	101
2.6.2	Tubing kits	108
2.6.3	Micro kit	110
2.6.4	Other accessories	116

Introduction

This section describes the holders, tubing kits and other available accessories.

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Other available accessories are for instance: the module panel, and the extension box.

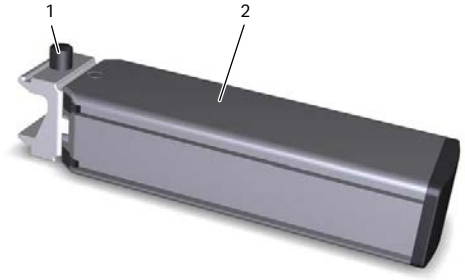
2.6.1 Holders

This section describes the holders that are available for ÄKTA pure.

Rail extension

The Rail extension rod can be used to attach accessories, eg., column holders or a Multi-purpose holder. The rod has extra rails on both sides. Push the button of the rod to attach it to a holder rail.

The illustration below shows the Rail extension rod.

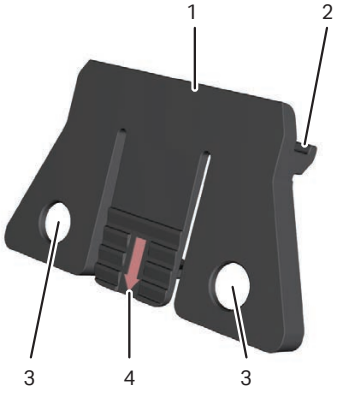


Part	Description
1	Button
2	Extension rod

Multi-purpose holder

The Multi-purpose holder can be used to attach accessories, eg., a Loop holder or a cassette. Attach the holder to a holder rail.

The illustration below shows the Multi-purpose holder.



Part	Description
1	Attachment point for accessories
2	Snap-in to holder rails
3	Attachment points for tubing holders
4	Tab

Loop holder

The Loop holder can be used to attach up to five 10 mL sample loops. Use two Multi-purpose holders to attach the holder to a holder rail.

The illustration below shows the Loop holder.

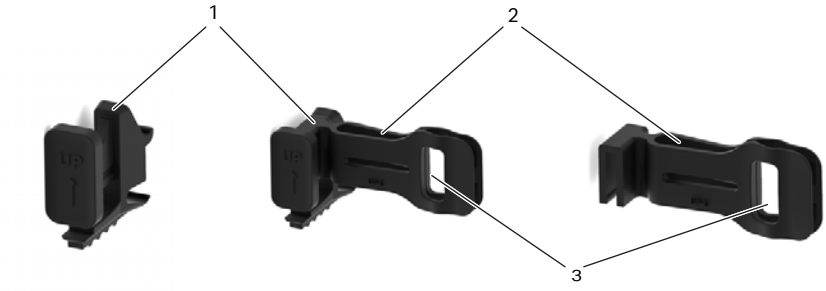


Part	Description
1	Upper attachment to multi-purpose holder
2	Lower attachment to multi-purpose holder

Multi-directional column clamp

The Multi-directional column clamp can be used to attach small sized columns with an outer diameter of 12 to 18 mm.

The illustration below shows the Multi-directional column clamp.



Part	Description
1	Base unit with snap-in to holder rails
2	Clamp unit
3	Position for column

The column can be mounted horizontally or vertically using the Multi-directional column clamp.

- Horizontal mounting: Two base units of the Multi-directional column clamp are used to mount a column horizontally on a long rail, as shown in the image below.



- Vertical mounting: One base unit and one clamp unit of the Multi-directional column clamp are used to mount a column vertically on a rail.

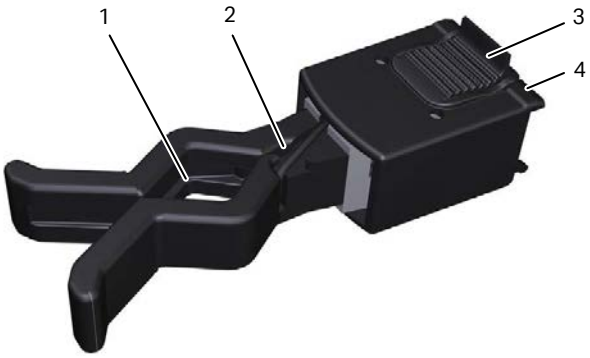
Tip: *Orient the clamp so that the name of the UV monitor,* **U9-M** *or* **U9-L***, can be read on the front of the clamp.*

- *For* **U9-M***: place the base clamp to the right of the column. Make sure that the text* **U9-M** *shows on the front of the clamp.*
- *For* **U9-T** *and* **U9-L***: place the base clamp to the left of the column. Make sure that the text* **U9-L** *shows on the front of the clamp.*

Column holder

The Column holder has one position for medium sized columns and one position for small sized columns. The Column holder can also be used for bottles. Use two holders to attach long columns.

The illustration below shows the Column holder.

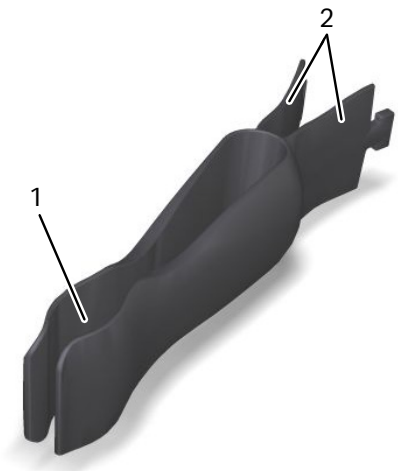


Part	Description
1	Position for a medium sized column or bottle
2	Position for a small sized column
3	Tab
4	Snap-in to holder rails

Column clamp

The column clamp can be used to attach small sized columns. Use two clamps to attach long columns.

The illustration below shows the Column clamp.

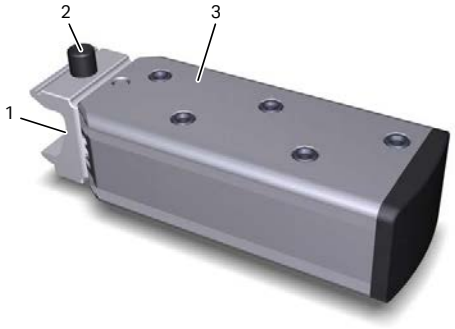


Part	Description
1	Position for a column
2	Inner end tabs

Column holder rod

The Column holder rod can be used to attach several HiTrap™ columns. The holder has threaded ports for HiTrap columns and tubing connectors. Push the button of the holder to attach the holder to a holder rail.

The illustration below shows the Column holder rod.

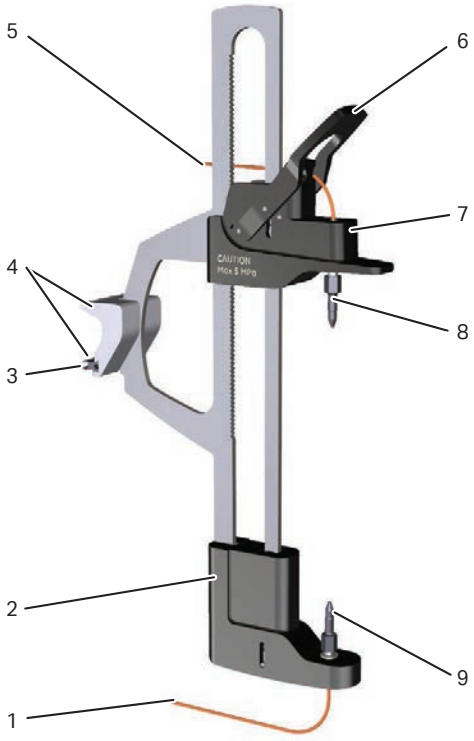


Part	Description
1	Snap-in to holder rails
2	Button
3	Column holder rod

Flexible column holder

The Flexible column holder can be used to attach, for example, HiScreen™ columns.

The illustration below shows the Flexible column holder.

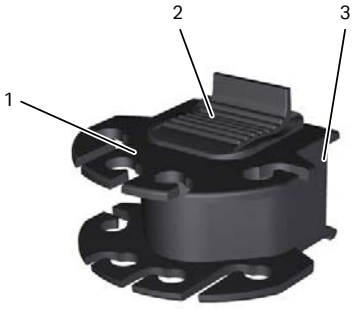


Part	Description
1	Lower tubing
2	Lower part
3	Snap-in-strips
4	Attachment part
5	Upper tubing
6	Lever
7	Upper part
8	Upper connector
9	Lower connector

Tubing holder spool

The Tubing holder spool is used to hold and arrange tubing.

The illustration below shows the Tubing holder spool.



Part	Description
1	Positions for tubing
2	Tab
3	Snap-in to holder rails

Tubing holder comb

The Tubing holder comb is used to hold and arrange tubing.

The illustration below shows the Tubing holder comb.



Part	Description
1	Positions for tubing
2	Tab
3	Snap-in to holder rails

Bottle holder

The Bottle holder is used for holding bottles. For example, the Bottle holder can be attached to the holder rails to hold a sample bottle.

The illustration below shows the Bottle holder.

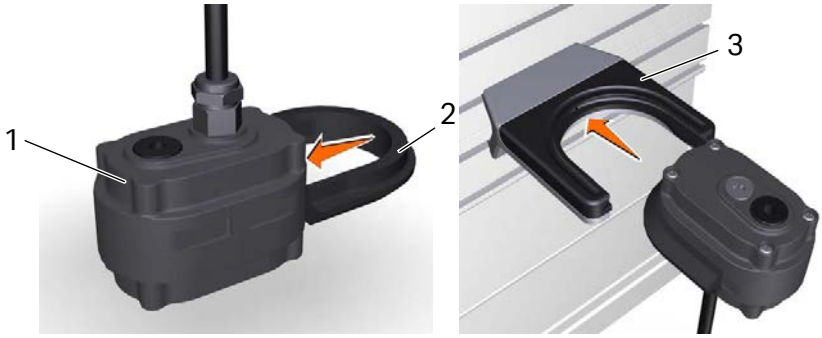


Part	Description
1	Position for bottle
2	Snap-in to holder rails

Adapter for air sensor

The adapter for air sensor is used to hold an optional air sensor.

The air sensor with adapter is connected to the Bottle holder, see illustrations below.



Part	Description
1	Air sensor
2	Air sensor adapter
3	Bottle holder

2.6.2 Tubing kits

This section describes the tubing kits that are available for ÄKTA pure.

Available tubing kits

The following tubing kits are available for ÄKTA pure.

Configuration	Tubing kit	Recommended flow rate[1]
ÄKTA pure micro	Red tubing kit (0.13 mm)	0.001 to 0.25 mL/min
	Blue tubing kit (0.25 mm)	0.25 to 2 mL/min
ÄKTA pure 25	Blue tubing kit (0.25 mm)	≤ 2 mL/min
	Orange tubing kit (0.5 mm)	1 to 10 mL/min
	Green tubing kit (0.75 mm)	≥ 10 mL/min
ÄKTA pure 150	Orange tubing kit (0.5 mm)	≤ 10 mL/min
	Green tubing kit (0.75 mm)	5 to 25 mL/min
	Beige tubing kit (1.0 mm)	≥ 25 mL/min

[1] The tubing kits can be used with other flow rates than the recommended flow rates described in this table.

Note: *To configure ÄKTA pure 25 M as ÄKTA pure micro, use the Micro kit.*

The Micro kit contains:

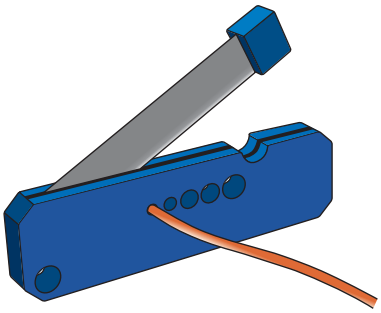
- *Pre-injection blue tubing (0.25 mm), from pumps to injection valve.*
- *Post-injection blue tubing (0.25 mm), for flow rates 0.25 to 2 mL/min.*
- *Post-injection red tubing (0.13 mm), for flow rates ≤ 0.25 mL/min.*

Replacing tubing kit

All components can be installed by the end users and if required, the instrument can later be restored to its original configuration.

Follow the steps below to replace a tubing kit.

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Unscrew the connectors, and disconnect the tubing.
3	If necessary, move or replace modules to optimize the flow path.
4	If the tubing has labels, remove the labels to be used with the new tubing later.

Step	Action
5	**CAUTION** **Cut injuries.** The tubing cutter is very sharp and must be handled with care to avoid injuries. Cut the new tubing to the same length as the old tubing. Use a tubing cutter to get a straight angle cut. 
6	Put the old labels on the new tubing.
7	Slide the fingertight connector onto the tubing.
8	Insert the tubing with the fingertight connector into the port. Make sure to insert the tubing all the way into the bottom of the port.
9	Tighten the connector fully. For areas difficult to access, use the fingertight wrench included in the accessory kit.

After installing the tubing kit, update the delay volume of the flow path. See *Set the delay volume, on page 203*.

2.6.3 Micro kit

The Micro kit is intended for use on ÄKTA pure 25 M with the multiple wavelength UV monitor **U9-M**. It improves the performance of the instrument and allows for micro-scale purification. To enable collection of fractions in microliter scale it is recommended to use the Fraction collector F9-R or F9-T in combination with the Micro kit.

Note: *ÄKTA pure micro is a system with the Micro kit pre-installed.*

Components included in the Micro kit

- Injection valve **V9M-J**
- UV flow cell **U9-2M**
- Conductivity monitor **C9M**
- Outlet valve **V9M-Os**
- Online filter
- Online filter clamp
- Mixer chamber 0.6 mL
- Tubing kit (pre-injection blue tubing, blue tubing kit and red tubing kit, see *Available tubing kits, on page 108*)
- 1/16 in male-male union and i.d. 0.25 mm fingertight union
- 1/16 in male-male union and i.d. 0.13 mm fingertight union
- Micro nozzle for Fraction collector F9-R
- Multi-directional column clamp
- Sample loops (10 µl and 50 µl)
- Injection kit

Note: *When using the Fraction collection F9-T, it is recommended to use the F9-T micro collection kit (29695762).*

Core modules of ÄKTA pure 25 M

The following core modules must be used with together with the Micro kit:

- Pumps **P9 A** and **P9 B**
- Pressure monitor **R9**
- Mixer **M9**
- UV monitor **U9-M** with detector **U9-D**
- Flow restrictor **FR-902**

Optional modules of ÄKTA pure 25 M

The following optional modules can be used for micro-scale purification:

- Fraction collector **F9-R** or **F9-T**
- Air sensor **L9-1.5** placed before pumps

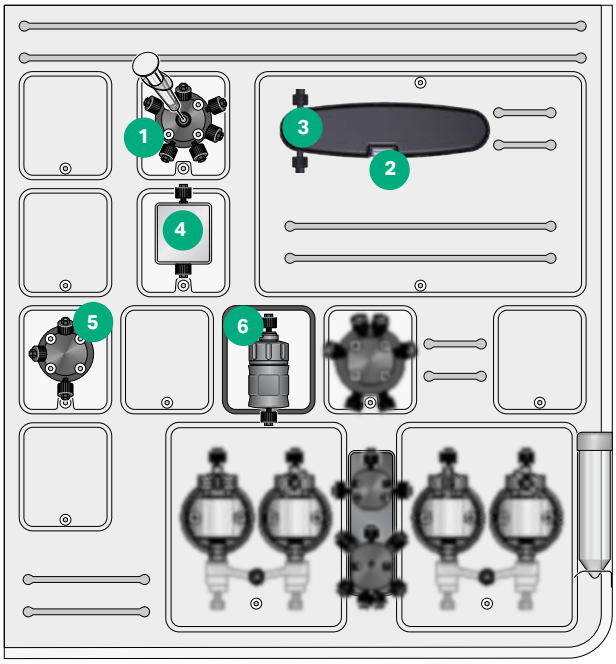
- Column valve **V9-Cs** and **V9-Cm**
- ALIAS™ Bio autosampler connected according to *Connect ALIAS Bio autosampler to lab scale ÄKTA systems Instructions (29040427)*

Note: *Using a column valve or an autosampler adds extra volume, and could decrease resolution for some columns.*

Note: *Other modules not listed here but supported by ÄKTA pure 25 M are not recommended for micro-scale purification due to large internal volumes that will decrease system performance, e.g. reduced resolution and increased peak broadening.*

Location of modules

The illustration below shows ÄKTA pure 25 M when the Micro kit is installed (without optional modules). This configuration for micro-purification optimizes the flow path and keeps dead volume to a minimum.



The table below shows the positions of the module panel.

Label	Component	Description
1	**V9M-J**	Injection valve
2	**U9-M**	UV monitor
3	**U9-2M**	UV flow cell
4	**C9M**	Conductivity monitor
5	**V9M-Os**	Outlet valve
6	**M9**	Mixer or Online filter

Install the tubing kit

ÄKTA pure micro is pre-configured with the red tubing kit.

For higher flow rates, the blue tubing is recommended, as it has a lower back pressure. The blue tubing kit is suitable for Capto™ HiRes or 10/300 SEC columns.

For lower flow rates, the red tubing is recommended, as it gives a better resolution. The red tubing kit is suitable for SEC 3.2/300 and 5/150 columns.

Note: *The 520 mm length of tubing* **5** *is for mounting 300 mm SEC columns. It can be shortened for other columns but the performance gain is insignificant.*

Connect tubing

Start the installation of tubing from the pump and continue with the tubing in numerical order. Make sure the tubing is installed as in the illustrations below. See *Replacing tubing kit, on page 108* for detailed instructions on how to replace a tubing kit.

Note: *Do not install a column until after the system performance test has been run.*

Note: *Connect tubing* **5** *directly to the UV flow cell when running the system performance test.*

Note: *Connect one of the supplied sample loops to the injection valve* ***V9M-J*** *(ports* **LoopE** *and* **LoopF***).*

Note: *Use fresh fingertight connectors. Tighten by hand and then use the wrench to further tighten circa ⅛ of a turn.*

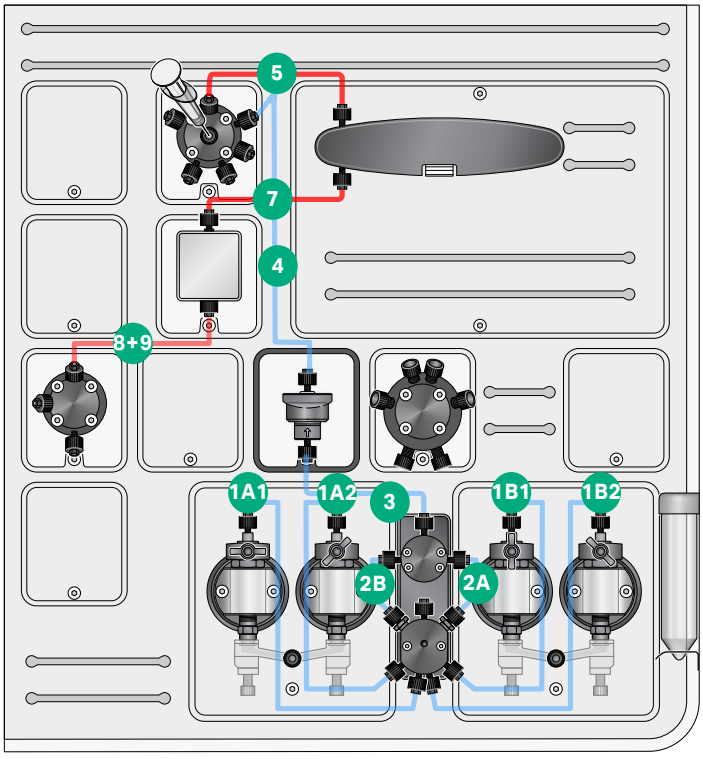
Note: *Check that all waste tubing (***W***,* **W1***,* **W2***) are connected as described in* *Waste tubing overview, on page 214**.*

The table below shows the pre-injection tubing.

Tubing label	Connection	Tubing	Connector	Tubing length
1A1	Pump head to pump restrictor (left side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	340 mm

Tubing label	Connection	Tubing	Connector	Tubing length
1A2	Pump head to pump restrictor (left side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	340 mm
1B1	Pump head to pump restrictor (right side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	340 mm
1B2	Pump head to pump restrictor (right side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	340 mm
2A	Pump restrictor (right side) to Pressure monitor (right side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	115 mm
2B	Pump restrictor (left side) to Pressure monitor (left side)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	115 mm
3	Pressure monitor to Online filter/Mixer	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	160 mm
4	Online filter/Mixer to Injection valve **V9M-J** (port **Pump**)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	250 mm

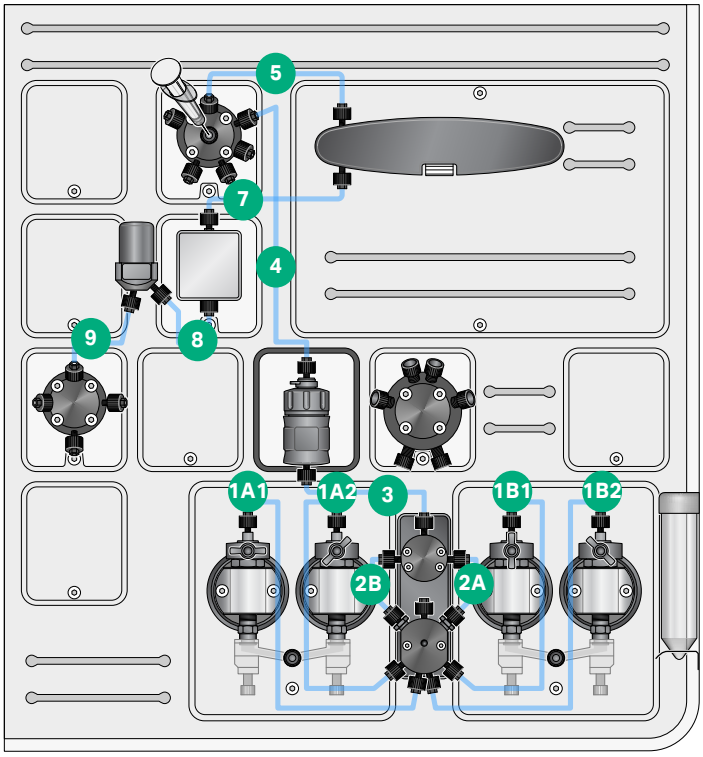
The illustration below shows how to install the red tubing kit.



The table below shows the components of the red tubing kit.

Tubing label	Connection	Tubing	Connector	Tubing length
5	Injection valve **V9M-J** (port **Col**) to Column	PEEK i.d. 0.13 mm	Fingertight connector,1/16"	520 mm
(6)	Column to UV monitor **U9-M**	M-M connector	Fingertight connector,1/16"	N/A
7	UV monitor **U9-M** to Conductivity monitor **C9M**	PEEK i.d. 0.13 mm	Fingertight connector,1/16"	170 mm
8 and **9**	Conductivity monitor **C9M** to Outlet valve **V9M-Os** (port **In**)	PEEK i.d. 0.13 mm	Fingertight connector,1/16"	175 mm
Frac	Outlet valve **V9M-Os** (port **Frac**) to Fraction collector nozzle	PEEK i.d. 0.13 mm	Fingertight connector,1/16"	400 mm

The illustration below shows how to install the blue tubing kit.



The table below shows the components of the blue tubing kit.

Tubing label	Connection	Tubing	Connector	Tubing length
5	Injection valve **V9M-J** (port **Col**) to Column or directly to UV monitor for running Performance test	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	520 mm
(**6**)	Column to UV monitor **U9-M**	M-M connector	Fingertight connector,1/16"	N/A
7	UV monitor **U9-M** to Conductivity monitor **C9M**	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	170 mm
8	Conductivity monitor **C9M** to Flow restrictor (port **IN**)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	95 mm
9	Flow restrictor to Outlet valve **V9M-Os** (port **In**)	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	135 mm
Frac	Outlet valve **V9M-Os** (port **Frac**) to Fraction collector nozzle	PEEK i.d. 0.25 mm	Fingertight connector,1/16"	400 mm

2.6.4 Other accessories

This section describes the Module Panel, and the Extension box.

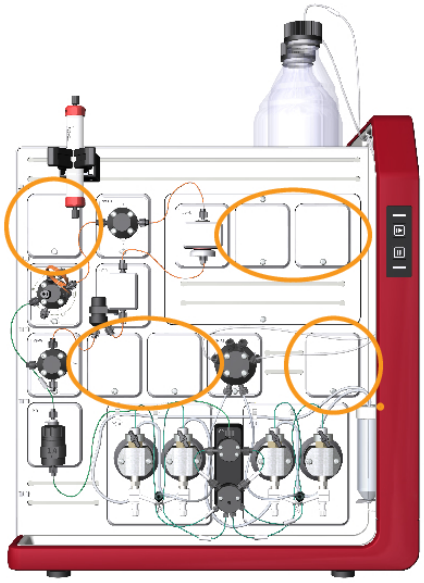
Module Panel

All positions in ÄKTA pure must be occupied. Positions not used for core or optional modules must be fitted with a Module Panel.

Module Panels are installed in the same way as the other optional modules and the cable inside must be connected to the Module Panel, see *Hardware installation of a module, on page 96*.

When an optional module is replaced by a Module panel, the removed module has to be deselected in the software configuration, see *Edit system properties, on page 175*.

The illustration shows the Module Panels installed in the example configuration used in this manual.



Extension box

The Extension box can be used to install extra modules on the ÄKTA pure instrument outside the system chassis when the positions on the chassis are filled. It is possible to install up to six Extension boxes with extra modules when using ÄKTA pure. See the Extension box instruction for more information.

The illustration below shows an Extension box with a module mounted on the side of ÄKTA pure.



The Extension box can be mounted in three ways.

- On the side of ÄKTA pure.
- Standing on top of or next to ÄKTA pure.
- On a Rail extension rod (29011352) on ÄKTA pure.

3 ÄKTA pure external modules

About this chapter

This chapter provides an overview of the external modules that can be connected to the ÄKTA pure instrument. A brief description of how to connect external modules is also provided.

In this chapter

Section		See page
3.1	External air sensors	119
3.2	Fraction collector F9-C	122
3.3	Fraction collector F9-R	135
3.4	Fraction collector F9-T	143
3.5	Sample pump S9 and S9H	152
3.6	I/O-box E9	157
3.7	Connection of external modules	167

3.1 External air sensors

This section describes the design and the function of the external air sensors.

In this section

Section		See page
3.1.1	Description	120
3.1.2	Tubing connections	121

3.1.1 Description

Introduction

Up to four external air sensors can be added to ÄKTA pure, and there are two different versions to choose from. They differ in internal diameter and optimal position on the instrument.

The air sensors can be attached to the instrument using the rails and holders, see *Adapter for air sensor, on page 107*.

In addition to be used for preventing air from entering the system, the external air sensors can be used together with System pump A, or an Sample pump to load the entire sample volume.

Air sensor L9-1.5

L9-1.5 has a 1.5 mm inner diameter and must be placed at the low pressure side before the pumps. Connect **L9-1.5** with the following tubing:

- For ÄKTA pure 25: i.d. 1.6 mm FEP tubing.
- For ÄKTA pure 150: i.d. 2.9 mm FEP tubing.

It is installed in the flow path before the system pumps or the sample pump and is used to prevent air entering the subsequent module.

Air sensor L9-1.2

L9-1.2 has a 1.2 mm inner diameter and is designed for o.d. 1/16" tubing at the high pressure side after the pumps. It is installed after the injection valve and is used to prevent air entering the column.

Note: **L9-1.2** *can be used in the same fashion as* **L9-1.5** *if O.D. 1/16" ETFE tubing is used between the air sensor and the inlet valve.*

3.1.2 Tubing connections

Air sensor L9-1.5

Connection between...	Tubing		Connector		Tubing length (mm)
	ÄKTA pure 25	ÄKTA pure 150	ÄKTA pure 25	ÄKTA pure 150	
L9-1.5 and inlet valves	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	Tubing connector, 5/16" + Ferrule (yellow), 1/8"	Tubing connector, 5/16" + Ferrule (blue), 3/16"	See note

Note: *When sample is loaded at high flow rate and the external air sensor is placed before the pump that is used for loading the sample, it is necessary to use longer tubing to make sure that no air reaches the pump. Use tubing with the minimum lengths given below between the valve located before the pump and the external air sensor. The length applies for maximum flow rate, but shorter tubing can be used at lower flow rates.*

- *Sample pump S9: 40 cm.*
- *Sample pump S9H: 20 cm.*

Air sensor L9-1.2

Connection between...	Tubing	Connector	Tubing length (mm)
Injection valve and **L9-1.2**	PEEK, o.d. 1/16"	Fingertight connector, 1/16"	100
L9-1.2 and Column valve or the column	PEEK, o.d. 1/16"	Fingertight connector, 1/16"	100

3.2 Fraction collector F9-C

About this section

This section shows an overview of Fraction collector F9-C.

For technical details, see *Fraction collector options, on page 533*.

In this section

Section		See page
3.2.1	Function	123
3.2.2	Fraction collector F9-C illustrations	125
3.2.3	Cassettes, Cassette tray and racks	129
3.2.4	Fraction collector tubes and bottles	132
3.2.5	Deep well plates	133

3.2.1 Function

Introduction

Fraction collector F9-C can collect fractions in deep well plates, tubes of different sizes or bottles. Up to six cassettes for deep well plates and tubes can be used. The cassettes can be used in any combination and are placed on the Cassette tray. A rack for 50 mL tubes and a rack for 250 mL bottles are also available.

Scanner functions detect which types of trays, racks, cassettes and deep well plates that are used in each run.

The Cassette tray or one of the racks is placed inside the fraction collector. A height exclusion bar makes sure that the bottles, tubes and deep well plates are correctly positioned and cannot damage the Dispenser head. The Tray catch and positioning discs on the floor of the fraction collector guide the Cassette tray or the rack into the correct position.

Scanning of Cassettes

When the door of the fraction collector is closed automatic scanning is performed. There are two types of scanning procedures:

- ***Full scan***: Scanning of Cassette type codes to determine which types of Cassettes are used, and scanning of rows and columns in deep well plates to identify which types of plates are used (24, 48, or 96 wells). Full scan is performed only when the system is in state ***Ready***.

  Note: *There is a delay between the end of a run and when the system goes into state **Ready**. The fraction collector will not perform a **Full scan**, when opening and closing the hatch before the system is in state **Ready**.*

- ***Quick scan***: Scanning of Cassette type codes to determine which type of Cassettes are used. Quick scan is performed during the run to make sure that correct Cassettes are placed in the Fraction collector.

Fractionation modes to avoid spillage

Three fractionation modes are available, all of which avoid spillage between wells or tubes during fractionation:

- ***Accumulator:*** The accumulator is used to collect liquid during movement between wells, tubes or bottles. The liquid is then dispensed in the next well or tube. Fractionation with accumulator can be used at all flow rates.
- ***DropSync:*** When using ***DropSync*** the sensors in the Dispenser head detect when a drop is released from the nozzle. The Dispenser head moves to the next well or tube just after a drop is released. Fractionation with ***DropSync*** can be used at flow rates up to 2 mL/min. If the cassettes are placed near the waste funnel the ***DropSync*** mode can be used at higher flow rates. Volatile solutions and solutions with low surface tension may require a lower flow.
- ***Automatic:*** The fraction collector uses the Drop Sync mode for flow rates up to 2 mL/min and automatically switches to Accumulator mode for higher flow rates.

Fractionation arm positions

- ***Home position:*** The home position is used when the fraction collector is idle. The Fractionation arm is positioned in the front of the interior of the fraction collector and the Dispenser head is positioned over the waste funnel. This position is called ***Waste (Frac)*** in UNICORN.
- ***Frac cleaning position:*** The Frac cleaning position is used for convenient cleaning of the Dispenser head. The Fractionation arm is positioned in the front of the interior of the fractionation collector and the Dispenser head is moved to the center of the Fractionation arm.

3.2.2 Fraction collector F9-C illustrations

Introduction

This section provides illustrations of Fraction collector F9-C. The main features and components are indicated.

Front view

The illustration below shows the main parts of the exterior of Fraction collector F9-C.



Part	Description
1	Fractionation indicator Symbol indicating that fractionation is ongoing. Do not open the door while the indicator is lit.
2	Door
3	Window
4	Door handle
5	Tubing connector for outlet valve tubing
6	Vents

Rear view

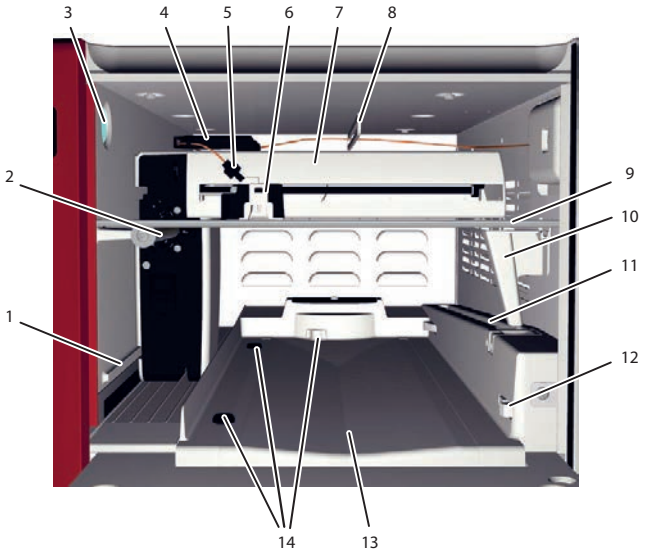
The illustration below shows the rear view of Fraction collector F9-C.



Part	Description
1	Vents
2	UniNet-9 D-type connector (for communication and power supply)
3	Waste tubing

Interior

The illustration below shows the main parts of the interior of Fraction collector F9-C.

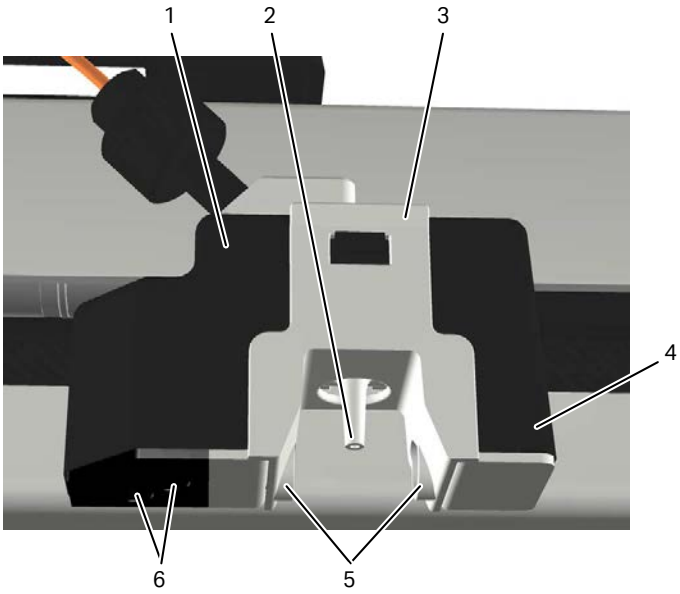


Part	Description
1	Fractionation arm guide rail
2	Fractionation arm main rail
3	Lamp
4	Tubing guide
5	Tubing connection
6	Dispenser head
7	Fractionation arm
8	Tubing guide
9	Height exclusion bar
10	Waste funnel
11	Waste tubing
12	Tray catch

Part	Description
13	Waste groove, in case of overflow
14	Tray guides

Dispenser head

The illustration below shows the Dispenser head of Fraction collector F9-C.



Part	Description
1	Dispenser head
2	Nozzle
3	Dispenser head cover
4	Accumulator (back part of Dispenser head)
5	Drop sync sensor
6	Type code reader

3.2.3 Cassettes, Cassette tray and racks

Introduction

Fractions can be collected in deep well plates and in tubes of different sizes. A number of cassettes and racks for different tubes and deep well plates are available. The cassettes are placed on a rack with six cassette positions. The Cassette type codes are scanned by the Cassette code reader to determine the type of Cassette.

Available cassettes, trays and racks

The following Cassettes and racks are available:

- Cassette 3 mL tubes (for 40 tubes)
- Cassette 5 mL tubes (for 40 tubes)
- Cassette 8 mL tubes (for 24 tubes)
- Cassette 15 mL tubes (for 15 tubes)
- Cassette 50 mL tubes (for 6 tubes)
- Cassette for deep well plate (24, 48, 96 wells)
- Cassette tray (for six cassettes)
- Rack for 50 mL tubes (for 55 tubes)
- Rack for 250 mL bottles (for 18 bottles)

For information on dimension requirements for tubes and deep well plates to be used in the fraction collector, see *Section 3.2.4 Fraction collector tubes and bottles, on page 132* and *Section 3.2.5 Deep well plates, on page 133* respectively.

Illustrations of Fraction collector F9-C tray and racks

The illustrations below show the Cassette tray, the Rack for 50 mL tubes and the Rack for 250 mL bottles.

The fronts of the tray and the racks are marked with the Cytiva logo.

In the Cassette tray, the cassette positions are marked 1 to 6.



Cassette tray



Rack for 50 mL tubes



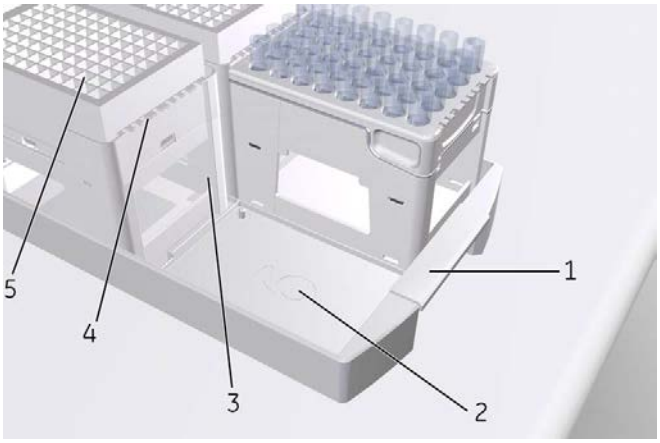
Rack for 250 mL bottles

Note: *The tray and racks are inserted into the fraction collector with the Cytiva logo facing outwards.*

Note: *Do not use the Cassette tray when a rack for tubes or bottles is placed in the fraction collector.*

Illustration of Cassettes on the Cassette tray

The illustration below shows Cassettes placed on the Cassette tray.



Part	Description
1	Cassette tray
2	Cassette position number
3	Cassette
4	Cassette type code
5	Tubes or deep well plates placed in a Cassette

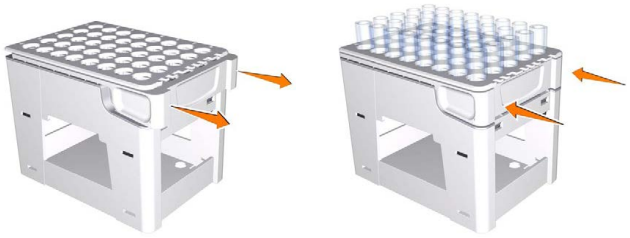
QuickRelease function

The Cassettes for the smaller tube sizes (3, 8, and 15 mL) have a built-in QuickRelease function. The QuickRelease function enables easy handling of tubes in the Cassettes. With the QuickRelease device in lock position the tubes are fastened in the Cassette and can easily be emptied. With the QuickRelease function in release position, the Cassette can easily be loaded with tubes and used tubes can easily be discarded.

Step	Action
1	Load the Cassette with tubes before fractionation:
	a. Pull the QuickRelease device to the release position.

Step	Action

b. Load the Cassette with tubes, and press the QuickRelease device to the locked position.



Release position Locked position

2 After fractionation, pull the QuickRelease device to the release position and remove the tubes containing the fractions of interest.

3 Empty and discard the remaining tubes:

a. Press the QuickRelease device to the lock position, and empty the remaining tubes.

b. Pull the QuickRelease device to the release position, and discard the tubes.



Empty the tubes Discard the tubes

3.2.4 Fraction collector tubes and bottles

The tubes and bottles used in Fraction collector F9-C must fulfill the requirements listed in the table below. Examples of manufacturers are also listed in the table.

Tube or bottle size (mL)	Diameter (mm)		Height (mm)		Examples of manufac-turers
	Min.	Max.	Min.	Max.	
3	10.5	11.5	50	56	Nunc
5	10.5	12	70	76	VWR
8	12	13.3	96	102	BD Biosciences, VWR
15	16	17	114	120	BD Biosciences
50	28	30	110	116	BD Biosciences
250 mL bottle	L: 55 W: 55[1]	L: 64.5 W: 64[1]	-	121	Nalgene, Kautex

[1] Length and width of the rectangular bottle base

Maximum flow rate

Fraction collection can be performed at different maximum flow rates depending on the size of the tubes that are used. The table below lists the maximum flow rates for the Fraction collector tubes.

Tube size (mL)	Maximum flow rate [mL/min]
3	15
8	25
15	50
50	150
250	150

3.2.5 Deep well plates

Requirements

The deep well plates used in Fraction collector F9-C must fulfill the requirements listed in the table below.

Property	Specification
Number of wells	24, 48, or 96
Shape of wells	Square, not cylindrical
Well volume	2, 5, 10 mL

Approved deep well plates

The plates listed in the table below are tested and approved by Cytiva to be used with Fraction collector F9-C.

Plate type	Manufacturer	Part no.
96 deep well plate	Cytiva	7701-5200 (Whatman™)
	BD Biosciences	353966
	Greiner™ Bio-One	780270
	Porvair Sciences	219009
	Seahorse Bioscience	S30009
	Eppendorf®	951033405/ 0030 501.306
48 deep well plate	Cytiva	7701-5500 (Whatman)
	Seahorse Bioscience	S30004
24 deep well plate	Cytiva	7701-5102 (Whatman)
	Seahorse Bioscience	S30024

Maximum flow rate

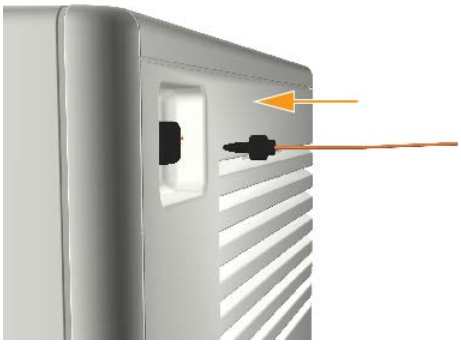
Fraction collection can be performed at different maximum flow rates depending on what type of deep well plates that are used. The table below lists the maximum flow rates for the different plate types.

Plate type	Maximum flow rate (mL/min)
96 deep well plate	10
48 deep well plate	15
24 deep well plate	25

Connect Fraction collector F9-C

Fraction collector F9-C is delivered with all internal tubing in place. The tubing between the fraction collector and the purification instrument needs to be installed.

Follow the instructions in the table below to connect the tubing from the ÄKTA pure instrument to the fraction collector.

Step	Action
1	Connect the tubing **Frac** to the **Frac** port on the Outlet valve on the ÄKTA pure instrument.
2	Connect the other end of the **Frac** tubing to the inlet port on the fraction collector.
	
3	Adjust the delay volume setting in UNICORN, see *Section 9.8.10 System settings - Tubing and Delay volumes, on page 585* for more details.

3.3 Fraction collector F9-R

About this section

This section shows an overview of Fraction collector F9-R.

For technical details, see *Fraction collector options, on page 533*.

In this section

Section		See page
3.3.1	Function	136
3.3.2	Illustrations	137
3.3.3	Connect the Fraction collector F9-R	139
3.3.4	Tubes and tube racks	140

3.3.1 Function

The fraction collector collects fractions from ÄKTA pure purification runs.

The fraction collector can be used for:

- Fixed volume fractionation
- Peak fractionation
- Combined fixed volume fractionation and peak fractionation

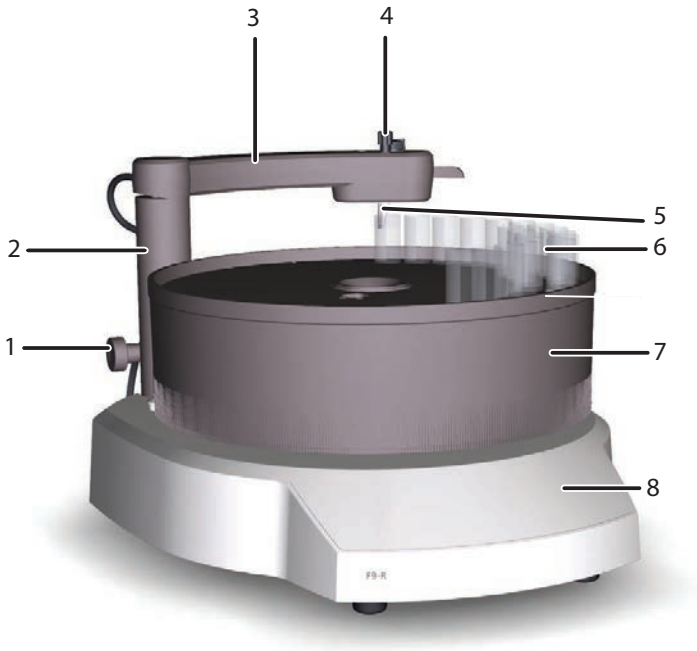
Fraction collector F9-R has the following function for reducing sample spill during fractionation:

- Drop Sync

3.3.2 Illustrations

Front view illustration

The illustration below shows the main parts of the Fraction collector.



Part	Description
1	Lock knob
2	Stationary part of delivery arm
3	Delivery arm
4	Tubing connector
5	Tube sensor
6	Collection tubes
7	Tube rack
8	Base unit

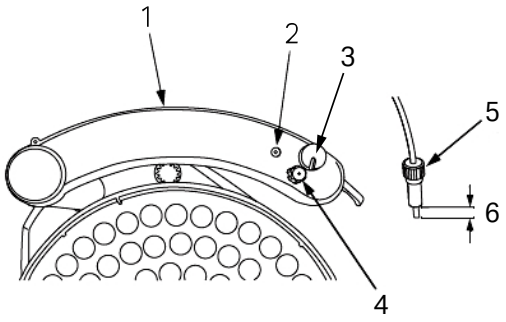
Connector panel illustration

The illustration below shows the main parts of the connector panel on the fraction collector.



Part	Description
1	Node ID switch
2	UniNet-9 F-type connector (for communication and power supply)

3.3.3 Connect the Fraction collector F9-R

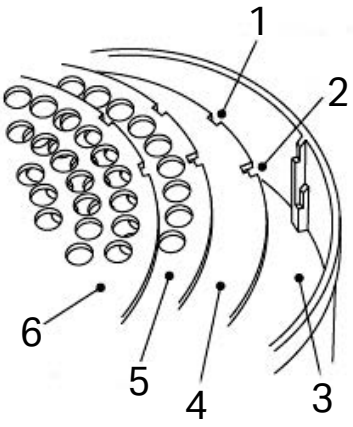
Step	Action
1	Lift out the Tubing holder (4) from the Delivery arm (1). 
2	Loosen the nut of the Tubing holder. Do not remove the Tubing holder nut (5) from the Tubing holder.
3	Insert the tubing through the Tubing holder.
4	Place the Tubing holder with the tubing over the Tube adjustment cavity (2) of the Delivery arm. Push the tubing down against the bottom of the Tube adjustment cavity, and then fingertighten the Tubing holder nut. This makes sure the correct length of the exposed tubing end (6).
5	Re-install the tubing holder in the Delivery arm.
6	For Fraction collector F9-R: Connect the tubing from the fraction collector to the port **Frac** on the outlet valve. For Fraction collector F9-R, 2nd: Connect the tubing from the second fraction collector to: • port **Out 10** on Outlet valve **V9-O** or **V9H-O** • port **Out 1** on Outlet valve **V9-Os** or **V9H-Os**
7	Adjust the delay volume setting in UNICORN to the volume of the tubing, see *Section 9.8.10 System settings - Tubing and Delay volumes, on page 585* for more details.

3.3.4 Tubes and tube racks

Available tubes

Fraction collector F9-R can collect fractions in tubes with diameters between 10 and 30 mm depending on the tube rack inserts that is used. The tubes can have a tube length between 50 and 180 mm.

Illustration of the Tube rack



Part	Description
1	Single cutout
2	L-shaped cutout
3	Bowl
4	Tube support
5	Tube guide
6	Tube holder

Note: *Note that the tube guide has both single and L-shaped cutouts, while the tube holder only has single cutouts.*

Tube rack inserts

Fraction collector F9-R is delivered with the 18 mm tube rack mounted. Each tube rack is made up of a combination of a Bowl, Tube support, Tube guide and Tube holder. Change the Tube holder and the Tube guide to collect fractions in 12 mm tubes or 30 mm tubes. The 12 mm tube rack is delivered with ÄKTA pure and the 30 mm tube rack is available as an accessory.

The table below describes inserts and corresponding fraction collection tubes.

Tube rack	Inserts	Tube diameter	Maximum number of tubes	Tube length
Tube rack 40 × 30 mm	12 mm Tube holder 12 mm Tube guide	30 mm	40	50 to 180 mm
Tube rack 95 × 10-18 mm[1]	18 mm Tube holder 18 mm Tube guide	• 10 mm • 12 mm • 18 mm	95	50 to 180 mm
Tube rack 175 × 12 mm	30 mm Tube holder 30 mm Tube guide	12 mm	175	50 to 180 mm

[1] This tube rack is supplied with the Fraction collector F9-R

Parameters for assembling a tube rack

When assembling a tube rack, different cutouts are used for the various inserts depending on the length of the collection tubes. See *Assemble the tube rack, on page 272*, for detailed instructions.

The table below shows which cutouts to use for the 12 mm and the 18 mm Tube rack inserts when using different tube lengths.

Inserts	Tube length	
	50 - 85 mm	85 - 180 mm
Tube support	L-shaped cutout	Not required
Tube guide	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout

The table below shows which cutouts to use for the 30 mm Tube rack inserts when using different tube lengths.

Inserts	Tube length		
	30 - 50 mm tubes[1]	50 - 85 mm tubes	85 - 180 mm tubes
Tube support	Single cutout	L-shaped cutout	Not required
Tube guide	Single cutout	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout	Single cutout

[1] For 30-50 mm tubes, first insert the tube guide from the 18 mm rack using the single cutout, before inserting the tube support for the 30 mm rack.

3.4 Fraction collector F9-T

About this section

This section shows an overview of Fraction collector F9-T.

For technical details, see *Fraction collector options, on page 533*.

In this section

Section		See page
3.4.1	Function	144
3.4.2	Illustrations and parts	145
3.4.3	Connecting the Fraction collector F9-T	148
3.4.4	Plates and tubes	149

3.4.1 Function

A Fraction collector is used for the collection of fractions from purification runs. The Fraction collector F9-T can be used for the following functions:

- Fixed volume fractionation
- Peak fractionation
- Combined fixed volume fractionation and peak fractionation

For a more detailed description of the fractionation functions see *Section 5.8.1 Fractionation overview, on page 261*.

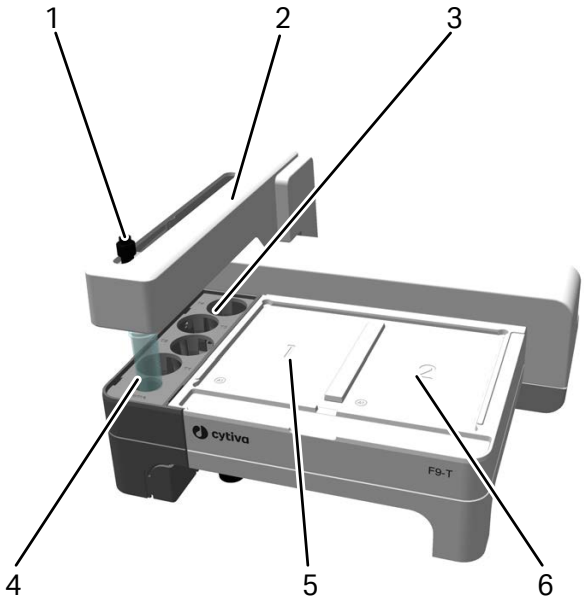
The fractions can be collected in plates or tubes, see the table in *Section 3.4.4 Plates and tubes, on page 149* for supported plates and tubes.

The Fraction collector F9-T can use the drop sync function to reduce risk of spillage during movement between fractions. Technical details are found in *Section 5.8.1 Fractionation overview, on page 261*.

3.4.2 Illustrations and parts

Front view

The illustration below shows the main parts of Fraction collector F9-T.

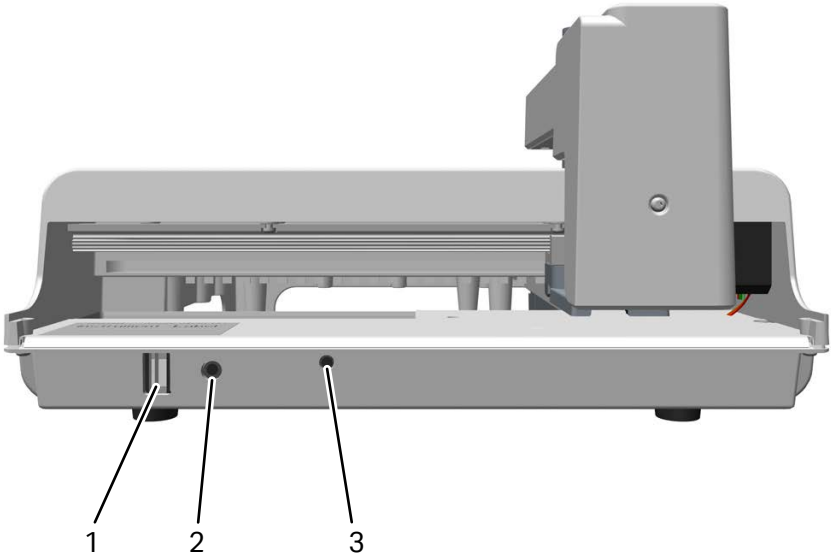


Part	Description
1	Nozzle
2	Fractionation arm
3	Tube rack for 50 mL tubes
4	Home position
5	Plate position 1
6	Plate position 2

Back view

The illustration below shows the back view of Fraction collector F9-T.

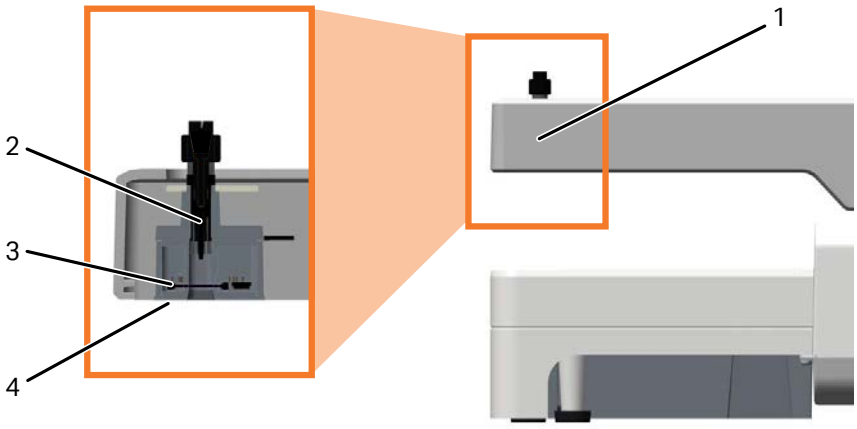
The Fraction collector is connected to the ÄKTA instrument via the UniNet-9 F-type connector port, both for communication and power supply.



Part	Description
1	UniNet-9 F-type connector port
2	Node ID
3	Status LED

Dispenser head

The illustration below shows the dispenser head of Fraction collector F9-T.



Part	Description
1	Dispenser head
2	Nozzle
3	Drop sync sensor
4	LED light

Drop sync function

To reduce sample spillage during fractionation, the fraction collector can use the Drop sync function. Technical details are found in *Section 5.8 Fractionation, on page 260*.

LED light

A LED light in the dispenser head indicate ongoing fractionation and illuminate the fractionation area. It can be turned on or off in ***System settings*** in UNICORN.

3.4.3 Connecting the Fraction collector F9-T

Connect the Fraction collector

The Fraction collector can be placed beside ÄKTA pure. The recommended tubing length for such a setup is 40 cm.

Note: *Longer tubing increases back pressure and peak broadening in the chromatographic process.*

Follow the steps below to connect the tubing from the ÄKTA pure instrument to the Fraction collector F9-T.

Step	Action
1	Connect one end of the tubing to the **Frac** port on the Outlet valve on the ÄKTA pure instrument using a fingertight connector.
2	Connect the other end to the selected nozzle.
3	Insert the nozzle into the fractionation arm.
4	Adjust the delay volume setting in the UNICORN software, see *Section 9.8.10 System settings - Tubing and Delay volumes, on page 585*. ***Note:*** *The default delay volume setting in UNICORN is defined for the 40 cm tubing with inner diameter 0.5 mm (orange tubing).*

Connect Fraction collector using a tunnel

To protect fractions, the Fraction collector F9-T can be placed inside an optional tunnel. Refer to *Fraction collector F9-T Operating Instructions (29478336)* for details.

Note: *ÄKTA pure cannot be placed on top of the tunnel.*

3.4.4 Plates and tubes

Plates

The tables below list available plate types and recommended plate manufacturers. Plates from other manufacturers can work if they are of the same quality and dimensions.

Plates	Maximum volume	Default fractionation volume
96 well microplate[1][2]	0.3 mL	0.1 mL
96 deep well plate[3]	2 mL	2 mL
48 deep well plate[4]	4.5 mL	4 mL
24 deep well plate[4]	9 mL	8 mL

[1] Microplate holder F9-T and micro nozzle are required.
[2] If microplates with maximum volume less than 0.3 mL is used, change to blue (i.d. 0.25 mm) tubing between the UV monitor and Fraction collector F9-T. Adjust delay volume accordingly.
[3] Only standard height 44 mm with square wells.
[4] Only standard height 44 mm with rectangular wells **A1** to **H6**.

Plates from the following manufacturers are recommended:

- Whatman™
- Corning
- Greiner
- Nunc

Tubes

The table below lists tubes and suitable holders.

For tube sizes 0.5 mL, 1.5 mL, and 2 mL, the manufacturer Eppendorf® is recommended. For 15 mL and 50 mL tube sizes, the manufacturer Falcon™ is recommended.

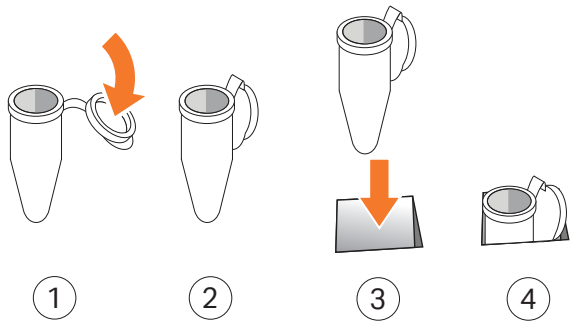
Tubes from other manufacturers can be used if they are of the same quality and dimensions.

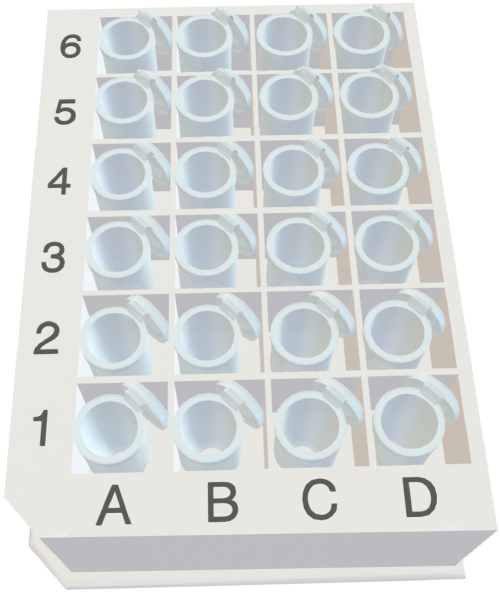
Tube size	Suitable holder
0.5 mL tubes	48 position rack
1.5 mL tubes	24 deep well plate[1]
2 mL tubes	24 deep well plate[1]
15 mL tubes[2]	Tube rack for 50 mL tubes
50 mL tubes	Tube rack for 50 mL tubes

[1] Only for tubes with an attached lid.
[2] Only for the **Home** position, not for fractionation.

Correct tube position in a 24 deep well plate

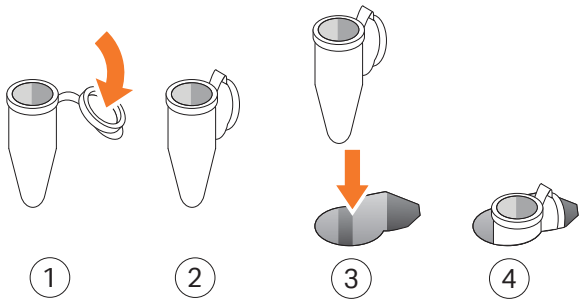
1.5 mL and 2.0 mL tubes are placed in a 24 deep well plate with their lids bent backward, and inserted in 45 degrees from the well structure with the lids in the upper right corners. For example, a tube in the **A1** position have the tube facing the outer corner, and the lid facing the opposite corner.





Correct tube position in a rack

0.5 mL tubes are placed in a rack with 48 positions with their lids bent backward. The tube is positioned in the round part of the hole, and the lid is positioned in the lid shaped part of the hole.





3.5 Sample pump S9 and S9H

Introduction

This section describes the design and function of Sample pump **S9** and **S9H**.

Function of the Sample pump

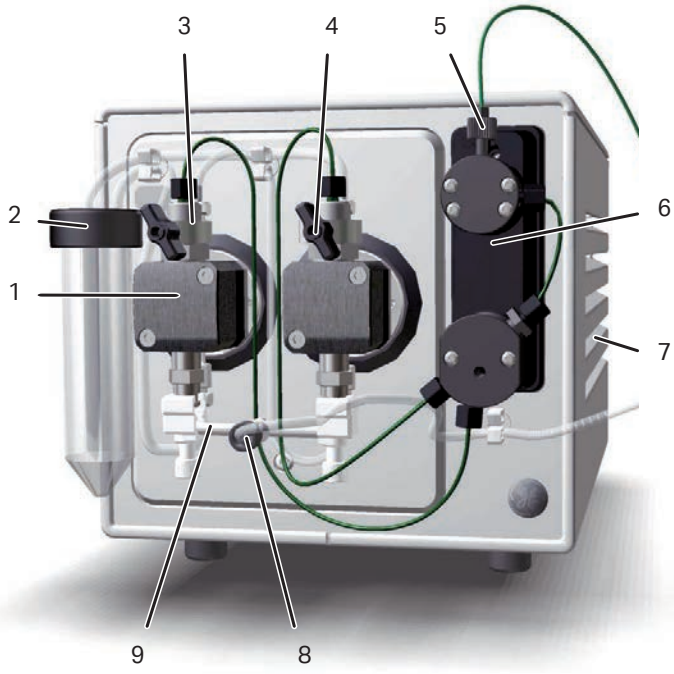
The Sample pump is dedicated to direct loading of sample onto a column, or to filling of sample loops or Superloops. The pump consists of two pump heads that work alternately to give a continuous, low pulsation, liquid delivery. There are two sample pump configurations available, one for ÄKTA pure 150 and one for ÄKTA pure 25. To make sure delivery of correct liquid volume, the pumps must be free from air. Each pump head is equipped with a purge valve that is used for this purpose.

The Sample pump also comprises a pressure monitor.

The table below contains the operating limits and labels of the sample pump configurations.

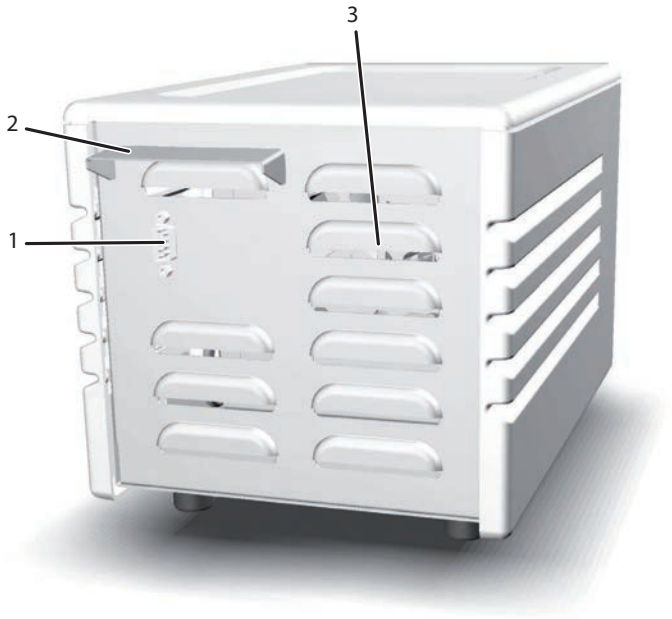
Configuration	Pump label	Pump type	Flow rate	Max. pressure
Sample pump **S9**, ÄKTA pure 25	**P9-S**	P9-S	0.001 to 50 mL/min	10 MPa
Sample pump **S9H**, ÄKTA pure 150	**P9H S**	P9H	0.01 to 150 mL/min	5 MPa

Front view



Part	Description
1	Pump head: Encapsulates the inner parts of the pump
2	Pump rinsing liquid tube holder
3	Outlet port with check valves
4	Purge valve: Used to remove air from the pump
5	Sample pump outlet port
6	Sample pressure monitor
7	Vents
8	Sample pump inlet port
9	Inlet manifold

Rear view



Part	Description
1	UniNet-9 type D port
2	IP cover: Protects pump electronics from liquid spill
3	Vents

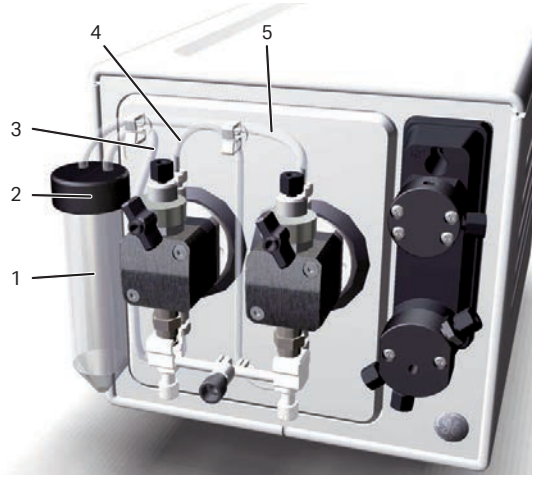
Sample pump piston rinsing system

A seal prevents leakage between the pump chamber and the drive mechanism. The seal is continuously lubricated by the presence of solvent. The pump piston rinsing system continuously flushes the low pressure chamber behind the piston with a low flow of 20% ethanol. This prevents salts from aqueous buffers to contaminate the pistons, and prolongs the working life of the seals.

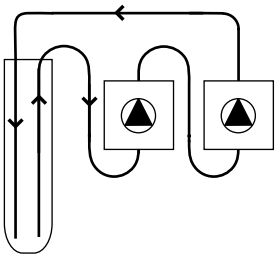
For instructions on how to fill the rinsing system, see *Section 7.6.1 Change pump rinsing solution, on page 444*.

Illustrations of the sample pump piston rinsing system

The illustrations below show the parts, tubing and flow path of the sample pump piston rinsing system.



Parts and tubing



Rinsing system flow path

Part	Description
1	Pump rinsing liquid tube
2	Pump rinsing liquid tube holder
3	Inlet tubing, from the rinsing liquid tube to the left pump head (lower rinsing system connection).
4	Tubing between the left pump head (upper rinsing system connection) and the right pump head (lower rinsing system connection).
5	Outlet tubing, from the right pump head (upper rinsing system connection) to the rinsing liquid tube.

Connect tubing to the ÄKTA pure instrument

The following table shows recommended tubing and connectors.

Tubing label	Connection	Tubing		Connector		Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150	ÄKTA pure 25	ÄKTA pure 150	
InS	Sample inlet valve port **Out** to sample pump inlet manifold	FEP o.d. 1/8", i.d. 1.6 mm	FEP o.d. 3/16", i.d. 2.9 mm	Tubing connector 5/16" with Ferrule (yellow), 1/8"	Tubing connector 5/16" with Ferrule (blue), 3/16"	580

Tubing label	Connection	Tubing		Connector		Tubing length (mm)
		ÄKTA pure 25	ÄKTA pure 150	ÄKTA pure 25	ÄKTA pure 150	
No label	Sample container to sample pump inlet manifold (sample inlet valve not used)	FEP o.d. 1/8", i.d. 1.6 mm	FEP o.d. 3/16", i.d. 2.9 mm	Tubing connector 5/16" with Ferrule (yellow), 1/8"	Tubing connector 5/16" with Ferrule (blue), 3/16"	Optional
3S	Sample pump pressure monitor to Inlet valve port **SaP**	PEEK, o.d. 1/16",i.d. 0.75 mm	PEEK, o.d. 1/16",i.d. 1 mm	Fingertight connector, 1/16"	Fingertight connector, 1/16"	530

Note: *The recommended location of the sample pump is on the bench to the left of the ÄKTA instrument. If the sample pump is placed elsewhere, longer tubing may be needed.*

3.6 I/O-box E9

About this section

This section describes the design and the function of the I/O-box **E9**.

In this section

Section		See page
3.6.1	Overview of the I/O-box	158
3.6.2	Analog connector and signals	160
3.6.3	Digital connector and signals	162
3.6.4	Connect external equipment to the I/O-box	164

3.6.1 Overview of the I/O-box

Function of the I/O-box

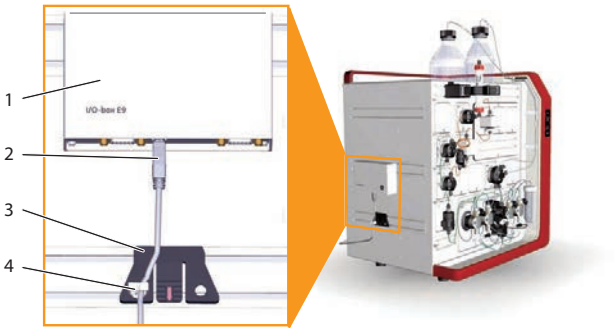
The I/O-box **E9** is used to interface other equipment in order to measure parameters such as refractive index, light scattering and fluorescence. See *Requirements on connected equipment, on page 164* for information on requirements of the equipment that can be connected to ÄKTA pure. The I/O-box can control external equipment by a digital output signal, as well as detecting the state of them by digital inputs. It is also possible to send out internal detector signals to external equipment.

Using two I/O-boxes

It is possible to install up to two I/O-boxes when using ÄKTA pure. If two I/O-boxes are to be used, the second I/O-box has to be configured as I/O-box E9, 2nd. The configuration is defined by the Node ID of the I/O-box. The Node ID is set by positioning the arrow of two rotating switches at the back of the I/O-box, see *Connectors, on page 159* and *Section 9.16 Node IDs, on page 645*.

Location

The illustration below shows the I/O-box, its recommended location and connection.



Part	Description
1	I/O-box
2	UniNet-9 F-type cable
3	Multi-purpose holder
4	Clip

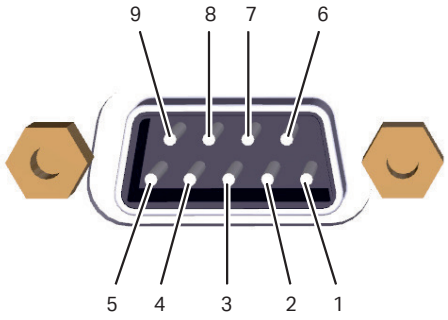
Connectors



Part	Description
Analog in/out	Signal connector for analog input and output signals.
UniNet-9	Connector used to connect the I/O-box to the ÄKTA pure instrument, for communication and power supply.
Status	Status indicator for service purposes.
Node ID	Switches used to configure I/O-box **E9** as primary or secondary I/O-box.
Digital in/out	Signal connector for digital input and output signals.

3.6.2 Analog connector and signals

Analog connector pins



Part	Description
1	Analog in signal 1 +
2	Analog in signal 1 - (or signal ground)
3	Shield, analog in (both ports)
4	Analog in signal 2 +
5	Analog in signal 2 - (or signal ground)
6	Calibration pin for service purposes Analog out signal (1.9 V) ***Note:*** *Do not use for other purposes.*
7	Analog out signal 1
8	Signal ground, analog out (both ports)
9	Analog out signal 2

Analog signals

All analog input and output signals are confined to the same **Analog in/out** connector.

Analog input signals

There are two analog input channels from which analog input signals can be used for peak detection, or data collection in UNICORN. It is possible to Autozero the input signals, which means that the current value will be displayed as 0 V in UNICORN. This can be done individually for the two analog input channels. The Autozero value is saved between runs and power-offs. The Autozero value can be reset.

Parameter	Description
Input signal range	-2000 to 2000 mV

Analog output signals

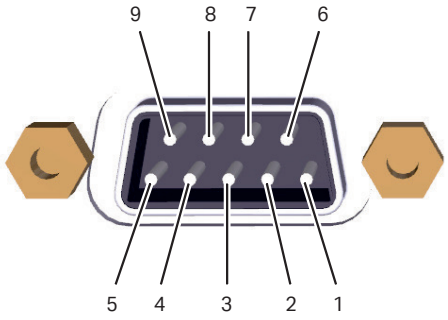
There are two analog output channels from which analog output signals and system parameters are transferred to the external connected equipment (e.g. light scattering detectors). Examples of analog output signals are UV, cond, conc B, temperature and pH.

Parameter	Description
Output signal range	-1000 to 1000 mV
Default output	The user defines the default output level for the selected signal.
Full scale output	The user sets the desired output signal value of, e.g., mAU, % or mS, which will corresponds to the full scale output voltage 1000 mV.
Negative full scale output	The corresponding negative full scale output value is set automatically. For example, if the full scale output is set to 2000 mAU, a UV value of 500 mAU will give an output voltage of 250 mV, and -100 mAU will give -50 mV output voltage. A signal value of 0 mAU, 0% or 0 mS will always give an output voltage of 0 mV.
Fix point	Zero is always a fix point. A desired output signal of, i.e., 0 mAU, 0% or 0 mS corresponds to an output voltage of 0 mV. At power-on, an output signal of 0 mV is transferred to the connected equipment, until the output signal range values are set.

Note: *No warning will be displayed in UNICORN if the analog output signal exceeds the set full scale output value or is less than the set negative full scale output value.*

3.6.3 Digital connector and signals

Digital connector pins



Part	Description
1	Digital in signal 1
2	Digital in signal 2
3	Digital in signal 3
4	Digital in signal 4
5	Signal ground
6	Digital out signal 1
7	Digital out signal 2
8	Digital out signal 3
9	Digital out signal 4

Digital signals

All digital input and output signals will be confined to the same D-sub connector and have a common ground. The four input signals will be scanned synchronously, and the outputs will be set synchronously.

Digital input signals

The digital in-signal can be used to monitor external equipment by registering, for example, error signals or event marks. An event mark can be used as a trigger for watches. The measured digital signals can be shown as a curve in UNICORN. The unit will handle both open/closed circuit and TTL-type voltage signals. An open circuit is interpreted as logical 1 and a closed circuit as logical 0.

Note: *A closed circuit is always closed against signal ground.*

Input connection	UNICORN interpretation
Open circuit	Logical 1
Applied voltage 3.5 to 5.0 V	
Closed circuit	Logical 0
Applied voltage 0 to 0.8 V	

Digital output signals

The digital output signal can be used to control external equipment that can receive digital signals, such as pumps or fraction collectors. The digital output signals define an open or closed circuit, where a logical 1 will result in an open circuit and a logical 0 will give a closed circuit. The default level, 1 or 0, is set by the user. The level can be changed by instructions either manually, in ***System Settings*** or by a method. It is possible to send pulses from the current level, with a pulse length of 0.1 s to 10 s.

Note: *A closed circuit is always closed against signal ground.*

Note: *The digital output signals do not output any voltage and cannot be used to drive something. It must always be connected to external equipment that supplies the voltage (max 5V).*

3.6.4 Connect external equipment to the I/O-box

Requirements on connected equipment

The signal characteristics for the connected equipment are described in the following tables. All connected equipment must have a common ground.

Analog input

Parameter	Value
Channels	2
Range	± 2000 mV
Input impedance	1 MΩ
Accuracy	± (0.1% + 0.2 mV)

Analog output

Parameter	Value
Channels	2
Range	± 1000 mV
Input impedance	100 kΩ
Accuracy	± (0.3% + 1 mV)

Digital input

Parameter	Value
Channels	4
Compatibility	TTL, open/closed circuit, max 5V

Digital output

Parameter	Value
Channels	4
Compatibility	Open/closed circuit, max 5V

Required material

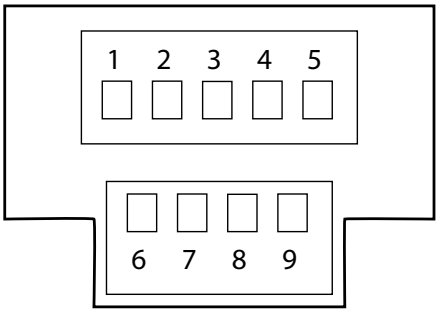
The following material is required:

- Flat-blade screwdriver, 2 mm
- Shielded cable with 9 conductors, 4 to 8 mm diameter
- Wire stripping tool

Instruction

Follow the instructions to connect one or two external cables to the supplied D-sub connectors.

Step	Action
1	Open the connector housing by removing housing screw and unlatch the housing top shell using a flat-blade screwdriver.
2	Locate the connection block mounted on the PCB board. The screw terminals has numbers corresponding to the connector contacts.



3	Strip the signal cable. • Strip-off 50 mm of the shield insulator. • Strip-off 4 mm of the single conductor insulation.
4	Loosen the strain relief clamp and insert the cable with the shield under the strain relief clamp. Fasten the strain relief clamp over the cable shield. ***Tip:*** *The connection block can be rotated inside the housing in order to position the screw terminals for left side or right side cable entry.*
5	Insert and fasten the single conductors in the screw terminals.
6	Close the housing top shell with the latch and screw the housing together.

System properties

Follow the instruction to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	In the ***Component types*** list, click ***Other***.
3	Select the ***I/O-box (E9)*** or ***I/O-box 2 (E9)*** check box in the ***Component selection*** list. Then click ***OK***. ***Note:*** ***I/O-box 2 (E9)*** *is only available in the Component selection list if* ***I/O-box (E9)*** *is already selected.*

System settings

Default values for digital out ports, noise reduction and configuration of analog out ports can be set.

Instruction name	Description
Digital out X	Sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Noise reduction analog in X	Filters the noise in the analog signal in port number X.
Alarm analog in X	Enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm digital in X	Enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in 'Value' occurs, an alarm will be triggered and the method will be paused.
Configure analog out X	Enables the user to send one of the pre-defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

Note: *The delay volume has to be updated if an external component is added to the flow path.*

3.7 Connection of external modules

Introduction

The external modules are not installed in the instrument cabinet, but are connected via a UniNet-9 cable at the back of the system. It is possible to install up to six external modules with F-type connectors and up to two external modules with D-type connectors at the same time.

Some of the external modules are also connected to the ÄKTA pure instrument by tubing. See the appropriate section in *Chapter 3 ÄKTA pure external modules, on page 118* for detailed instructions on how to install this tubing, if applicable.

F-type and D-type connectors

The illustrations below show the F-type and D-type connectors.



F-type



D-type

Constraints

The table below indicates usage constraints for the different external modules.

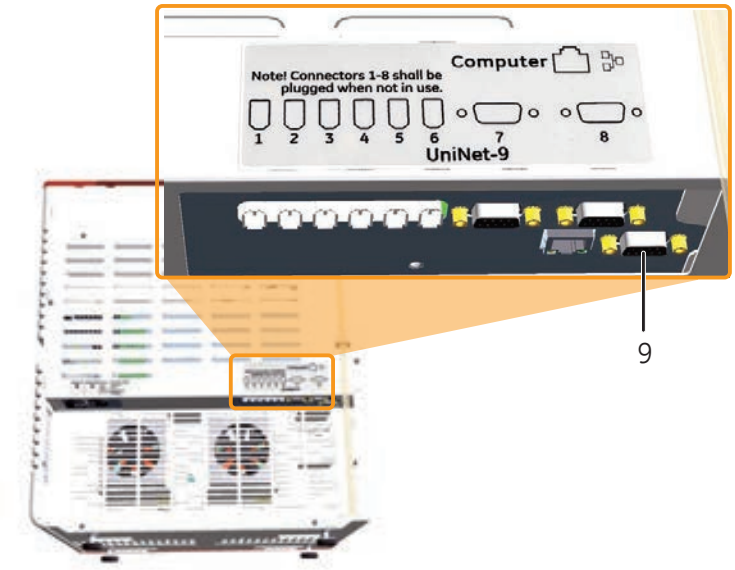
External module	Connector	Constraints
I/O-box E9	F-type	I/O-box E9 has no constraints.
I/O-box E9, 2	F-type	I/O-box E9, 2 requires I/O-box E9.
External air sensor L9	F-type	External air sensor L9 has no constraints.
External Air sensor L9, 2	F-type	External Air sensor L9, 2 requires External Air sensor L9.
External Air sensor L9, 3	F-type	External Air sensor L9, 3 requires External Air sensor L9, 2.
External Air sensor L9, 4	F-type	External Air sensor L9, 4 requires External Air sensor L9, 3.
Fraction collector F9-C	D-type	Fraction collector F9-C requires an Outlet valve.

External module	Connector	Constraints
Fraction collector F9-R	F-type	Fraction collector F9-R requires an Outlet valve.
Fraction collector F9-T	F-type	Fraction collector F9-T requires an Outlet valve.
Fraction collector F9-R, 2nd	F-type	Fraction collector F9-R, 2nd requires Fraction collector F9-C, F9-R or F9-T.
Sample pump S9 and S9H	D-type	Cannot be used at the same time as Mixer valve V9-M or V9H-M.

Note: *To optimize signal quality, the total cable length connecting all external modules to the ÄKTA pure instrument must not exceed 10 m.*

ÄKTA pure connector plate

The connector plate with the UniNet-9 connectors is located on the back of the ÄKTA pure instrument.



Connector	Connector name	Description
1 to 6	F-type connector	Connector point for optional modules.
7 to 8	D-type connector	Connector point for optional modules.
9	**Test**	Connector point for service.

NOTICE

Do not connect any module to the connector **Test** on the ÄKTA pure instrument.

Note: *Plug all unused UniNet-9 ports (ports 1 to 8) on the ÄKTA pure instrument with jumpers.*

Note: *The connector* **Test** *must be protected by a plastic lid. Do not plug the connector with a jumper.*

4 System configuration

About this chapter

This chapter describes hardware configuration of the ÄKTA pure instrument and how to install optional modules and add them in UNICORN.

In this chapter

Section		See page
4.1	Configuration overview	171
4.2	Configure modules	178
4.3	Configure ÄKTA pure micro	201
4.4	General system settings	203

4.1 Configuration overview

Introduction

ÄKTA pure is a flexible system that allows the user to configure both hardware and software to meet many purification needs. The instrument can be easily extended with additional valves, detectors and fraction collectors. There are a large number of different hardware modules to choose from in order to customize the number of columns, inlets, outlets, detectors and ways to apply and collect samples.

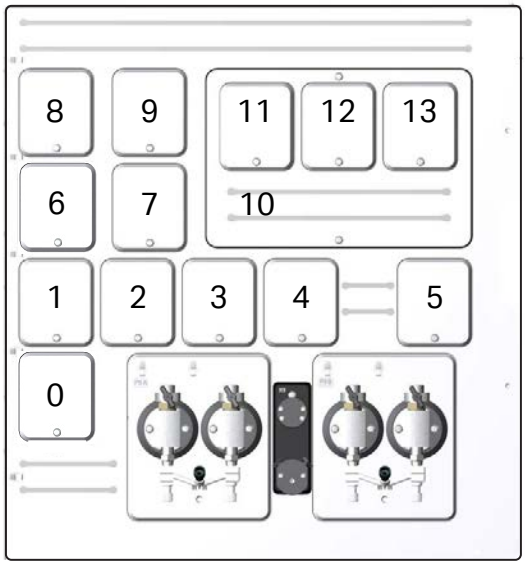
System configuration steps

The system configuration consists of four main steps:

1. Select modules and positions
2. Install the module(s)
3. Edit system properties
4. Edit system settings

Select modules and positions

Optional modules can be placed in any of the available positions in the cabinet. Its function is determined by the module type and node ID. However, to achieve an optimal flow path for a purification run, most modules have a dedicated location in the cabinet. The dedicated location for all modules and, if applicable, any constraints are described in the table below. Each location is marked with a number, which can be seen in the illustration below.



All positions in ÄKTA pure must be occupied. Positions not used for core or optional modules must be fitted with a Module Panel.

Recommended module position

Module	Recommended location	Constraints
Column valve **V9-C** or **V9H-C**	• 9 • 8 (if combined with **V9-C2** or **V9H-C2**)	Cannot be used at the same time as Column valve **V9-Cs**, **V9H-Cs**, or **V9-Cm**.
Second column valve **V9-C2** or **V9H-C2**	9	Requires **V9-C** or **V9H-C**.
Column valve **V9-Cm**	9	Cannot be used at the same time as Column valve **V9-C**, **V9H-C**, **V9-Cs** or **V9H-Cs**.
Column valve **V9-Cs** or **V9H-Cs**	9	Cannot be used at the same time as Column valve **V9-C**, **V9H-C**, or **V9-Cm**.
Conductivity monitor **C9** or **C9M**	7	Conductivity monitor **C9** has no constraints. **C9M** is used when configuring the instrument for microscale purification.
Conductivity monitor **C9, 2nd**	None	Requires **C9** or **C9M**.
Injection valve **V9-Inj**	6	Core module for ÄKTA pure 25.
Injection valve **V9H-Inj**	6	Core module for ÄKTA pure 150.
Injection valve **V9M-J**	9	**V9M-J** is used when configuring the instrument for microscale purification.
Inlet valve **V9-IAB** or **V9H-IAB**	• 4 • 5 (if used in combination with Inlet valve **V9-IA** or **V9H-IA**)	No constraints. For possible combinations, see *Combination of Inlet valves, on page 47*.
Inlet valve **V9-IA** or **V9H-IA**	4	No constraints. For possible combinations, see *Combination of Inlet valves, on page 47*.

Module	Recommended location	Constraints
Inlet valve **V9-IB** or **V9H-IB**	5	No constraints. For possible combinations, see *Combination of Inlet valves, on page 47*.
Sample inlet valve **V9-IS** or **V9H-IS**	8	Cannot be used at the same time as Mixer valve **V9-M** or **V9H-M**.
Inlet valve ***V9-X1*** or ***V9H-X1***	None	No constraints. No support in the method phases.
Inlet valve ***V9-X2*** or ***V9H-X2***	None	Inlet valve ***V9-X2*** or ***V9H-X2*** requires Inlet valve ***V9-X1*** or ***V9H-X1***, respectively. No support in the method phases.
Loop valve **V9-L** or **V9H-L**	8	Loop valve **V9-L** or **V9H-L** has no constraints, but is connected with the injection valve by replacing the loop.
Mixer **M9**	• 0 • 3 (for configuration with micro kit)	Core module.
Mixer valve **V9-M** or **V9H-M**	3	Cannot be used at the same time as Sample pump **S9** or **S9H**.
Outlet valve **V9-Os** or **V9H-Os**	1	Cannot be used at the same time as Outlet valve **V9-O**, **V9H-O**, or **V9M-Os**.
Outlet valve **V9-O** or **V9H-O**	1	Cannot be used at the same time as Outlet valve **V9-Os**, **V9H-Os**, or **V9M-Os**.
Outlet valve **V9M-Os**	1	**V9M-Os** is used when configuring the instrument for microscale purification. Cannot be used at the same time as Outlet valve**V9-O**, **V9H-O**, **V9-Os**, or **V9H-Os**.
pH valve **V9-pH** or **V9H-pH**	2	Must always be connected after the column due to pressure constraints and fractionation control. The delay volume calculations will be effected if the valve is located elsewhere. Normally it is situated between the monitor and the Outlet valve.
Versatile valve **V9-V** or **V9H-V**	None	No constraints.
Versatile valve ***2***	None	Requires versatile valve **V9-V**.

Module	Recommended location	Constraints
Versatile valve ***3***	None	Requires versatile valve ***2***.
Versatile valve ***4***	None	Requires versatile valve ***3***.
UV monitor **U9-M**	10	Cannot be used at the same time as UV monitor **U9-T**, or **U9-L**.
UV monitor **U9-T**	11	• Cannot be used at the same time as UV monitor **U9-M**, or **U9-L**. • Requires the Multi-module panel.
UV monitor **U9-T**, 2nd	None	Requires UV monitor **U9-M**, **U9-T**, or **U9-L**. For possible combinations, see *Using two UV monitors, on page 189*.
UV monitor **U9-L**	11	• Cannot be used at the same time as UV monitor **U9-M**, or **U9-T**. • Requires the Multi-module panel.
UV monitor **U9-L**, 2nd	None	Requires UV monitor **U9-M**, **U9-T**, or **U9-L**. For possible combinations, see *Using two UV monitors, on page 189*.

Install the modules

Detailed information about installation of the modules is found in *Chapter 2 The ÄKTA pure instrument, on page 16* and *Chapter 3 ÄKTA pure external modules, on page 118*.

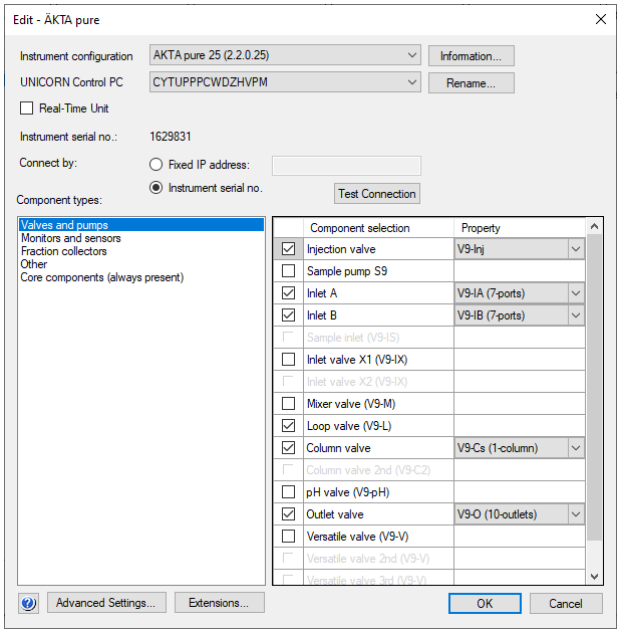
Below is a quick guide of how to perform a module hardware installation.

Step	Action
1	Switch off the power.
2	Set the node ID of the module. See *Section 9.16 Node IDs, on page 645* for detailed instructions.
3	Install the module. See *Section 2.5 Installation of internal modules, on page 96* for detailed instructions.
4	Connect the tubing.

Edit system properties

When a new module has been installed, the system properties have to be updated in UNICORN. The system will restart automatically when the configuration has been changed in ***System Properties*** and the system can be reconnected.

The following instruction gives a general description of how to update the system properties in UNICORN.

Step	Action
1	In the ***Administration*** module, choose ***Tools*** →***System Properties*** or click the ***System Properties*** icon to open the dialog box.
2	Select the system of interest in the ***System Properties*** dialog box.
3	Click ***Edit***. ***Note:*** *Only active systems can be edited.* *Result:* The ***Edit*** dialog box is displayed. 
4	Select the component type of interest from the ***Component types*** list. ***Note:*** *Instrument modules are referred to as* ***Components*** *in UNICORN. There are five main types of modules:* • *Valves and pumps*

Step	Action
	• *Monitors and sensors* • *Fraction collectors* • *Other (e.g., I/O-box)* • *Core components (always present)* *Result:* All available components are shown in the ***Component selection*** list.
5	Click the checkbox to select the added ***Component***. ***Note:*** *Some **Component selection** choices (e.g. a second UV monitor) cannot be selected unless the first one has been selected.*
6	When applicable, choose the appropriate ***Property***. ***Note:*** *The **Property** of a module (e.g. the type of UV monitor) cannot be selected unless the **Component** has been selected.*
7	Click ***OK*** to apply the changes.

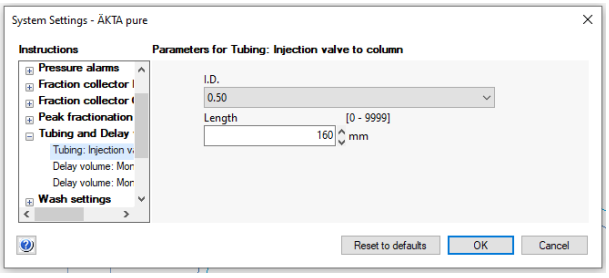
The components selected in ***System Properties*** will reflect which instructions and phase properties are available in ***Method Editor***.

Edit system settings

It may be necessary to edit the ***System Settings*** when the configuration of the system is changed. For example, if the change in configuration affects the delay volume following the UV monitor (or other monitor connected via the I/O-box) the appropriate system settings for ***Tubing and delay volumes*** have to be updated. This is to make sure that the fractions marked in the chromatogram corresponds to the actual collected fractions.

Other system settings might also need to be edited for some optional modules.

The ***System Settings*** dialog box in which to edit the system settings is shown below.



In this dialog box it is possible to set the parameters for the available instructions.

All system settings available for ÄKTA pure are found in *Section 9.8 System settings, on page 567*.

4.2 Configure modules

About this section

This section describes the software configuration that must be set for the individual modules that are to be used for a specific run.

A general description of how to update the system properties are found in *Edit system properties, on page 175*.

A general description of how to edit system settings are found in *Edit system settings, on page 176*.

For a description of how to configure ÄKTA pure 25 M for microscale purification with the Micro kit see *Section 2.6.3 Micro kit, on page 110*.

In this section

Section		See page
4.2.1	Configuration of injection valves	179
4.2.2	Configuration of inlet valves	180
4.2.3	Configuration of mixer valves	182
4.2.4	Configuration of loop valves	183
4.2.5	Configuration of column valves	184
4.2.6	Configuration of versatile valves	186
4.2.7	Configuration of pH valves	187
4.2.8	Configuration of outlet valves	188
4.2.9	Configuration of UV monitors	189
4.2.10	Configuration of conductivity monitor	191
4.2.11	Configuration of external air sensors	192
4.2.12	Configuration of fraction collectors	194
4.2.13	Configuration of I/O-box	199

4.2.1 Configuration of injection valves

System properties

Follow the instruction below to update the system properties for ÄKTA pure 25.

Note: *For ÄKTA pure 150, the Injection valve* **V9H-Inj** *is always selected.*

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select the properties according to the following table:

Valve	Component selection	Property
Standard	Injection valve	**V9-Inj**
Micro	Injection valve	**V9M-J**

4.2.2 Configuration of inlet valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select components and properties according to the following table:

Valve	Component selection	Property
Inlet valve **V9-IA**	***Inlet A***	***V9-IA (7-ports)***
Inlet valve **V9H-IA**	***Inlet A***	***V9H-IA (7-ports)***
Inlet valve **V9-IB**	***Inlet B***	***V9-IB (7-ports)***
Inlet valve **V9H-IB**	***Inlet B***	***V9H-IB (7-ports)***
Inlet valve **V9-IAB** used for inlet A	***Inlet A***	***V9-IAB part A (2-ports)***
Inlet valve **V9H-IAB** used for inlet A	***Inlet A***	***V9H-IAB part A (2-ports)***
Inlet valve **V9-IAB** used for inlet B	***Inlet B***	***V9-IAB part B (2-ports)***
Inlet valve **V9H-IAB** used for inlet B	***Inlet B***	***V9H-IAB part B (2-ports)***
Sample inlet valve **V9-IS**	***Sample inlet (V9-IS)***	N/A
Sample inlet valve **V9H-IS**	***Sample inlet (V9H-IS)***	N/A
Inlet valve **V9-IX**	***Inlet valve X1 (V9-IX)***	N/A
Inlet valve **V9H-IX**	***Inlet valve X1 (V9H-IX)***	N/A
Inlet valve **V9-IX**, 2nd	***Inlet valve X2 (V9-IX)***	N/A

Step	Action

Valve	Component selection	Property
Inlet valve **V9H-IX**, 2nd	***Inlet valve X2 (V9H-IX)***	N/A

System settings

There are no system settings available for the inlet valves.

4.2.3 Configuration of mixer valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select ***Mixer valve (V9-M)*** or ***Mixer valve (V9H-M)*** in the ***Component selection*** list.

System settings

The flow rate for mixer by-pass wash can be set.

Instruction name	Description
Mixer by-pass wash settings	Sets the flow rate used during ***Mixer by-pass wash*** and defines wash volumes for mixer by-pass wash options. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*

4.2.4 Configuration of loop valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select ***Loop valve (V9-L)*** or ***Loop valve (V9H-L)*** in the ***Component selection*** list.

System settings

The flow rate for loop wash can be set.

Instruction name	Description
Loop wash settings	Sets the flow rate used during ***Loop wash***. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*

4.2.5 Configuration of column valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	From the ***Component selection*** list select:

Column valve	Select
Primary	***Column valve***
Secondary	***Column valve 2nd***

4	Select the properties of the primary column valve according to the following table:

Valve	Property
Column valve **V9-C**	***V9-C (5-columns)***
Column valve **V9H-C**	***V9H-C (5-columns)***
Column valve **V9-Cm**	***V9-Cm (3-column)***
Column valve **V9-Cs**	***V9-Cs (1-column)***
Column valve **V9H-Cs**	***V9H-Cs (1-column)***

Note:

The property of the secondary column valve is automatically selected:

- *ÄKTA pure 25: Column valve* **V9-C2**
- *ÄKTA pure 150: Column valve* **V9H-C2**

System settings

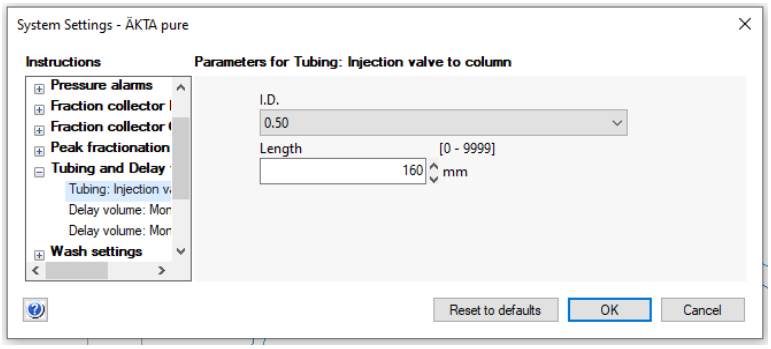
After selecting Column valve **V9-Cs**, **V9H-Cs**, **V9-Cm**, or no column valve, in UNICORN, the tubing length has to be defined.

The instructions ***Tubing: Injection valve to column*** and ***Tubing: Sample pump to injection valve*** are used in calculations of pre-column pressure when Column valve **V9-C** or **V9H-C** (5-columns) is not mounted onto the system. In such cases, there is no pre-column pressure sensor present. The estimated pre-column pressure is calculated using the measured system pressure and the length of the tubing between the system pressure sensor and the Column.

These instructions (***Tubing: Injection valve to column*** and ***Tubing: Sample pump to injection valve***) are available only when the Column valve **V9-C** or **V9H-C** (5-columns) is not selected in the component list. The instruction ***Tubing: Sample pump to injection valve*** is in addition only available if the sample pump is selected in the list.

Follow the steps below to set the tubing length between the Injection valve and the column, and between the Sample pump and the Injection valve.

Step	Action
1	In ***System Control*** select ***System*** →***Settings***.
2	Select ***Tubing and Delay Volumes*** and select ***Tubing: Injection valve to column***.
3	Select the ***I. D.*** from the drop-down list.
4	Type in the length of the tubing in the ***Length*** field and click ***OK***.



5	Perform step 3 for the instruction ***Tubing: Sample pump to injection valve***.

Note: *The built-in pressure sensors for Column valves* **V9-C** *and* **V9H-C** *have to be re-calibrated after installation. See* *Calibrating the pressure sensors, on page 351*.

Note: *A pre-column pressure alarm must always be set to protect the column. Column valves* **V9-Cs**, **V9H-Cs**, *and* **V9-Cm** *do not contain pressure sensors. See* *Section 5.6 Pressure alarms, on page 238*.

4.2.6 Configuration of versatile valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select the used Versatile valve in the ***Component selection*** list. A Versatile valve (**V9-V** or **V9H-V**) can only be selected if the requirements in the table below are met.

Valve	Constraints
Versatile valve	Versatile valve has no constraints.
Versatile valve 2	Versatile valve 2 requires Versatile valve.
Versatile valve 3	Versatile valve 3 requires Versatile valve and Versatile valve 2.
Versatile valve 4	Versatile valve 4 requires Versatile valve, Versatile valve 2, and Versatile valve 3.

System settings

If the versatile valve is placed in the flow path between the UV monitor and the outlet valve, the delay volume must be set. See *Set the delay volume, on page 203*.

4.2.7 Configuration of pH valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select ***pH valve (V9-pH)*** or ***pH valve (V9H-pH)*** in the ***Component selection*** list.

System settings

If the pH valve is placed in the flow path between the UV monitor and the outlet valve, the delay volume must be set. See *Set the delay volume, on page 203*.

Note: *It is recommended not to alter the default values for restrictor and pH cell delay volumes when standard modules and standard tubing for flow restrictor are used.*

4.2.8 Configuration of outlet valves

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Valves and pumps*** from the ***Component types*** list.
3	Select components and properties according to the following table:

Valve	Component selection	Property
Outlet valve **V9-O**	***Outlet valve***	***V9-O (10-outlets)***
Outlet valve **V9H-O**	***Outlet valve***	***V9H-O (10-outlets)***
Outlet valve **V9-Os**	***Outlet valve***	***V9-Os (1-outlet)***
Outlet valve **V9H-Os**	***Outlet valve***	***V9H-Os (1-outlet)***
Outlet valve **V9M-Os**	***Outlet valve***	***V9-Os (1-outlet)*** ***V9M-Os*** is not listed, but selected as ***V9-Os***

System settings

If the outlet valve is not placed in the recommended position using the standard tubing kit, the delay volume must be set. See *Set the delay volume, on page 203*.

4.2.9 Configuration of UV monitors

Using two UV monitors

The UV monitors **U9-T**, and **U9-L** can be used in two configurations, as primary or secondary UV monitor. The secondary UV monitors are called respectively ***U9-T 2nd*** and ***U9-L 2nd*** in UNICORN.

It is possible to use two UV monitors in ÄKTA pure, the six possible combinations are listed in the table below. The configuration of each UV module is defined by the Node ID.

Primary UV monitor	Secondary UV monitor
U9-M	**U9-T**
U9-M	**U9-L**
U9-T	**U9-T**
U9-T	**U9-L**
U9-L	**U9-T**
U9-L	**U9-L**

The secondary UV monitor (***U9-T 2nd*** or ***U9-L 2nd***) can be located anywhere in the flow path and is therefore shown in the ***Process Picture*** in UNICORN as a component without a fixed place. This means that it is possible to place the secondary UV monitor before the primary UV monitor in the flow path.

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Monitors and sensors*** from the ***Component types*** list.
3	Select components and properties according to the following table:

Monitor	Component selection	Property
UV monitor **U9-M**	***UV monitor***	***U9-M***
UV monitor **U9-T**	***UV monitor***	***U9-T***
UV monitor **U9-T**, 2nd	***UV monitor 2nd***	***U9-T 2nd***
UV monitor **U9-L**	***UV monitor***	***U9-L***

Step	Action

Monitor	Component selection	Property
UV monitor **U9-L**, 2nd	***UV monitor 2nd***	***U9-L 2nd***

Note:

The secondary UV monitor is only available in the ***Component selection*** *list when the primary UV monitor is already selected.*

For a system with two UV monitors, also set the following:

- The primary wavelength of the primary UV monitor is by default used for peak fractionation. This can be changed in ***System control*** and in ***Method Editor***.

  In ***Method Editor***, go to ***Phase Properties*** →***Fractionation Settings*** →***Peak Frac Settings*** or ***Text instructions***.

  In ***System control***, go to ***Manual instructions*** or ***System settings***.
- If the secondary UV monitor is placed on the high pressure side of the column, pressure limits have to be considered. See *UV monitor options, on page 530* for pressure limits.
- The delay volumes need to be updated, see *Set the delay volume, on page 203*.

System settings

If the UV monitor is not placed in the recommended position using the standard tubing kit, or a secondary UV monitor is used, the delay volume must be set. See *Set the delay volume, on page 203*.

The flow cell length must be set for UV monitor **U9-T**, and **U9-L**. This is done as a calibration. See *Performing the calibration, on page 361*.

4.2.10 Configuration of conductivity monitor

Using two conductivity monitors

The Conductivity monitor **C9** can be used in two configurations, as primary or secondary conductivity monitor. The secondary conductivity monitor is called ***Conductivity monitor 2nd (C9)*** in UNICORN. The configuration is defined by the Node ID of the module.

The secondary conductivity monitor (***Conductivity monitor 2nd (C9)***) can be located anywhere in the flow path and is therefore shown in the ***Process Picture*** in UNICORN as a component without a fixed place. This means that it is possible to place the secondary conductivity monitor before the primary conductivity monitor in the flow path.

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Monitors and sensors*** from the ***Component types*** list.
3	From the ***Component selection*** list select:

Conductivity monitor	Select
Primary	***Conductivity monitor (C9)***
Secondary	***Conductivity monitor 2nd (C9)***

Note:

Conductivity monitor 2nd (C9) *is only available in the* ***Component selection*** *list when* ***Conductivity monitor (C9)*** *is already selected.*

Note:
The conductivity monitor **C9M** *for ÄKTA pure micro is selected as* ***Conductivity monitor (C9)*** *and* ***Conductivity monitor 2nd (C9)*** *in UNICORN.*

System settings

If the conductivity monitor is used for peak fractionation, or if a secondary conductivity monitor is used after the UV monitor, the delay volume must be changed. See *Set the delay volume, on page 203*.

4.2.11 Configuration of external air sensors

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Monitors and sensors*** from the ***Component types*** list.
3	Select the used external Air sensor in the ***Component selection*** list. An external Air sensor (**L9-1.5** or **L9-1.2**) can only be selected if the requirements in the table below are met.

Sensor	Constraints
External air sensor L9	External air sensor L9 has no constraints.
External Air sensor L9, 2	External Air sensor L9, 2 requires External Air sensor L9.
External Air sensor L9, 3	External Air sensor L9, 3 requires External Air sensor L9, 2.
External Air sensor L9, 4	External Air sensor L9, 4 requires External Air sensor L9, 3.

4	Select ***Property*** according to where the air sensor is placed. ***Note:*** *The available system properties are the same, regardless of which type of air sensor that is used,* **L9-1.2** *or* **L9-1.5***.*

System settings

The sensitivity of the external air sensor can be set.

Parameter	Air volume detected		Usage
	ÄKTA pure 25	ÄKTA pure 150	
Normal (default)	30 µL	100 µL	Detect empty buffer/ sample vessels
High	10 µL	30 µL	Detect even small air bubbles

Note: *The sensitivity must be set to **Normal** when the external air sensor is located before the System pump (**Air sensor ext - Before pump A** or **Air sensor ext - Before pump B**). Due to higher pressure and risk of small air bubbles, the sensitivity must be set to **High** when the air sensor is located after the Injection valve (**Air sensor ext - after Injection valve**).*

4.2.12 Configuration of fraction collectors

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Fraction collectors*** from the ***Component types*** list.
3	Select components and properties according to the following table:

Fraction collector	Component selection	Property
Fraction collector F9-C	***Fraction collector***	***F9-C***
Fraction collector F9-R	***Fraction collector***	***F9-R***
Fraction collector F9-R, 2nd	***Fraction collector F9-R 2nd***	N/A
Fraction collector F9-T	***Fraction collector***	***F9-T***

Note: *__Fraction collector 2nd__ is only available in the __Component selection__ list if __Fraction collector__ is already selected.*

System settings

If non-standard tubing is used between the outlet valve and the fraction collector, the delay volume must be set. See *Set the delay volume, on page 203*.

Fraction settings and numbering mode can be set.

Fraction collector F9-C

Instruction name	Description
Fractionation settings	Comprises fractionation mode and fractionation order. ***Fractionation mode*** : • ***Automatic*** • ***Accumulator*** • ***DropSync*** ***Fractionation order***: • ***Row-by-row*** • ***Column-by-column*** • ***Serpentine-row*** • ***Serpentine-column*** For fractionation mode ***DropSync***, only the ***Serpentine*** option is available.
Last tube filled	Action when last tube is filled: • Pause • Direct the flow to one of outlets • Direct the flow to waste
Cassette configuration	***Automatic*** or ***Manual*** configuration. • If ***Automatic*** is selected, a Quick scan or a Full scan will be performed when the door of the fraction collector is closed to determine which type of cassettes and plates are used. • If ***Manual*** is selected, used plates and tubes in each tray position are entered.
Fraction collector lamp	Lamps in the fraction collector chamber on or off.
Peak fractionation parameters	Set the detection parameters for peak collection, that is they decide when a peak starts and ends. This information is used by the instructions ***Peak fractionation*** and ***Peak frac in outlet valve*** in order to start and end the peak collection.

Fraction collector F9-R

Instruction name	Description
Fractionation settings	***Drop sync*** synchronises tube change to drop release. The available settings are on or off. It is recommended to use ***Drop sync*** for flow rates below 2 mL/min. Higher flow rates can however be used, depending on the properties (for example viscosity) of the liquid.
Fractionation numbering mode	Determines whether the fraction number is reset at the end of a method or not. ***Note:*** *The default setting is* ***Reset****.*
Fractionation settings frac 2nd	***Drop sync*** on or off. It is recommended to use this setting for flow rates below 2 mL/min. Higher flow rates can however be used, depending on the properties (e.g. viscosity) of the liquid.
Fractionation numbering mode frac 2nd	Determines whether fraction number for the second fraction collector is reset at the end of a method or not. ***Note:*** *The default setting is* ***Reset****.*
Peak fractionation parameters	***Peak fractionation parameters*** sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends. This information is used by the instructions ***Peak fractionation***, ***Peak fractionation frac 2nd*** and ***Peak frac in outlet valve*** in order to start/end the peak collection.

Fraction collector F9-T

Instruction name	Description
Drop sync	Drop sync synchronize movement after drop release. The available settings are ***On***, ***Off***, and ***Auto***. The Auto setting turns the drop synchronization on for flow rates below 3 mL/min, and turns it off for flows above 3 mL/min. This is done for all plate types except 96 deep well, where the limit is 5 mL/min. Do not use the Auto setting together with a micro nozzle because the micro nozzle only form drops up to 1.5 mL/min.

Instruction name	Description
Collection of pre-fractionation volume	The options for ***Collection of pre-fractionation volume*** are: • ***Auto*** sets the pre-fractionation volume to be collected in a separate well/tube position if the fraction volume is below 5 mL. If the fraction volume is 5 mL or more, the pre-fractionation volume will be collected in the same tube/well as the first fraction. • ***Combined with first*** fraction is used when the pre-fractionation volume shall be collected in the same position as the first fraction. ***Note:*** *If the sum of pre-fractionation plus fraction volume exceed the maximum volume for the well/tube, the pre-fractionation volume will automatically be collected in a separate well/tube.* • ***Separate position*** is used when the pre-fractionation volume shall be collected in a position that is different from the first fraction.
Last position filled action	***Last position filled action*** sets system behavior after the last position has been filled during fractionation. The options for ***Last position filled action*** are: • ***Out 1*** directs the flow to outlet 1 after the last position has been filled. • ***Pause*** sets the method to pause after the last position has been filled. • ***Waste*** directs the flow to waste after the last position has been filled. ***Tip:*** *It is possible to enable collection in more than two plates when* ***Last position filled action*** *is set to* ***Pause****.*

Instruction name	Description
Flow when moving from plate 1 to plate 2	***Flow when moving from plate 1 to plate 2*** sets system behavior when moving between plates. The options for ***Flow when moving from plate 1 to plate 2*** are: • ***Keep flow***, keeps the flow rate when the fractionation arm is moving from plate 1 to plate 2. For high flow rates, drops may be lost during movement. • ***Direct flow to waste,*** directs the flow to waste when the fractionation arm is moving from plate 1 to plate 2. When the fractionation arm reaches its position the outlet valve will turn to frac again. • ***Auto*** chooses ***Keep flow*** or ***Direct flow to waste*** depending on flow rate. ***Keep flow*** is selected for flow rates below 1.5 mL/min, ***Direct flow to waste*** for flow rates above or equal to 1.5 mL/min.
Frac behavior when ending a run	***Frac behavior when ending a run*** sets system behavior after the run has ended. The options for ***Frac behavior when ending a run*** are: • ***Go home without reset*** moves the fractionation arm to its home position without resetting the plate(s). • ***Go home and reset*** moves the fractionation arm to the home position and resets the plate(s). • ***Remain without reset*** keeps the fractionation arm at its last position without resetting the plate.
LED mode	***LED mode*** switches the green light in the fractionation arm on or off during fractionation.
Peak fractionation parameters	***Peak fractionation parameters*** sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends.

4.2.13 Configuration of I/O-box

System properties

Follow the instruction below to update the system properties.

Step	Action
1	Open the system properties ***Edit*** dialog box.
2	Select ***Other*** from the ***Component types*** list.
3	From the ***Component selection*** list select:

Conductivity monitor	Select
Primary	***I/O-box (E9)***
Secondary	***I/O-box 2 (E9)***

Note:

I/O-box 2 (E9) *is only available in the* ***Component selection*** *list when* ***I/O-box (E9)*** *is already selected.*

System settings

Default values for digital out ports, noise reduction and configuration of analog out ports can be set.

Instruction name	Description
Digital out X	Sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Noise reduction analog in X	Filters the noise in the analog signal in port number X.
Alarm analog in X	Enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm digital in X	Enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in 'Value' occurs, an alarm will be triggered and the method will be paused.

Instruction name	Description
Configure analog out X	Enables the user to send one of the pre-defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

Note: *The delay volume has to be updated if an external module, that is connected to the I/O box, is added to the flow path after the fractionation source.*

4.3 Configure ÄKTA pure micro

Select and configure the instrument

Follow the steps below to update the ***System Properties*** for the Micro kit.

Note: *Instrument modules are referred to as* ***Components*** *in UNICORN.*

Step	Action
1	Go to ***System Properties***, then select the system.
2	Click ***Edit***.
3	Select the relevant instrument configuration in the ***Instrument configuration*** drop-down menu.
4	Go through all ***Component types*** and select the installed modules. ***Note:*** *The Conductivity monitor* **C9M** *for ÄKTA pure micro is selected as Conductivity monitor* ***C9***.
5	Select ***Injection valve***, with property **V9M-J**.
6	Select ***Outlet valve***, with property **V9-Os** (1-outlet). ***Note:*** *The Outlet valve* **V9M-Os** *for ÄKTA pure micro is selected as Outlet valve* ***V9-Os***.
7	Click ***OK*** to apply the changes.

Adjust *System Settings*

This section describes which changes need to be made in the ***System Settings*** after installing the Micro kit.

Tubing and delay volumes

In ***UNICORN***, select ***System Control*** →***System*** →***Settings*** →***Tubing and Delay volumes***.

The following table shows the settings for the red tubing kit.

Setting	Input value
Tubing: Injection valve to Column	i.d. 0.13 mm and length 520 mm
Delay volume: Monitor to outlet valve	12 µL
Delay volume: Monitor to frac	18 µL

The following table shows the settings for the blue tubing kit.

Setting	Input value
Tubing: Injection valve to Column	i.d. 0.25 mm and length 520 mm
Delay volume: Monitor to outlet valve	38 µL
Delay volume: Monitor to frac	60 µL

Wash settings

Select in ***UNICORN*** →***System Control*** →***System*** →***Settings*** →***Wash settings***.

- Set ***System wash flow rate*** to 10 mL/min
- Set ***System pump wash flow rate*** to 10 mL/min
- Set ***System pump wash volume*** to 10 mL

Note: *Adjust the flow rate during the system wash to avoid high system pressure if a column valve is used.*

Fractionation settings

Fraction collector	Setting
Fraction collector **F9-R**	In UNICORN, select ***System Control*** →***System*** →***Settings*** →***Fraction collection*** →***Fractionation setting*** and set ***Drop Sync*** to ***On***.
Fraction collector **F9-T**	In UNICORN, select ***System Control*** →***System*** →***Settings*** →***Fraction collection*** and set ***Drop Sync*** to ***On***. ***Note:*** *Do not set* ***Drop sync*** *to* ***Auto****.*

4.4 General system settings

Set the delay volume

Adjust the delay volume in the ***System Setting*** dialog box in UNICORN when:

- A module has been installed after the module that is set as the peak fractionation source in the flow path.
- Another monitor is used as peak fractionation source than the default setting. The default peak fractionation source is the primary wavelength of the primary UV monitor.

Delay volumes can be set for the options ***Monitor to outlet valve***, ***Monitor to frac***, ***Monitor to frac 2***, and ***pH valve***. Depending on the system configuration used, different delay volume options will be available for selection in the ***System Setting*** dialog box. The delay volume has to be set for all displayed options.

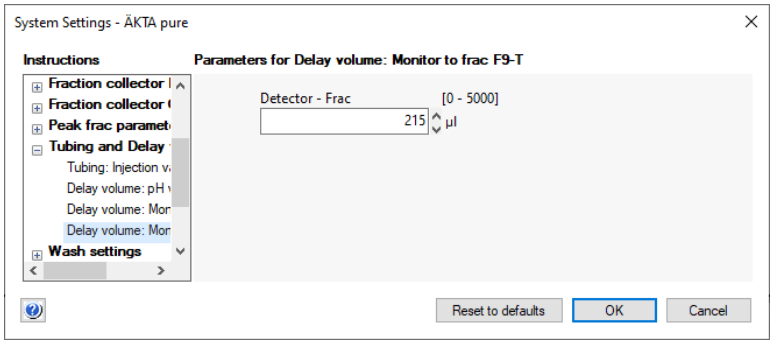
Note: *The system will use the delay volume appropriate to the configuration used and ignore other settings (e.g. the value for* ***Monitor to outlet valve*** *will be ignored when the fraction collector is used). It is however recommended to set all delay volumes so that the volumes remain correct when the fractionation method is changed.*

Delay volumes for modules and standard tubing configurations are found in *Section 9.13 Delay volumes, on page 635*. Delay volumes per tubing length are found in *Tubing types, on page 536*.

Follow the steps below to check or set the delay volumes:

Step	Action
1	Check that the system is in state ***Ready***.
2	In ***System Control***, select ***System*** →***Settings***.
3	Select ***Tubing and Delay Volumes*** and set tubing I.D. and length so that it corresponds to the configuration you are using. Click ***OK***.

Step	Action
4	Select ***Tubing and Delay Volumes*** and select the delay volume option of interest.

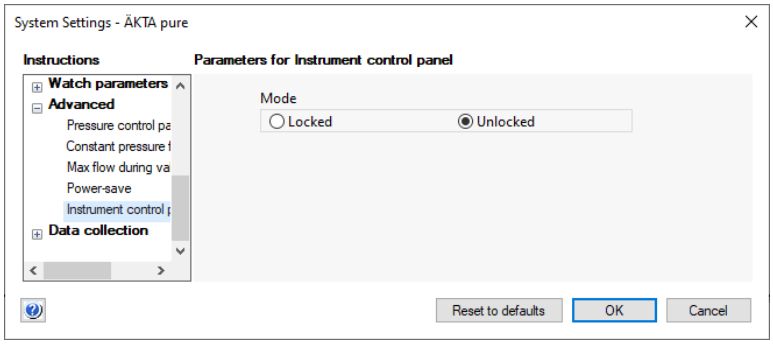


5	Check the delay volume, and write a new value in the volume field if necessary.
6	Click ***OK***.

Lock or Unlock function

Follow the instruction below to lock or unlock the **Pause** and **Continue** buttons of the Instrument control panel from UNICORN.

Step	Action
1	In ***System Control***, select ***System*** →***Settings***.
2	In the ***System Settings*** dialog box: **a.** Select ***Advanced*** →***Instrument control panel***. **b.** Select ***Locked*** or ***Unlocked***. **c.** Click ***OK***.



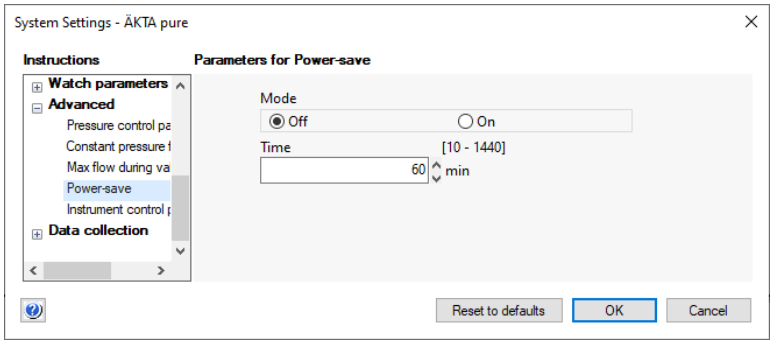
Power-save

ÄKTA pure has a power-save mode. The instrument enters ***Power-save*** after having been in the ***Ready*** state for a set period of time. The system enters the ***Ready*** state when a method run, a method queue or a manual run ends.

To enable ***Power-save***, a system must be connected and in state ***Ready***.

Follow the steps below to activate ***Power-save***.

Step	Action
1	Select ***System*** →***Settings*** in the ***System Control*** module.
2	Go to ***Advanced*** →***Power-save***.



3	**a.** Select ***On*** in the ***Mode*** field. **b.** Type the number of minutes in the ***Time*** field. ***Note:*** *This is the time the instrument will be in state **Ready** before power-save mode is entered.* **c.** Click ***OK***.

5 Operation

About this chapter

This chapter describes the steps involved when operating ÄKTA pure.

In this chapter

Section		See page
5.1	Before preparing the system	207
5.2	Prepare the flow path	208
5.3	Start UNICORN and connect to system	217
5.4	Prime inlets and purge pump heads	220
5.5	Connect a column	233
5.6	Pressure alarms	238
5.7	Sample application	242
5.8	Fractionation	260
5.9	Create a method and perform a run	284
5.10	ÄKTA pure micro or Micro kit operation	299

5.1 Before preparing the system

Introduction

It is important to prepare the system in accordance with the settings in the method to be run. Before preparing the system, check the settings in the ***Method Editor*** and make sure that all accessories to be used are available.

Checklist

Make sure the system is prepared in accordance with the settings in the method to be run.

Depending on configuration, remember to check the following:

- which valve ports to use for inlets and outlets
- which column type to use
- which column position to use
- which buffers and samples to prepare
- which sample application technique to use
- which monitors and sensors to use

Note: *Make sure that all used monitors and sensors are calibrated according to the maintenance program. See* *Section 7.3 Calibration procedures, on page 348**.*

5.2 Prepare the flow path

About this section

This section describes how to prepare the flow path before a run.

In this section

Section		See page
5.2.1	Overview	209
5.2.2	Select mixer chamber	211
5.2.3	Select UV flow cells	212
5.2.4	Prepare tubing	213
5.2.5	Waste	214

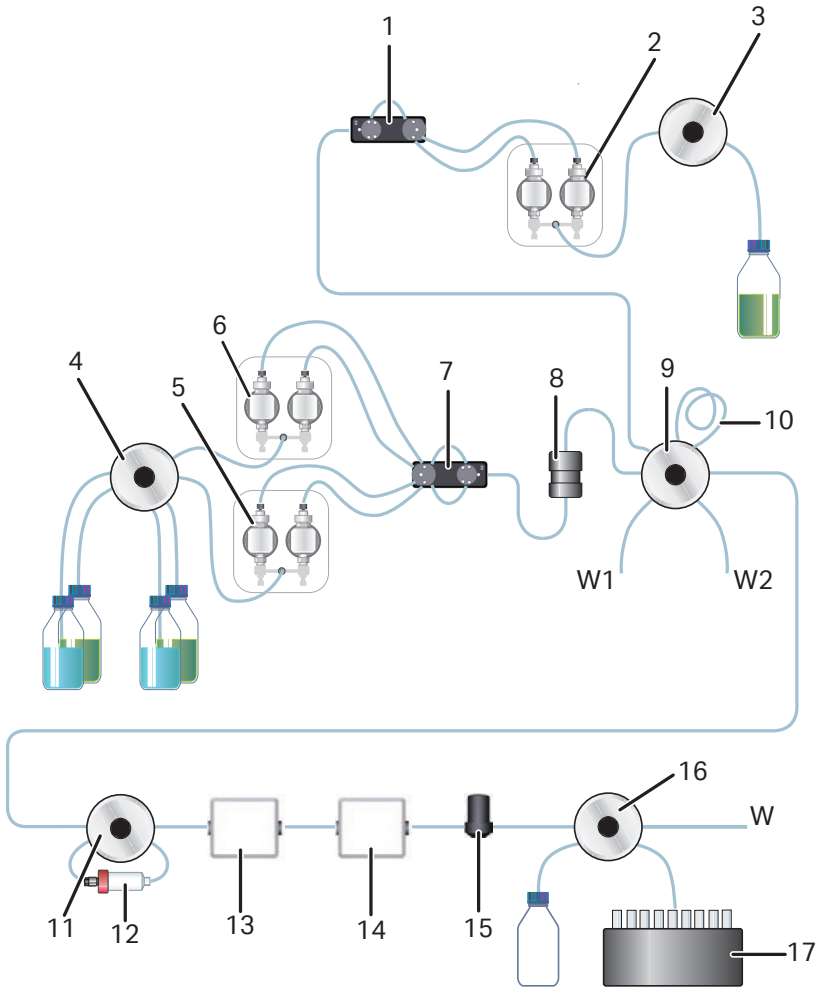
5.2.1 Overview

Introduction

The flow path is defined by the user and may contain tubing, valves, pumps and monitors. This section gives an overview of a flow path.

Illustration of the flow path

The illustration below shows the flow path for a typical system configuration. The individual instrument modules are presented in the following table. The configuration of the system is defined by the user.



Part	Description
1	Pressure monitor
2	Sample pump
3	Sample inlet valve
4	Inlet valve
5	System pump B
6	System pump A
7	Pressure monitor
8	Mixer
9	Injection valve
10	Sample loop or Superloop
11	Column valve
12	Column
13	UV monitor
14	Conductivity monitor
15	Flow restrictor
16	Outlet valve
17	Fraction collector
W, W1, W2	Waste

5.2.2 Select mixer chamber

To obtain a homogeneous buffer composition, it is important to use a Mixer chamber suitable for the flow rate of the method. The tables below show what Mixer chambers to use in ÄKTA pure at different flow rates.

If the liquids are difficult to mix, use a larger Mixer chamber to achieve optimal mixing.

Note: *Using a larger Mixer chamber distorts and delays the gradient.*

The default mixer size is 1.4 mL for both systems. ÄKTA pure 150 also includes a 5 mL mixer. Other mixer sizes are available as accessories.

Mixer chamber volume (mL)	Flow rate (mL/min) for a binary gradient	
	ÄKTA pure 25	ÄKTA pure 150
0.02 (online filter)	0.05 to 0.25 with micro configuration	Not recommended
0.6	0.1 to 5	Not recommended
1.4	0.5 to 15	0.5 to 15
5	2 to 25	2 to 50
15	Not compatible	15 to 150

CAUTION

Risk of explosion. Do not use mixer chamber 15 mL with an ÄKTA pure 25 configuration. The maximum pressure for mixer chamber 15 mL is 5 MPa.

Note: *In ÄKTA pure 25, at low flow rates (0.25 mL/min or lower), a gradient of sufficient quality can be achieved without using the mixer. For low flow rates in ÄKTA pure 150, the 0.6 mL mixer chamber can be used.*

Note: *The 1.4 mL mixer chamber in ÄKTA pure 25, or 5 mL mixer chamber in ÄKTA pure 150 can work up to the maximum flow rate, provided that the buffers are easily mixed.*

For information on how to install a Mixer chamber, refer to *Section 7.5.4 Replace the mixer, on page 408*.

5.2.3 Select UV flow cells

General considerations

Flow cells with shorter path lengths are suitable to use when high protein concentrations are used. Flow cells with longer path lengths are suitable to use when low protein concentrations are used.

UV flow cells for UV monitor U9-M

UV flow cells are available with three different path lengths; 0.5 mm, 2 mm (default) and 10 mm.

The real cell path length of the UV cell is automatically recognized by the monitor when a cell is fitted. The UV data is normalized to the nominal path length. This allows UV data from runs made with different UV flow cells (but with the same nominal path length) to be directly compared.

UV flow cells for UV monitor U9-T and U9-L

UV flow cells are available with three different path lengths; 0.4 mm, 2 mm (default), and 5 mm. When replacing a UV flow cell, the path length must be set in the ***System Control*** module, in ***System*** →***Calibrate***. Use the nominal flow cell length if the UV flow cell is replaced but not calibrated. See *Updating the cell path length, on page 363* to set the flow cell path length.

The path length of the UV flow cell might differ from the nominal length, which leads to incorrect results in the calculation of protein concentration in the eluate. The UV data is normalized to the nominal path length. This allows UV data from runs made with different UV flow cells (but with the same nominal path length) to be directly compared. To achieve normalized absorbance, the path length of the UV flow cell must be calibrated and the calculated flow cell path length set manually. See *Calibration of the UV monitor U9-T and U9-L flow cell length, on page 360*.

5.2.4 Prepare tubing

Prepare the inlet tubing

Connect inlet tubing to the inlet ports that are to be used, and place all inlet tubing that is to be used during the method run in the correct buffers.

Note: *When using high viscosity buffers/samples in combination with high flow rates it is recommended to increase the tubing I.D. and/or shorten the length.*

Prepare the outlet tubing

Connect outlet tubing to the outlet ports of Outlet valve that are to be used. If a fraction collector is to be used, make sure that tubing is connected as described in *Connect Fraction collector F9-C, on page 134*, *Section 3.3.3 Connect the Fraction collector F9-R, on page 139*, or *Section 3.4.3 Connecting the Fraction collector F9-T, on page 148*. Then, prepare the fraction collector as described in *Section 5.8.3 Prepare Fraction collector F9-C, on page 266*, *Section 5.8.4 Prepare Fraction collector F9-R, on page 272*, or *Section 5.8.5 Prepare Fraction collector F9-T, on page 278*. Otherwise, place the outlet tubing in suitable tubes or flasks.

5.2.5 Waste

Waste tubing overview

The table below lists the waste tubing of the instrument and where it is located. Make sure that the waste tubing is connected to the correct positions on the modules.

Module	Tubing connections	Location of tubing
Injection valve	Waste ports **W1** and **W2**	Front of the ÄKTA pure instrument.
pH valve (optional)	Waste port **W3**	Front of the ÄKTA pure instrument.
Outlet valve (optional)	Waste port **W**	Front of the ÄKTA pure instrument.
Buffer tray (Rescue drainage)	Drainage hole of the Buffer tray	Rear of the ÄKTA pure instrument.
Fraction collector F9-C (optional)	Fraction collector waste outlet	Rear of the fraction collector.

Prepare waste tubing

Follow the steps below to prepare the waste tubing.



CAUTION

Fasten the waste tubing. During operation at high pressure the ÄKTA pure instrument may release bursts of liquid in the waste tubing. Securely fasten all waste tubing to the ÄKTA pure instrument and to the waste vessel.



CAUTION

Make sure that the waste vessel will hold all the produced volume of the run. For ÄKTA pure, a suitable waste vessel must typically have a volume of 2 to 10 liters.

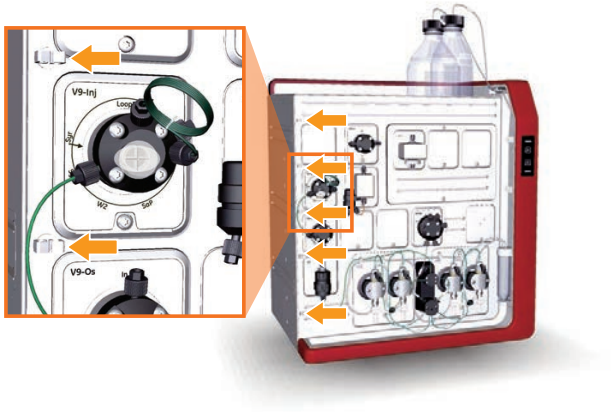


NOTICE

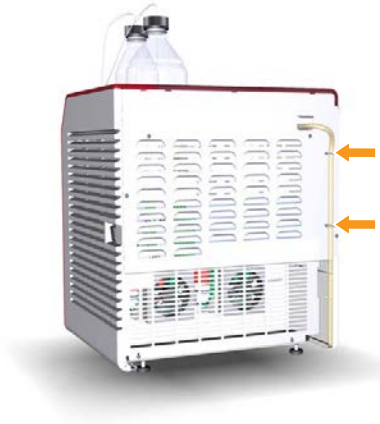
The maximum level of the waste vessel must be lower than the bottom of the ÄKTA pure instrument.

Step	Action
1	Insert the waste tubing from all installed modules, in this example Injection valve (**W1** and **W2**), Outlet valve (**W**) and the fraction collector, in a vessel.
2	Make sure that the tubing is securely fastened to the ÄKTA pure instrument:

a. Fasten waste tubing from the valves with the clips on the front of the system.



b. Fasten waste tubing from the Buffer tray with the clips on the rear of the system.



Step	Action
3	Cut the waste tubing to appropriate length. It is important that the tubing is not bent and will not be submerged in liquid during the run.



Note:

If the tubing is too short, replace it with new tubing. Do not lengthen the tubing as this might cause obstruction of the tubing.

4	Fasten all waste tubing securely to the waste vessel.

Plug unused valve ports

It is recommended to plug all unused valve ports with stop plugs before starting a run. See *Tubing connectors, on page 537* for information about connectors.

5.3 Start UNICORN and connect to system

Introduction

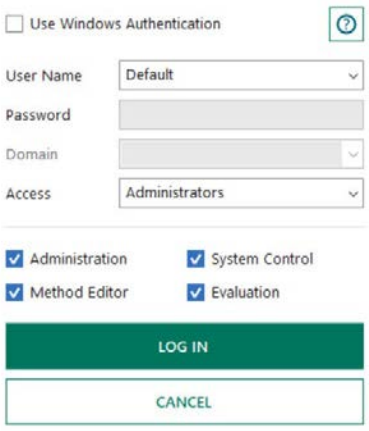
This section describes how to start and log on to UNICORN and how to connect the instrument to UNICORN.

Start UNICORN and log on

A valid e-license must be available for the workstation. See *UNICORN Administration and Technical Manual* for more information about e-licenses.

Follow the steps below to start and log on to UNICORN.

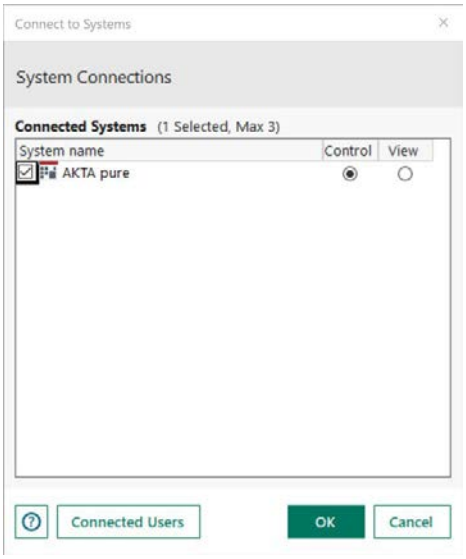
Step	Action
1	Double-click the UNICORN icon on the desktop. ***Note:*** *If there is no connection to the database it is still possible to log on to UNICORN and control a running system. The **Log On** dialog box will give the option to start **System Control** without a database. Click **Start System Control** to proceed to the next **Log On** dialog box.*
2	In the ***Log On*** dialog box, select a user name in the ***User Name*** list and enter the password in the ***Password*** field. ***Note:*** *The password requirement used to log on to UNICORN can be disabled in the user settings.* ***Note:*** *It is also possible to select the **Use Windows Authentication** check box and enter a network ID in the **User Name** box.*



Step	Action
3	Select which UNICORN modules to start, then click ***OK***.

Connect to system

Follow the instructions to connect the instrument to UNICORN.

Step	Action
1	In the ***System Control*** module, click the ***Connect to Systems*** button  *or* click ***Connect to Systems*** on the ***System*** menu. *Result:* The ***Connect to Systems*** dialog box opens. 
2	In the ***Connect to systems*** dialog box, select the checkbox in front of the system name.
3	To control the selected system, click ***Control***, then click ***OK***. *Result:* The instrument control panel displays a white, rapidly flashing light followed by a steady white light when the system is ready. The selected instrument can now be controlled by the software.

Step	Action
	Note: *Instruments that are turned off or disconnected from the network appear dimmed and cannot be connected.* ***Tip:*** *To view the users currently connected to systems, either in control or view mode, click the* ***Connected Users*** *button.* ***Tip:*** *Result UNICORN states* ***Connection = Connected in control*** *or* ***Connection = Connected in view*** *in the status bar in the lower part of the* ***System Control*** *window.* 

5.4 Prime inlets and purge pump heads

About this section

This section describes how to prime inlets and purge the pump heads of the system pumps and the sample pump (the sample pump is an optional module).

Note: *Before usage of a pump, it is important to:*

- *Prime the inlets (fill the buffer inlets with liquid).*
- *Purge the pump (remove air from the pump heads).*

Note: *The procedures described in this section may have to be adapted if your system configuration differs from the one described in this manual.*

In this section

Section		See page
5.4.1	System pumps	221
5.4.2	Sample pump	228

5.4.1 System pumps

Introduction

This section describes how to prime inlets and purge System pumps **P9-A**, **P9-B**, **P9H-A**, and **P9H-B**.

Overview

The procedure consists of the stages described in the table below.

Stage	Description
1	Prime all inlet tubing to be used during the run
2	Purge System pump B
3	Validate purge of System pump B
4	Purge System pump A
5	Validate purge of System pump A
6	End the run

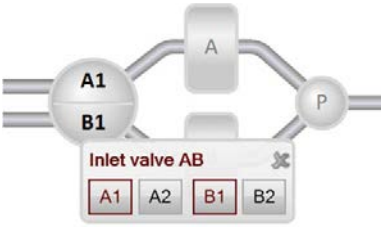
Tip: *The procedures for purging the pump heads and priming the inlets using the **Process Picture**, are described below. It is also possible to perform the procedures from the **Manual instructions** dialog box.*

Prime inlet tubing

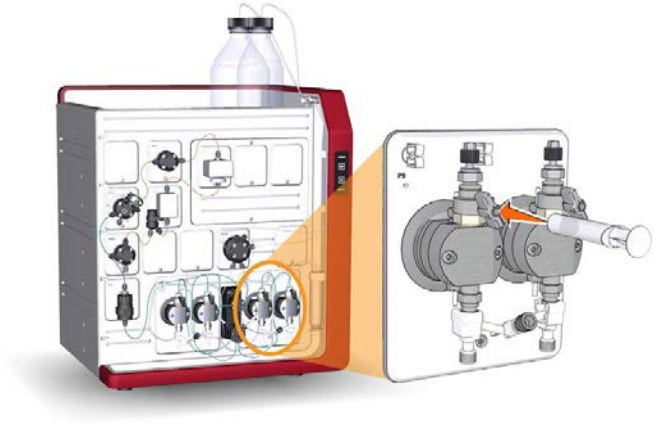
Follow the steps below to fill all A and B inlet tubing to be used in the run with appropriate buffer or solution.

Step	Action
1	Check if all inlet tubing that is to be used during the method run is placed in the correct buffer.
2	Open the ***System Control*** module.
3	**a.** In the ***Process Picture***, click on the buffer inlets.

Step	Action
	b. Select the position of the inlet to be filled. Select the positions in reverse alphabetical order and start with the highest number. For example, if all the four inlets in ***Inlet valve AB*** are to be filled, fill them in the following order: ***B2***, ***B1***, ***A2***, ***A1***.



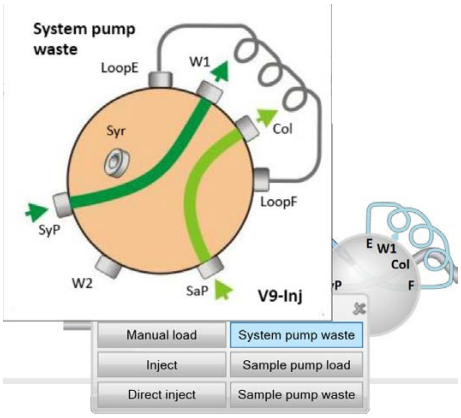
4	Connect a 25 to 30 mL syringe to the purge valve of one of the pump heads of the pump that is being prepared. Make sure that the syringe fits tightly into the purge connector.



5	Open the purge valve by turning it counter-clockwise about three quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.
6	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
7	Repeat steps 3 to 6 for each piece of inlet tubing that is to be used during the run.

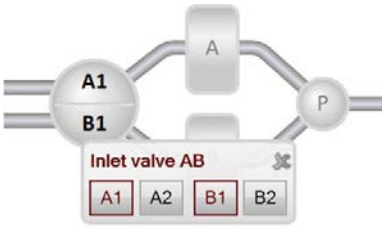
Purge System pump B

Follow the instruction below to purge both pump heads of System pump B.

Step	Action
1	Make sure that the piece of waste tubing connected to the Injection valve port **W1** is placed in a waste vessel.
2	In the ***Process Picture***, click on the ***Injection valve*** and select ***System pump waste***. For injection valve **V9M-J** select position ***Waste***. 
3	**a.** In the ***Process Picture***, click on the pumps. **b.** Set ***Conc % B*** to 100% B.  **c.** Click ***Set % B***. *Result:* Only System pump B is active.
4	**a.** In the ***Process Picture***, click on the buffer inlets.

Step	Action

b. Select the position of one of the inlets that will be used at the beginning of the run.



5

a. In the ***Process Picture***, click on the ***Pumps***.

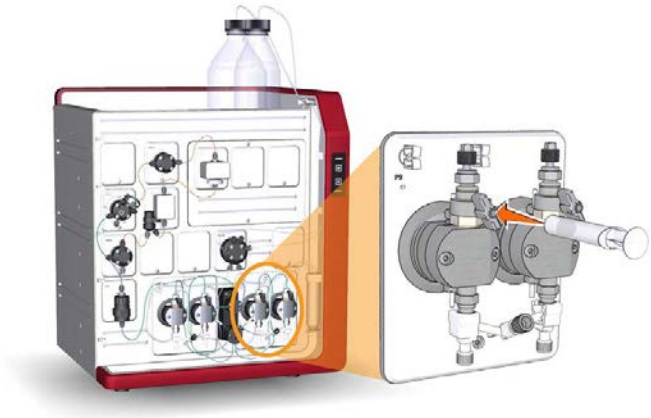
b. Set the ***System flow*** to 1.0 mL/min for ÄKTA pure 25, 10.0 mL/min for ÄKTA pure 150, or 0.25 mL/min for ÄKTA pure micro.

c. Click ***Set flow rate***.

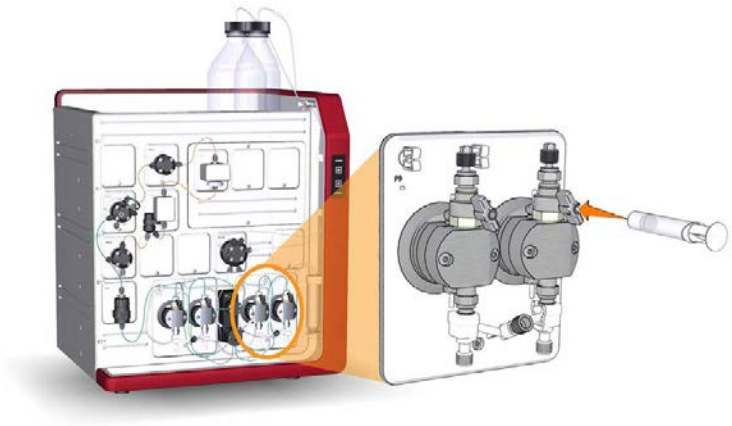


6

Connect a 25 to 30 mL syringe to the purge valve of the left pump head of System pump B. Make sure that the syringe fits tightly into the purge connector.



Step	Action
7	Open the purge valve by turning it counter-clockwise about three quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.
8	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
9	Connect the syringe to the purge valve on the right pump head of System pump B, and repeat steps 6 to 8. Keep the system flow running.

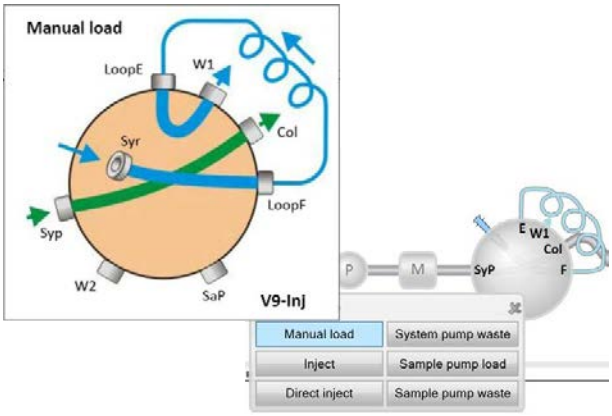


Validate purge of pump B

Follow the steps below to check that there is no air left in the pump after performing a purge.

Step	Action
1	In the ***Process Picture***, click on the ***Injection valve*** and select ***Manual load***.

Step	Action



Note:

For injection valve **V9M-J** *select position* ***Load****.*

2 Make sure that the pump flow is on.

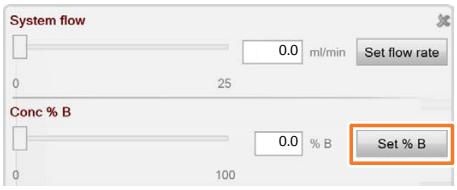
3 In the ***Chromatogram*** pane, check the ***PreC pressure*** curve. If the ***PreC pressure*** do not stabilize within a few minutes there may be air left in the pump. See *Section 8.6 Troubleshooting: Pumps, on page 490* for a trouble-shooting guide.

Purge System pump A

Purge both pump heads of System pump A by following the same procedure as in *Purge System pump B, on page 223*, but replace step 3 with the following actions:

In the ***Process Picture***:

1. Click on the pumps.
2. Set ***Conc % B*** to 0% B.
3. Click ***Set % B***



Result:

Only System pump A is active.

Validate purge of pump A

Follow the procedure described in *Validate purge of pump B, on page 225* to check if there is air left in the pump.

End the run

Click the ***End*** button in the ***System Control*** toolbar to end the run.



5.4.2 Sample pump

Introduction

This section describes how to prime inlets and purge Sample pumps **S9** and **S9H**.

Overview

The procedure consists of the stages described in the table below.

Stage	Description
1	Prime all sample inlet tubing to be used during the run
2	Purge the Sample pump
3	Validate purge of Sample pump
4	End the run

Tip: *The procedures for purging the pump heads and priming the inlets using the **Process Picture**, are described below. It is also possible to perform the procedures from the **Manual instructions** dialog box.*

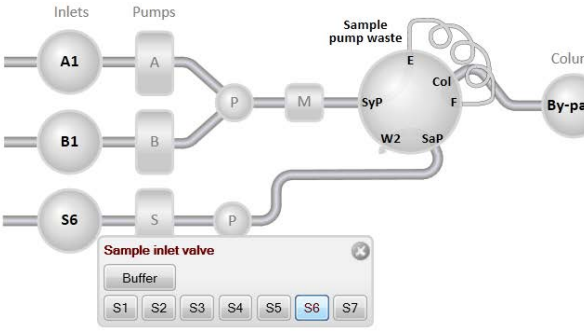
Prime sample inlets

Follow the steps below to fill all sample inlet tubing, to be used in the run, with appropriate buffer or solution. Skip steps three and four if no sample inlet valve is used.

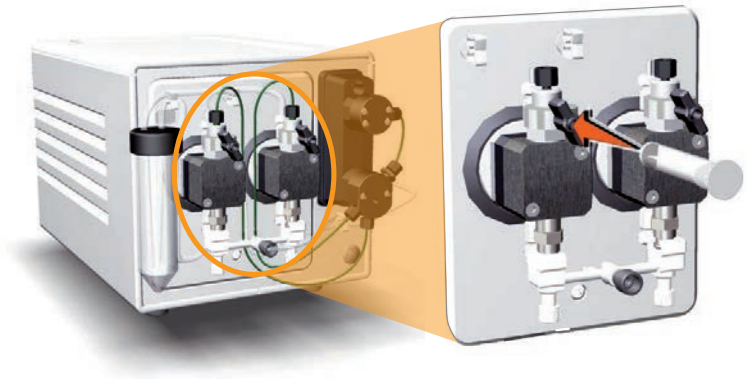
Step	Action
1	Make sure that all sample inlet tubing that is to be used during the method run is immersed in the correct buffers.
2	Open the ***System Control*** module.
3	**a.** In the ***Process Picture*** , click on ***Sample inlet valve***.

Step	Action

b. Select the position of the inlet to be filled. Start at the inlet position with the highest number and end at the position with the lowest number.



4 Connect a 25 to 30 mL syringe to one of the purge valves of the pump heads of the Sample pump. Make sure that the syringe fits tightly into the purge connector.



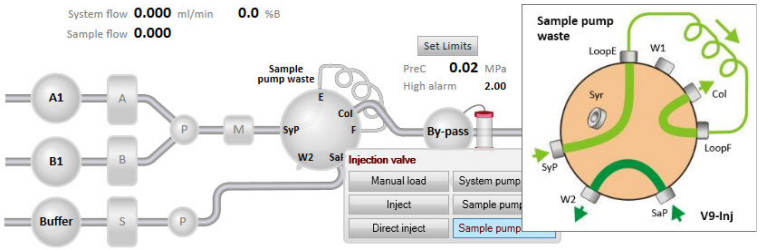
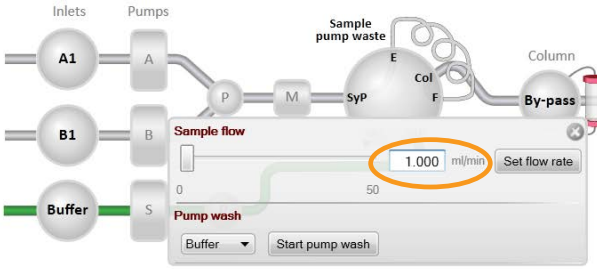
5 Open the purge valve by turning it counter-clockwise about three-quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the sample pump.

6 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.

7 Repeat steps 4-5 for each sample inlet that is to be used in the method run.

Purge the Sample pump

Follow the instruction below to purge both the pump heads of the Sample pump.

Step	Action
1	Make sure that the waste tubing connected to Injection valve port **W2** is immersed in a waste vessel.
2	Open the ***System Control*** module.
3	In the ***Process Picture***, click on ***Injection valve*** and select ***Sample pump waste*** . 
4	**a.** If Sample inlet valve **V9-IS** or **V9H-IS** is used, click on the ***Sample inlet valve*** icon in the ***Process Picture***. Then, select the ***Buffer*** inlet. **b.** Click on the ***Sample pump*** icon in the ***Process Picture***. Then, set the ***Sample flow*** to 1.0 mL/min for ÄKTA pure 25 or 10.0 mL/min for ÄKTA pure 150.  **c.** Click ***Set flow rate*** and close the pop-up window.

Step	Action
5	Connect a 25 to 30 mL syringe to the left purge valve of the Sample pump. Make sure that the syringe fits tightly into the purge connector.

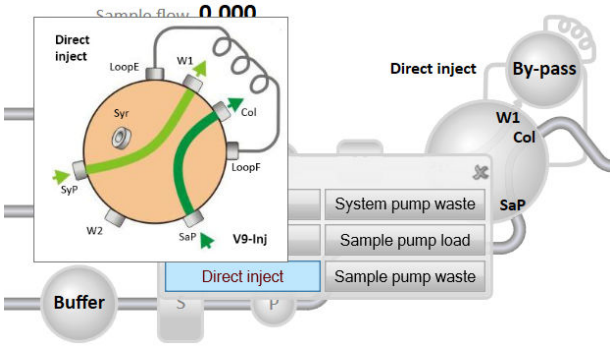


6	Open the purge valve by turning it counter-clockwise about three-quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.
7	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
8	Connect the syringe to the right purge valve on the Sample pump, and repeat step 6 to step 7.



Validate purge

Follow the steps below to check that there is no air left in the pump after performing a purge.

Step	Action
1	In the ***Process Picture***, click on the ***Injection valve*** and select ***Direct inject***. 
2	Make sure that the pump flow is on.
3	In the ***Chromatogram*** pane, check the ***PreC pressure*** curve. If the ***PreC pressure*** do not stabilize within a few minutes there may be air left in the pump. See *Section 8.6 Troubleshooting: Pumps, on page 490* for a trouble-shooting guide.

End the run

Click  in the ***System Control*** toolbar to end the run.

5.5 Connect a column

Introduction

This section describes how to connect a column to the instrument using a column holder and without introducing air into the flow path. Several types of column holders are available for ÄKTA pure.



WARNING

To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure of the column. Before connecting a column to the ÄKTA pure instrument, read the instructions for use of the column.

Methods automatically include a pressure alarm based on the specifications of the chosen column type. However, for manual runs set the pressure limits in the ***Process Picture*** or as a ***Manual Instruction***. To protect the chromatography resin, special settings need to be set. See *Section 5.6 Pressure alarms, on page 238* for more information on pressure alarms.



NOTICE

Do not overtighten when connecting columns. Overtightening might rupture the connectors or squeeze the tubing and thereby result in high back pressure.

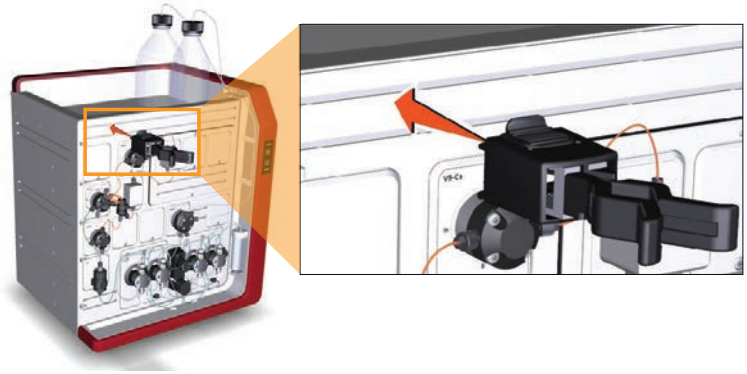
Note: *If no column valve is used, remove the column from the system before running a system wash. The pressure during a system wash may become too high for the column.*

Attach a column holder and connect a column

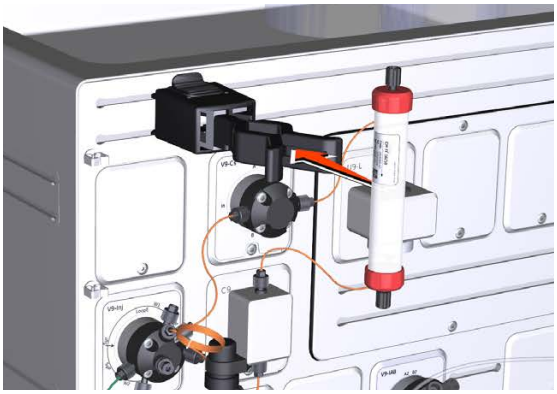
Always use a column holder. If a column valve is used, connect the column to the appropriate A and B ports on the valve. If no column valve is used, connect the column directly to the flow path tubing. Use appropriate tubing and connectors. The instructions show a system configured with Column valve **V9-Cs**.

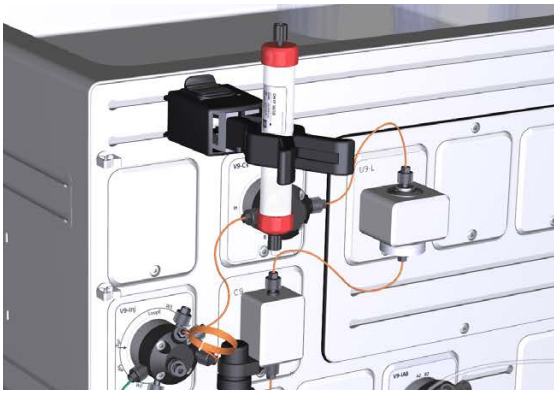
Follow the steps below to connect a column to the instrument.

Step	Action
1	Attach an appropriate column holder to the rail on the instrument.

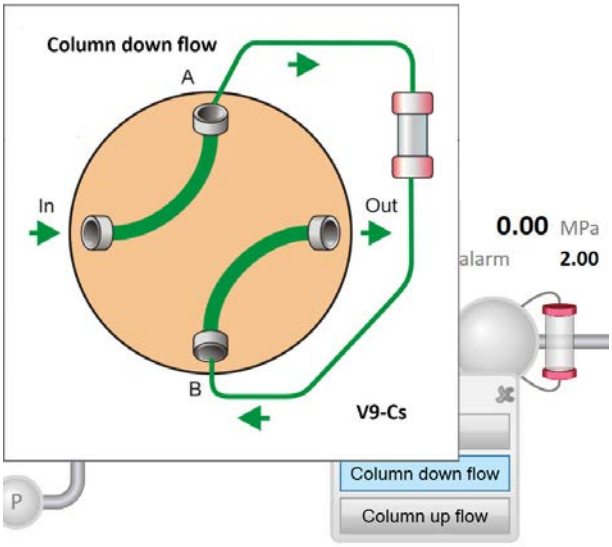


Step	Action
2	Attach the column to the column holder.





Step	Action
3	Connect a suitable tubing to a Column valve port, in this example port **1A**. If no column valve is used, connect the tubing to the previous module in the flow path.
4	Open the ***System Control*** module.
5	If a column valve is used: a. Go to the ***Process Picture***, and click on the ***Column valve***. b. Select ***Column down flow***.



6	In the ***Process Picture***: a. Click on the ***Pumps***. b. Enter a low ***System flow*** that is recommended for the used column. ***Tip:*** *The recommended flow rate and maximum pressure of a column are described in the* ***Column Handling*** *dialog. Click on the* ***Column Handling*** *icon (*  *) to open the* ***Column Handling*** *dialog.* c. Click ***Set flow rate***.

Step	Action



7 When buffer leaves the tubing in a continuous mode and the top part of the column is filled with buffer, connect the tubing to the top of the column.



8 Connect a piece of tubing to the bottom of the column.



Step	Action
9	When buffer leaves the tubing at the bottom of the column in a continuous mode, connect this piece of tubing to the Column valve. Use the port opposite to the one already connected to the column, in this example port **1B**. If no column valve is used, connect the tubing to the next module in the flow path.
	
10	Click the ***End*** icon in the ***System Control*** toolbar to end the run.
	

5.6 Pressure alarms

Introduction

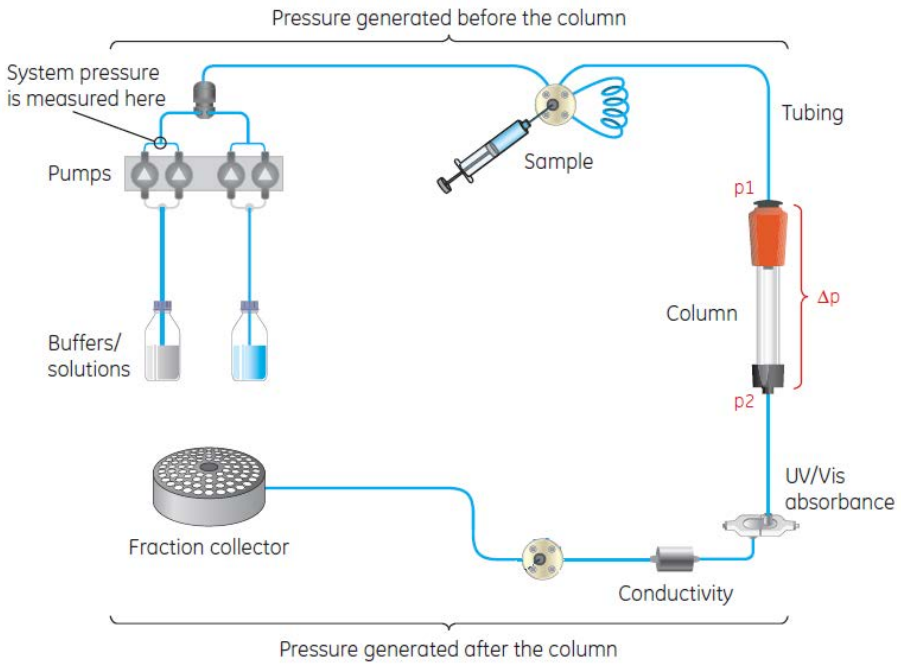
The columns can be protected by two different types of pressure alarms:

- The pre-column pressure alarm protects the column hardware
- The delta-column pressure alarm (only available when **V9-C** or **V9H-C** is installed) protects the chromatography resin

Column valves **V9-C** and **V9H-C** have built-in pressure sensors that automatically measure the pre-column and delta-column pressure. If Column valve **V9-C** or **V9H-C** is not used (the column is connected without a column valve or to Column valve **V9-Cs**, **V9H-Cs**, or **V9-Cm**), the pre-column pressure value is calculated based on the system pressure and the pressure drop between the system pressure sensor and the column. This takes into account temperature and tubing dimensions.

See the instructions below to set the pressure alarm for the column to be used in the run and, if applicable, to set the parameters for the tubing dimensions.

Description of pressure measurement



System pressure, generated by the complete system flow path, is measured in ÄKTA chromatography systems at the system pump. Δp = p1 (pressure generated after and by the column itself) – p2 (pressure generated after the column).

The pressure at the pump, System pressure, is always the highest pressure in the system. It can be divided into different part as:

$P_{system} = P_{before} + P_{delta\text{-}column} + P_{after}$

The pressure values related to columns are defined as:

$P_{pre\text{-}column} = P_{delta\text{-}column} + P_{after}$

$P_{post\text{-}column} = P_{after}$

$P_{delta\text{-}column} = P_{pre\text{-}column} - P_{post\text{-}column}$

The $P_{pre\text{-}column}$ value needs to be monitored to protect the column hardware. The $P_{delta\text{-}column}$ value needs to be monitored to protect the packed resin.

Setting pressure alarms

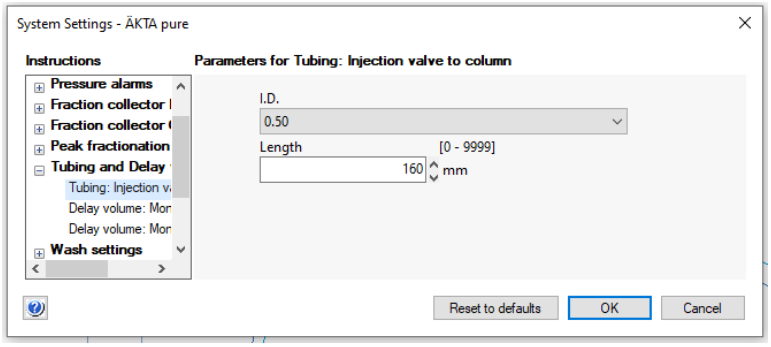
Only the $P_{pre\text{-}column}$ pressure alarm can be set in UNICORN when the column valve V9-C with pressure sensors is not installed. To protect the packed bed, the $P_{delta\text{-}column}$ pressure limit from the column list needs be used and set as $P_{pre\text{-}column}$ pressure alarm value. This is done automatically in UNICORN for predefined methods or phases for most glass columns.

Set tubing dimension parameters to calculate pre-column pressure

For instruments without a pre-column pressure sensor (that is without a Column valve, or with a **V9-Cm**, **V9-Cs**, or **V9H-Cs** Column valve) the pre-column pressure is calculated from the system pressure and tubing dimensions.

Follow the steps below to set the tubing dimension parameters.

Step	Action
1	Select ***System*** →***Settings*** in the ***System Control*** module.
2	Select ***Tubing and Delay volumes*** →***Tubing: Injection valve to column***.



3	**a.** Select the inner diameter of the tubing between the injection valve and the column from the ***I.D.*** drop-down list.

Step	Action
	b. Select the ***Length*** box and type the tubing length.
4	If the sample pump is used: **a.** Select ***Tubing: Sample pump to injection valve***. **b.** Select the inner diameter of the tubing between the injection valve and the column from the ***I.D.*** drop-down list. **c.** Select the ***Length*** box and type the tubing length.
5	Click ***OK***.

Pre-column pressure alarms

It is important that the pre-column pressure alarm is set during all runs where a column is used.

The pre-column pressure alarm can be set in:

- the method to be run
- the ***System Settings*** dialog box
- during a manual run

Pre-column pressure alarm limits are automatically set in the method when a column from the column list is selected in the method. Refer to *UNICORN Method Manual* for more information on pressure alarms.

For some columns, the max delta-column pressure (resin) is significantly lower than the max pre-column pressure (hardware). To protect the chromatography resin if a delta-column pressure measurement is not available (that is, when column valve **V9-C** or **V9H-C** is not used), the pre-column pressure alarm must be manually set to the value in the column list that is the lowest of the max pre-column pressure and the max delta-column pressure. This is done automatically in UNICORN for predefined methods or phases for some columns.

Delta-column pressure alarms

If column valve **V9-C** or **V9H-C** is installed, the delta-column pressure will be measured.

The delta-column pressure alarm can be set in:

- the method to be run
- the ***System Settings*** dialog box
- during a manual run

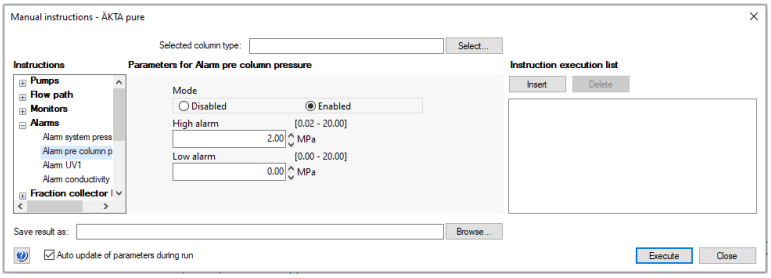
Delta-column pressure alarm limits are automatically set in the method when a column from the column list is selected in the method. Refer to *UNICORN Method Manual* for more information on pressure alarms.

Set pressure alarms

Pressure alarm limits can be set manually in ***System Control***. The example below describes how to set the high pressure limit for the column. Other alarms are set in a corresponding way.

Tip: *The procedures for setting the pressure alarms using the **Manual Instructions** dialog, are described below. It is also possible to perform the procedures from the **Process Picture**.*

Step	Action
1	Select ***Manual*** →***Execute Manual Instructions...*** in the ***System Control*** module.
2	Select ***Alarms*** →***Alarm pre column pressure***.



3	Select ***Enabled*** in the ***Mode*** field.
4	Type the high pressure limit in the ***High alarm*** field.
5	Click ***Execute***.

5.7 Sample application

In this section

Section		See page
5.7.1	Overview	243
5.7.2	Sample application using direct injection onto the column	245
5.7.3	Sample application using Superloop	249
5.7.4	Sample application using a sample loop	256

5.7.1 Overview

This section describes the different sample application techniques that can be used with ÄKTA pure. The table below shows the alternatives for sample application available in the ***Sample application*** phase of a method.

Sample application	Using	Compatible loops
Inject sample directly onto column	• Sample pump • System pump A • System pump A and Air sensor • Sample pump and Air sensor	• Not applicable
Inject sample from loop	• Syringe • Sample pump • System pump A • ***Reinject counted volume***[1]	• Sample loop • Superloop, 10 mL • Superloop, 50 mL • Superloop, 150 mL

[1] The collected peak from a previous run, that included the ***Peak to Loop*** setting, is re-injected from selected sample loop or Superloop.

Note: *In order to avoid sample carry-over when switching techniques for loading samples, wash the injection valve with buffer between the loading of two different samples. For example, when switching from loading sample in the loop to loading sample directly onto the column with the valve in* ***Direct inject*** *position. See Cleaning Injection valve V9-Inj or V9H-Inj, on page 371 or Cleaning Injection valve V9M-J, on page 372.*

To properly fill the loops and to avoid cross-contamination, use the phase ***Manual Loop Fill*** from the phase library. Run this phase in a separate method, or add it as the first phase in a method. If running a scouting run, it is recommended to run the phase ***Manual Loop Fill*** in a separate method.

When using a pump for sample application, it is important to prime inlets and purge the pump before using the pump to load the sample.

- For Sample pump, follow the instructions in *Section 5.4.2 Sample pump, on page 228*.
- For System pump A, follow the instructions in *Section 5.4 Prime inlets and purge pump heads, on page 220*.

When loading sample using System pump A and an external air sensor, the sensor must be installed according to *Adapter for air sensor, on page 107*.

Note: *When sample is loaded at high flow rate and the external air sensor is placed before the pump that is used for loading the sample, it is necessary to use longer tubing to make sure that no air reaches the pump. Use tubing with the minimum lengths given below between the valve located before the pump and the external air sensor. The length applies for maximum flow rate, but shorter tubing can be used at lower flow rates.*

- *Sample pump S9: 40 cm.*
- *Sample pump S9H: 20 cm.*

5.7.2 Sample application using direct injection onto the column

Introduction

There are two ways to load sample directly onto a column:

- a fixed volume is loaded, or
- all the sample is loaded.

To inject all the sample, one of the following configurations is required:

- system pump, external air sensor, inlet valve A and the mixer valve, or
- sample pump and sample inlet valve.

Minimize sample loss

To minimize sample loss during direct injection of sample onto the column, sample remaining in the flow path will be pushed onto the column with buffer from the inlet valve. This step is called ***Finalize sample injection*** in the text instructions of the sample application phase of the method to be used. Refer to *Section 5.9 Create a method and perform a run, on page 284* for more information on methods and phases.

When preparing to inject...	Then...
A fixed volume of sample	1. Manually prime the sample inlet tubing with sample, see *Prime sample inlets, on page 228*. 2. In the ***Method editor***, make the following selections for the ***Sample Application*** phase of the method to be run: a. Select ***Pump injection***, b. Select ***Inject fixed sample volume*** and set the volume to be injected. 3. Make sure that the flow path from the sample inlet valve up to the injection valve will be filled with an appropriate buffer: a. Make sure that the buffer inlet tubing of the inlet valve is immersed in buffer, and b. Enable the function ***Wash sample flow path with buffer*** in the ***Sample application*** phase.

When preparing to inject...	Then...
All the sample	1. In the ***Method editor***, make the following selections for the ***Sample Application*** phase of the method to be run: a. Select ***Pump injection***, and b. Select ***Inject all sample using air sensor***. 2. Manually prime the sample inlet tubing with sample or buffer, see *Prime sample inlets, on page 228*, and make sure that the tubing is immersed in sample before starting the run. 3. Make sure that the flow path from the sample inlet valve up to the injection valve is filled with sample or an appropriate buffer and that the buffer inlet tubing is immersed in buffer.

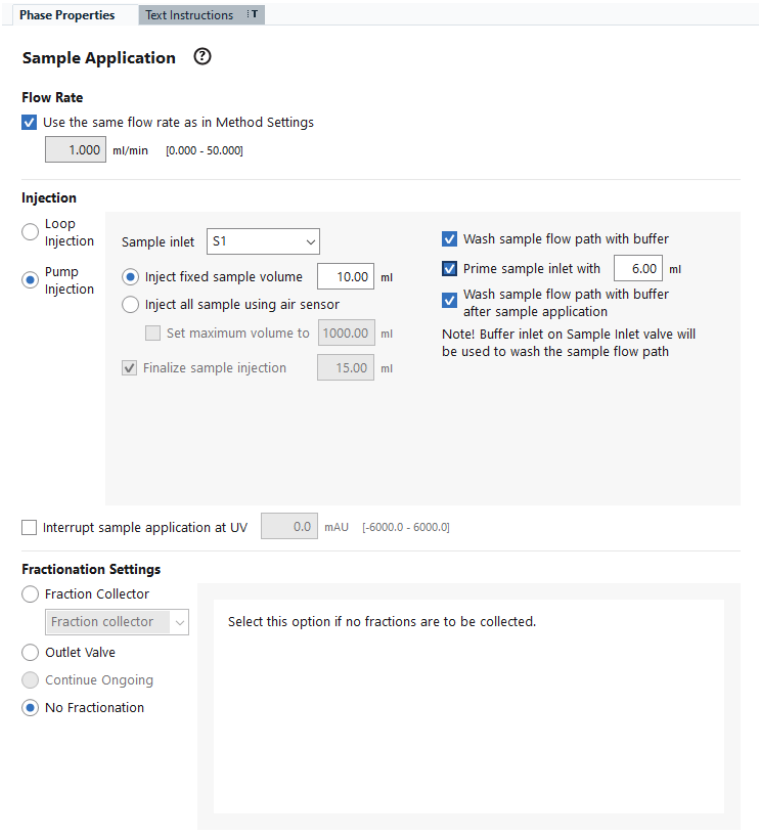
Maximize precision and accuracy

To achieve full precision and accuracy when a volume of sample is injected directly onto the column, certain selections need to be made in the ***Sample Application*** phase of the method. See *Section 5.9 Create a method and perform a run, on page 284* for more information on methods and phases.

Follow the steps below to set up the ***Sample Application*** phase.

Step	Action
1	In ***Method Editor***, select the ***Sample Application*** phase, and go the ***Text instructions***.

Step	Action
2	Select ***Pump injection***.



3	Select ***Inject fixed sample volume*** and set the volume of the sample to be injected onto the column.
4	Enable the function ***Prime sample inlet with***, and set the volume to be used for priming. ***Note:*** *If manual priming of the flow path up to the injection valve is preferred, enable* ***Prime sample inlet with*** *but set the volume to 0 mL.* *Result:* The step ***Finalize sample injection*** is automatically deactivated in order to maximize precision and accuracy. See *Minimize sample loss, on page 245* for more information.

Step	Action
5	If the system pump is used to load the sample: enable the function ***Wash sample flow path with buffer after sample application*** in order to make sure that the correct sample volume is loaded onto the column.

5.7.3 Sample application using Superloop

Introduction

Superloop allows injection of large sample volumes onto the column. Superloop can also be used for multiple injections, for example in a scouting experiment when the same application conditions are required. Superloop models are available in 10 mL, 50 mL and 150 mL sizes.

Superloop can be connected to either the Injection valve or the Loop valve. When using the Loop valve, up to five loops can be connected simultaneously.

Note: *After loading Superloop, always plug the* **Syr** *port on the Injection valve with a Stop plug. With Superloop connected to the valve, an over-pressure may be created during injection.*

Prepare Superloop

To avoid injecting air into the system flow path, Superloop must be pre-filled with buffer manually, before fitting Superloop to the system.

Note: *Read the instructions for Superloop before installing, operating, or maintaining Superloop.*

Connect Superloop

Follow the instruction below to connect Superloop to the Injection valve or to the Loop valve.

Step	Action
1	Attach Superloop to the instrument using a Column holder.
2	Connect a piece of tubing from the top of Superloop to: • port **LoopE** on the Injection valve or • a **E** port, eg., **1E**, on the Loop valve
3	Connect a piece of tubing from the *bottom* of Superloop to:

Step	Action
	• port **LoopF** on the Injection valve



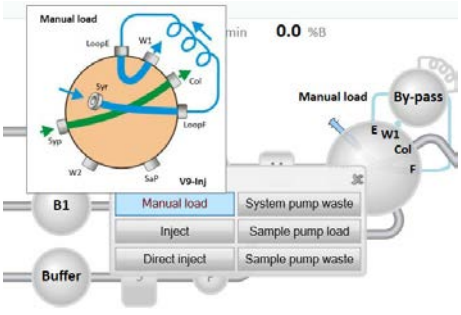
or

- the **F** port corresponding to the connected **E** port, eg., **1F**, on the Loop valve

Fill Superloop using a syringe

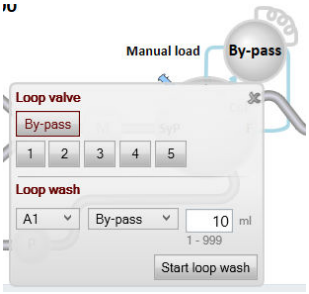
Follow the instruction below to fill Superloop using a syringe.

Step	Action
1	Check if the system is in state ***Ready***.
	• If yes: The Injection valve is in position ***Manual Load*** per default. Continue to step 3.
	• If no: Continue to step 2 to position the valve.
2	In the ***Process Picture***, click the ***Injection valve*** and select ***Manual Load***.



3	If the loop is connected to:
	• the Injection valve, continue to step 5.
	• the Loop valve, continue to step 4.

Step	Action
4	In the ***Process Picture***, click ***Loop valve*** and select the position the loop is connected to, for example ***Position 1***.



5	Fill a syringe with sample.
6	Connect the syringe to Injection valve port **Syr**.
7	Load sample into Superloop by emptying the syringe into the Injection valve.
8	Disconnect the syringe and plug the **Syr** port with a Stop plug.

Fill Superloop using the Sample pump

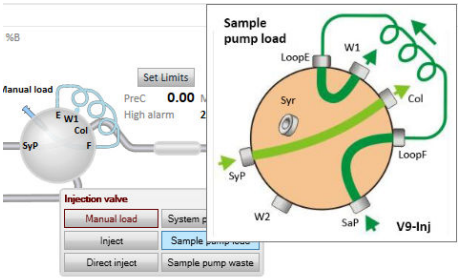
Follow the instruction below to fill Superloop using the Sample pump.

CAUTION

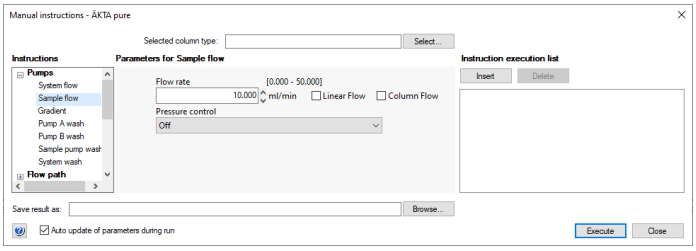
Glass tube splinter. Make sure to set the sample pressure below the max pressure of Superloop before executing a flow in the Manual instructions dialog box when Superloop is connected.

Tip: *Superloop can also be filled as part of a method run, as set in the **Sample Application** phase in the **Method Editor**. For multiple injections, it may be more convenient to fill Superloop once, as described in the instruction below.*

Step	Action
1	In the ***Process Picture***, click the ***Injection valve*** and select ***Sample pump load***.



2	Make sure that the sample inlet tubing from the sample vessel is connected to the Sample inlet valve.
3	In the ***Manual instructions*** dialog box: **a.** Select ***Alarms: Alarm sample pressure***. **b.** Set ***Mode*** as ***Enabled***. **c.** Set a ***High alarm*** level that is below the maximum pressure of Superloop. **d.** Click ***Execute***.
4	In the ***Manual instructions*** dialog box: **a.** Select ***Pumps →Sample flow***. **b.** Set ***Flow rate*** to an appropriate value for Superloop size, in this example 10 mL/min. **c.** Click ***Execute***.



5	When Superloop is filled with as much volume as is needed, click the ***End*** button in the ***System Control*** toolbar.



Fill Superloop using System pump A

The **Mixer valve** has to be installed to fill Superloop using System pump A.

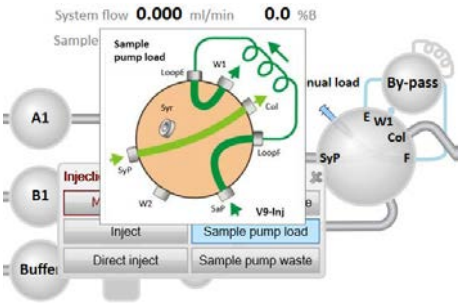
Follow the instruction below to fill Superloop using the System pump A.

NOTICE

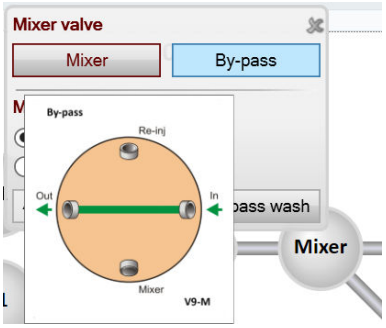
Glass tube splinter. Make sure to set the system pressure below the max pressure of Superloop before executing a flow, when Superloop is connected.

Tip: *Superloop can also be filled as part of a method run, as set in the **Sample Application** phase in the **Method Editor**. For multiple injections, it may be more convenient to fill Superloop once, as described in the instruction below.*

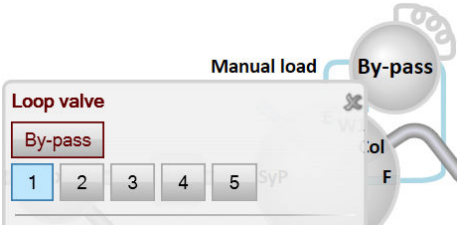
Step	Action
1	**a.** In the ***Manual instructions*** dialog box, select ***Alarms: Alarm system pressure***. **b.** Set ***Mode*** as ***Enabled***. **c.** Set a ***High alarm*** level that is below the maximum pressure of Superloop. **d.** Click ***Execute***.
2	In the ***Process Picture***, click the ***Injection valve*** and select ***Sample pump load***.



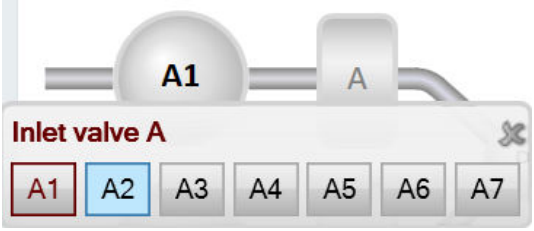
Step	Action
3	In the ***Process Picture***, click the ***Mixer valve*** and select ***By-pass***.



4	• If the loop is connected to the Injection valve, continue to step 6. • If the loop is connected to the Loop valve, continue to step 5.
5	**a.** In the ***Process Picture***, click the ***Loop valve***. **b.** Select the position the loop is connected to, for example ***Position 1***.

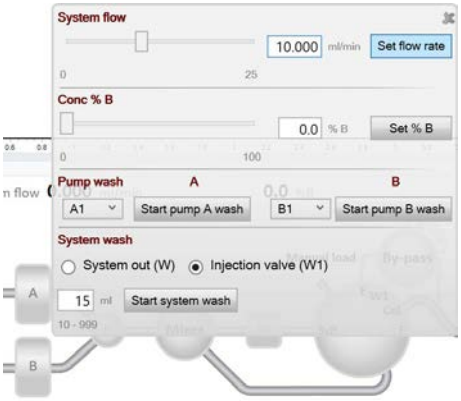


6	**a.** In the ***Process Picture***, click on the buffer inlet valve A. **b.** Select the inlet position to be used for sample injection, for example ***A2***.



7	**a.** In the ***Process Picture***, click on the ***Pumps***. **b.** Set the ***System flow*** to an appropriate value for Superloop size, in this example 10 mL/min.

Step	Action
	c. Click ***Set flow rate***.



8	When Superloop is filled with as much volume as is needed, click the End button in the ***System Control*** toolbar.



5.7.4 Sample application using a sample loop

Introduction

A sample loop is recommended for injection of smaller sample volumes onto the column.

A sample loop can be connected to either the Injection valve or the Loop valve. When using the Loop valve, up to five loops can be connected simultaneously.

Note: *Sample loop is called capillary loop in UNICORN.*

For micro scale applications, also see *Section 5.10 ÄKTA pure micro or Micro kit operation, on page 299*.

Fill the sample loop

Follow the steps below to fill the sample loop with sample.

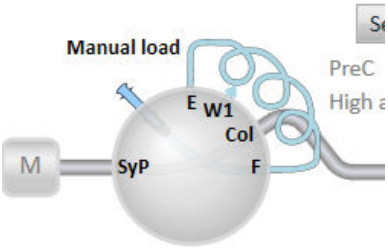
Step	Action
1	Connect a suitable sample loop to Injection valve ports **LoopF** (fill) and **LoopE** (empty).
2	Fill a syringe with sample.



Step	Action
3	Connect the syringe to the Injection valve port **Syr**.

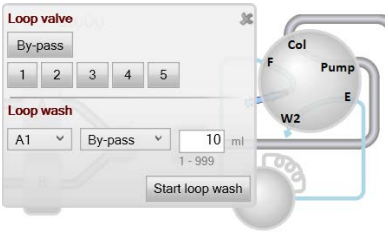


4	In the ***Process Picture***, click on the ***Injection valve*** and check that ***Manual load*** is selected.



5	• If the loop is connected to the Injection valve, continue to step 7. • If the loop is connected to the Loop valve, continue to step 6.
6	**a.** In the ***Process Picture***, click the ***Loop valve***.

Step	Action
	b. Select the position the loop is connected to, for example ***Position 1***.



7	Load sample into the sample loop. ***Note:*** *To avoid sample loss due to siphoning, leave the syringe in the Injection valve port* **Syr** *during the entire run.* ***Tip:*** *It is recommended to overfill the loop to make sure that the loop is completely filled. Excess of sample will leave the valve through port* **W1**.

Fill the sample loop using the Sample pump

Most often the sample loop is filled using a syringe. However, to fill it using the sample pump, follow the instructions for filling the Superloop, see *Fill Superloop using the Sample pump, on page 251*.

Note: *It is not necessary to set the Alarm Sample Pressure when filling a sample loop.*

Note: *It is recommended to overload the loop to make sure that the loop is completely filled.*

Note: *Set* ***Flow rate*** *to an appropriate value for the loop size.*

Note: *After loading, plug the* **Syr** *port on the Injection valve with a Stop plug.*

Fill the sample loop using System pump A

Most often the sample loop is filled using a syringe. However, to fill it using System pump A, follow the instructions for filling the Superloop, see *Fill Superloop using System pump A, on page 253*.

Empty the loop

General considerations

During the method run, the sample is automatically injected onto the column. The loop is emptied and washed out using buffer from the system pumps. The total buffer volume to be used for emptying and washing the loop is set in the Method Editor.

Using a sample loop connected to Loop valve V9-L or V9H-L

For maximum reproducibility, use complete loop fill when loading the loop, that is, overfill the loop with a sample volume of up to 3-5 times the volume of the loop. For minimum sample loss, use partial loop fill, that is, fill only up to 50% of the loop volume. Empty the loop with 3-5 times the volume of the loop.

Volume used to empty a loop connected to Loop valve V9-L or V9H-L

To minimize the risk for carry over and to make sure that the complete sample volume reaches the column, the loop must be emptied with an excess of buffer. The tubing between the Loop valve port **E** and the Injection valve port **E** holds a small volume. If the loop is emptied with a volume equal to, or less than the loop volume this needs to be taken into account. It is also important to use a low flow rate to make sure that the correct volume is added to the column when injecting a small volume.

Note: *Partially emptying the loops that are attached to Loop valve V9-L or V9H-L can increase the risk for carry over from one loop position to the next.*

Cleaning the injection valve

After sample application using a sample loop loaded with a syringe, the injection valve retains a small amount of sample. Clean the injection valve to prevent carry-over to the next sample application.

For detailed instructions see, *Cleaning Injection valve V9-Inj or V9H-Inj, on page 371* and *Cleaning Injection valve V9M-J, on page 372*.

5.8 Fractionation

Introduction

Fraction collectors F9-C, F9-R, and F9-T collect fractions from ÄKTA pure purification runs. The fraction collectors are connected to ÄKTA pure and controlled by UNICORN. Control of the fraction collector can be achieved automatically in a method run, or manually.

In this section

Section		See page
5.8.1	Fractionation overview	261
5.8.2	Delay volume and pre-fractionation volume	263
5.8.3	Prepare Fraction collector F9-C	266
5.8.4	Prepare Fraction collector F9-R	272
5.8.5	Prepare Fraction collector F9-T	278
5.8.6	Fractionation settings	282

5.8.1 Fractionation overview

Fractionation types

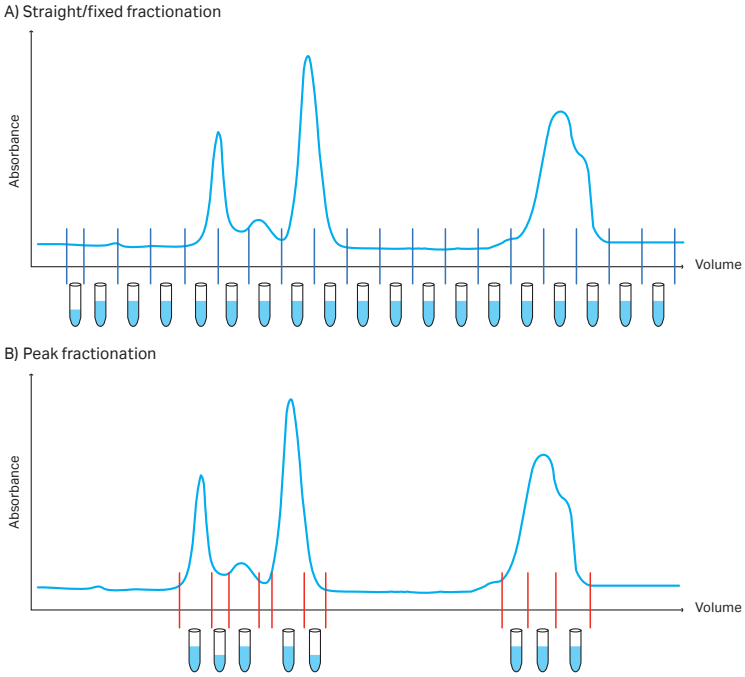
The table below lists the types of fractionation that the fraction collectors can be used for.

Type	Description
Fixed volume fractionation	During fixed volume fractionation the fraction collector continuously switches tubes according to the set volume throughout the entire fractionation. This type of fractionation is also known as straight fractionation.
Peak fractionation	Peak fractionation can be used to further increase the purity of the collected protein peaks and minimize the number of tubes used. The monitor signal is used to determine when to switch the tubes. See *Section 9.8.12 System settings - Watch parameters, on page 589* for information about different watch options.
Combined fixed volume fractionation and peak fractionation	The two fractionation types listed above can be used in combination. Combination of fixed volume and peak fractionation allows fractions collected by fixed volume fractionation and fractions collected by peak fractionation to be directed to different collection tubes.

To be able to analyze different parts of the peak, the fraction size during elution is usually set to a value smaller than the expected peak volume.

Illustration

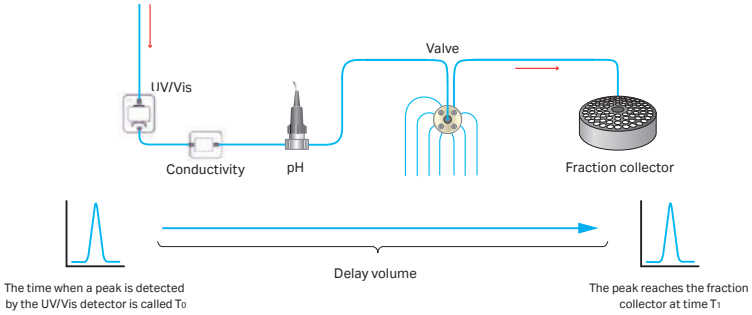
The illustration below shows examples of fractionation using fixed volume fractionation and fractionation using peak fractionation.


A) Straight/fixed fractionation
Absorbance
Volume
B) Peak fractionation
Absorbance
Volume

5.8.2 Delay volume and pre-fractionation volume

Description

The delay volume settings are used to make sure that the fractions collected during fractionation, using the outlet valve or the fraction collector, correspond to the fractions indicated in the chromatogram. The delay volume is the volume between the detector selected as fractionation source and the fraction collector or outlet that is used. The illustration below shows and example of the delay volume between the UV/Vis monitor and the fraction collector. See *Section 9.13 Delay volumes, on page 635* for more information.

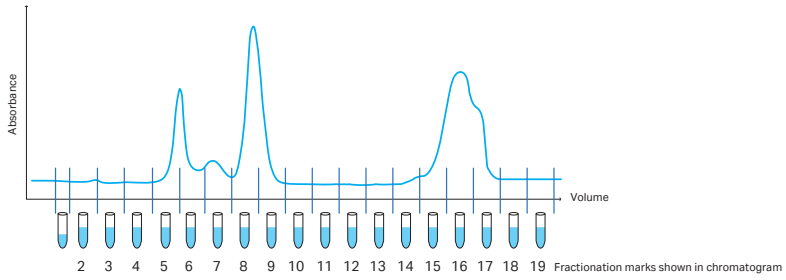


As the delay volume is affected by the length and diameter of the tubing, it must be set according to the tubing and modules used. Update the delay volume if necessary. See *Set the delay volume, on page 203* for detailed instructions.

The volume collected before the first fraction, i.e. the content in the system flow path to the fraction collector when fraction collection starts, is referred to as pre-fractionation volume.

Illustration of fraction marking using fixed volume fractionation

The following illustration shows the fractions collected, and the numerical marking of fractions, when fixed volume (straight) fractionation is used with Fraction collector F9-R.



When fixed volume fractionation is used, the pre-fractionation volume is collected at the beginning of fractionation.

Note: *In Fraction collector F9-R the pre-fractionation volume is called Delay volume.*

Fraction collector F9-R saves the pre-fractionation (Delay) volume in the first fraction (fraction 1), see the example illustration above. Pre-fractionation volumes are not indicated with a numerical fraction mark on the chromatogram.

In Fraction collector F9-T the pre-fractionation volume can be collected in three ways:

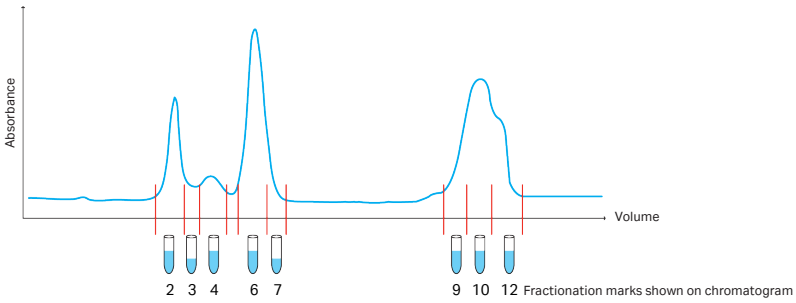
- ***Auto*** sets the Pre-fractionation volume in a separate well/tube position for all plate and tube types, except for 24 deep well plates and 50 mL tubes, where collection of pre-fractionation volume will be together with the first fraction.
- ***Combined with first*** is used to collect the pre-fractionation volume in the same position as the first fraction.
- ***Separate position*** is used to collect the pre-fractionation volume in a position that is different from the first fraction.

Fraction collector F9-C does not save the pre-fractionation volume as a fraction, instead the pre-fractionation volume is disposed of in the waste funnel.

Note: *If two fraction collectors are used, the fractions collected by the second Fraction collector are indicated by the suffix 2: (i.e. fractions **1 (2), 2 (2), 3 (2)** etc.).*

Illustration of fractions and fraction marking using peak fractionation

The following illustration shows the fractions collected, and the numerical marking of fractions, when peak fractionation is used with Fraction collector F9-R.



When peak fractionation is used, Fraction collector F9-R collects the pre-fractionation volumes in the fractions preceding each peak. In the chromatogram above, pre-fractionation volumes are collected in fractions 1, 5 and 8. The numerical fraction marks for the pre-fractionation volume fractions are not indicated on the chromatogram.

In Fraction collector F9-T the pre-fractionation volume can be collected in three ways:

- ***Auto*** sets the Pre-fractionation volume in a separate well/tube position for all plate and tube types, except for 24 deep well plates and 50 mL tubes, where collection of pre-fractionation volume will be together with the first fraction.

- ***Combined with first*** is used to collect the pre-fractionation volume in the same position as the first fraction.
- ***Separate position*** is used to collect the pre-fractionation volume in a position that is different from the first fraction.

Fraction collector F9-C does not save the pre-fractionation volumes as fractions, instead the pre-fractionation volumes are disposed of in the waste funnel.

Note: *If two fraction collectors are used, the fractions collected by the second Fraction collector are indicated by the suffix 2: (i.e. fractions **1 (2), 2 (2), 3 (2)** etc.).*

5.8.3 Prepare Fraction collector F9-C

About this section

This section describes how to prepare and assemble Fraction collector F9-C before a run.

The fraction collector is connected to ÄKTA pure and controlled by UNICORN. Control of the fraction collector can be achieved automatically in a method run, or manually.



CAUTION

Fire Hazard. Do not fractionate flammable liquids using Fraction collector F9-C. When running RPC methods, or other procedures using organic solvents, collect fractions through the outlet valve, Fraction collector F9-R, or Fraction collector F9-T.



NOTICE

The tray and racks can tilt slightly when not fully inserted into the fraction collector and may harm the fractionation arm. The tilt is due to the height difference between the door and the floor of the fraction collector. The tendency to tilt is affected by the placement and weight of the cassettes, tubes or bottles.

Prepare the fraction collector

Before starting to prepare Fraction collector F9-C, check the fractionation settings in the method to be run. Perform the steps described below according to the settings in the method.

- Insert the Cassette tray or a rack for tubes or bottles.
- Change the ***System Settings*** in UNICORN to set the fractionation mode and other settings for fraction collection.

How to insert a tray or a rack is shown below.

For information on how to change the ***System Settings*** before a run, see *UNICORN System Control Manual*. The available ***System Settings*** are described in *Section 9.8.9 System settings - Fraction collection, on page 581*.

Prepare and insert the Cassette tray

Follow the steps below to add cassettes to the Cassette tray and insert the tray into the fraction collector.

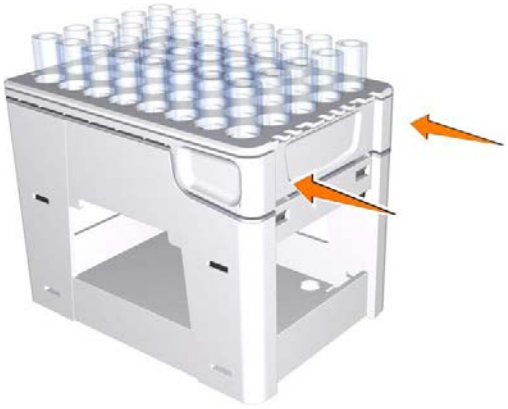
Step	Action
1	For cassettes with the QuickRelease function, open the cassettes. For more information on the QuickRelease function see *QuickRelease function, on page 130*.



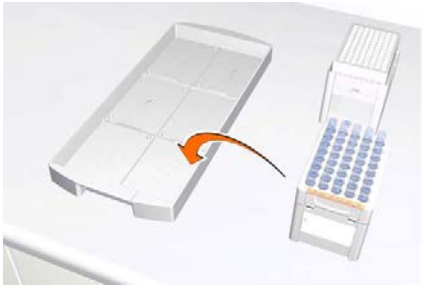
2	Place the tubes and deep well plates in the cassettes. Make sure that the deep well plates are rotated so that the well marked **A1** is positioned above the **A1** marking on the cassette.



Step	Action
3	Close the cassettes that have the QuickRelease function.



4	Place the cassettes on the Cassette tray. Make sure that the cassette type code (see illustration below) faces the front of the tray marked with the Cytiva logo.



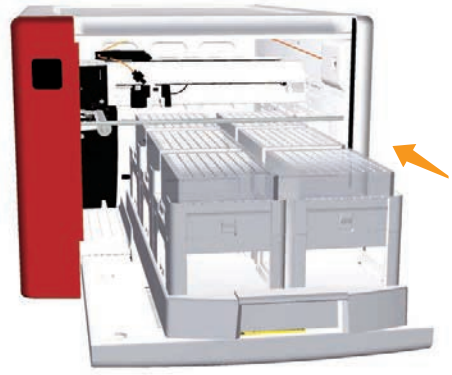


5	Open the door of the fraction collector using the handle.
6	Insert the tray into the fraction collector.

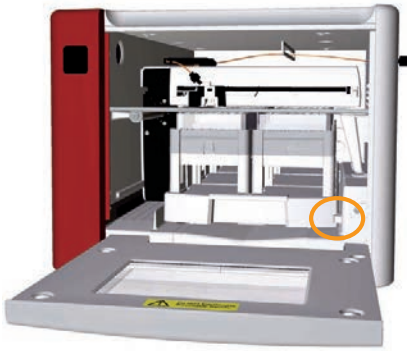
a. Make sure that the front of the tray (marked with the Cytiva logo) faces outwards.

Step	Action

b. Position the tray and slide it into the fractionation collector until it reaches the end, as in the image shown below.



c. Make sure that the Tray catch snaps into closed position, as in the image shown below.



Note:

A height exclusion bar makes sure that the tubes or deep well plates are correctly positioned and cannot damage the Dispenser head.

7 Close the door. Make sure that it closes properly.

Result:

After the door has been closed, the fractionation arm scans the cassette type code of each cassette to identify the cassette types. If deep well plates are used, the instrument also identifies the types of deep well plates.

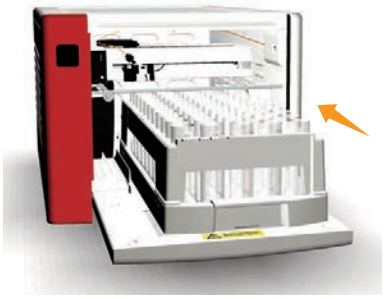
Note:

If the tray is inserted with the front of the tray facing the wrong way it will not be possible to close the door.

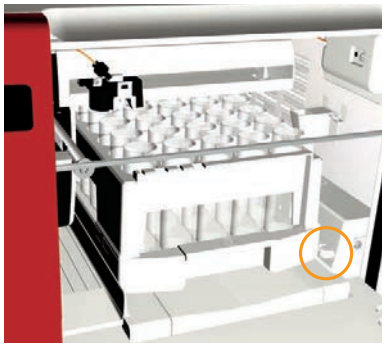
Prepare and insert a rack for tubes or bottles

Follow the steps below to insert a rack for tubes or bottles into the fraction collector.

Step	Action
1	Place the tubes or bottles in the rack.
2	Open the door of the fraction collector using the handle.
3	Insert the rack into the fraction collector: **a.** Make sure that the front of the rack (marked with the Cytiva logo) faces outwards. **b.** Position the rack and slide it into the fractionation collector until it reaches the end.



c. Make sure that the Tray catch snaps into closed position, as shown below.



Note:
A height exclusion bar makes sure that the tubes or bottles are correctly positioned and cannot damage the Dispenser head.

Step	Action
	Note: *Do not use the Cassette tray when the Rack for 50 mL tubes or the Rack for 250 mL bottles is placed in the fraction collector.*
4	Close the door. Make sure that it closes properly. ***Note:*** *If the rack is inserted with the front of the rack facing the wrong way it will not be possible to close the door.*

5.8.4 Prepare Fraction collector F9-R

Introduction

This chapter describes how to prepare and assemble Fraction collector F9-R before a run.

Fraction collector F9-R is connected to ÄKTA pure and controlled by UNICORN. Control of the fraction collector can be achieved automatically in a method run, or manually.

Prepare the fraction collector

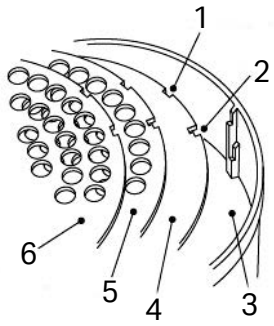
Before starting to prepare the Fraction collector F9-R, check the fractionation settings in the method to be run. Perform the steps described below according to the settings in the method.

1. Assemble the Tube rack.
2. Insert collection tubes.
3. Adjust the Delivery arm.
4. Update ***System Settings*** in UNICORN.

Assemble the tube rack

Follow the steps below to assemble the Tube rack.

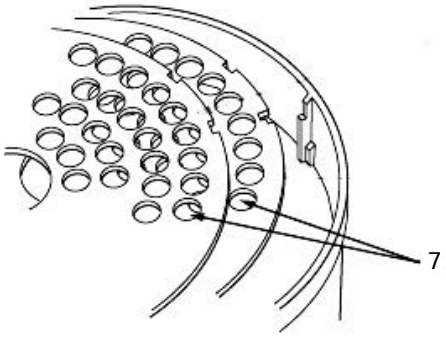
Step	Action
1	Insert the Tube support (4), if required, into the bowl (3). The circular marks on the Tube support must face down.



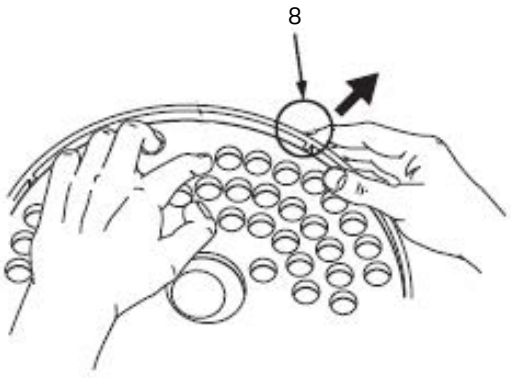
Note:

When assembling a Tube rack, Single cutouts (1) and L-shaped cutouts (2), are used for various inserts depending on the length of the collection tubes. See Parameters for assembling a tube rack, on page 141 for detailed information.

Step	Action
2	Insert the Tube guide (5) with the tube position numbers upwards. The Tube guide must rest about 1 cm above the Tube support.
3	Insert the Tube holder (6) with the tube position numbers upwards: Check that tube position 1 (7) is directly above tube position 1 (7) of the Tube guide.



4	**a.** Push the flexible bowl out at each rib and snap the Tube holder under the top lip of the rib (8).

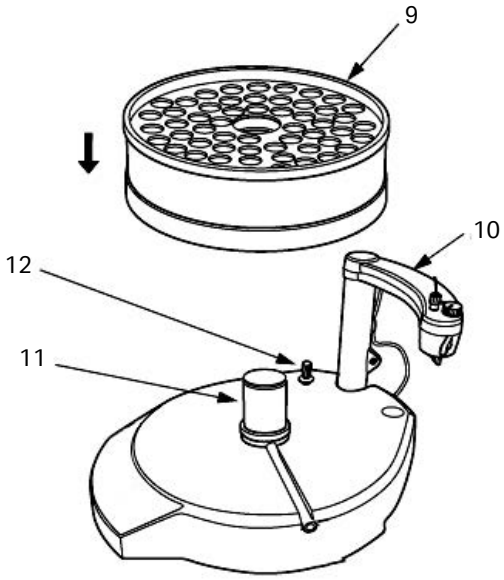


Note:
Do not force the tube holder into place as this may damage the lip.

b. Check that the surface of the Tube holder is level.

5	Gently move the Delivery arm (10) out to the outer stop.

Step	Action
6	Place the Tube rack (9) over the Central spindle (11) and pull the spring loaded Drive sleeve (12) out so the Tube rack comes to rest.



Insert collection tubes

Insert a sufficient number of collection tubes into the Tube rack, starting at position 1, pushing each one down as far as they will go. All the tubes must be of the same length and diameter and there must be no spaces in the sequence.

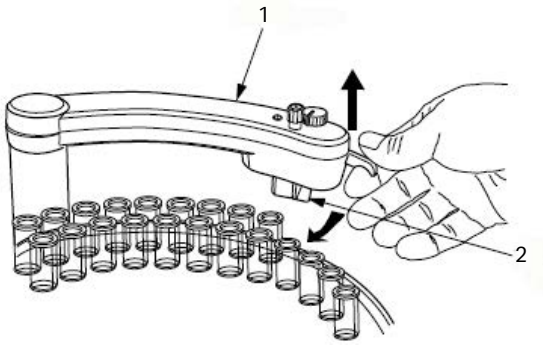
Adjust the delivery arm

Follow the steps below to adjust the height of the delivery arm.

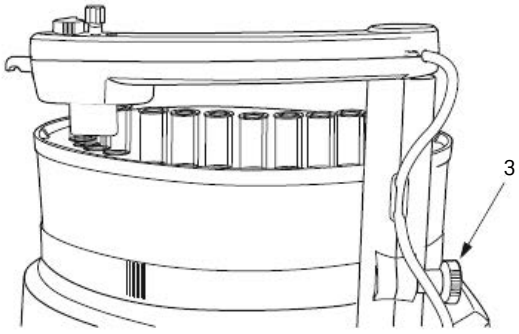
Step	Action
1	**a.** Lift and then lower the delivery arm (**1**).

Step	Action

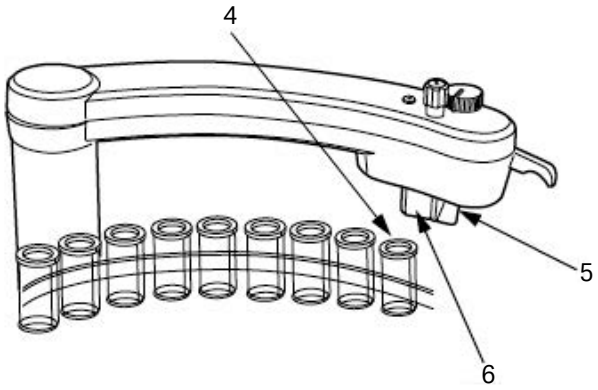
b. Allow the delivery arm to move so that the tube sensor (**2**) touches the collection tubes in the outer track.



2 Loosen the lock knob (**3**).

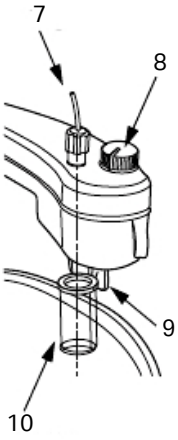


Step	Action
3	**a.** Adjust the height so that the horizontal mark (**5**) on the tube sensor (**6**) is at the same level as the top of the flat collection tubes and approximately 2 mm over the top of the flanged collection tubes (**4**).



b. Lock the delivery arm at this height with the lock knob.

4 Check that the tube sensor (**9**) is in the correct position for the tubes used (**10**). The eluent tubing (**7**) must be above the center of the collection tube.



5 Use the Sensor control (**8**) to position the tube holder over the center of the collection tube.

NOTICE

Never lift Fraction collector F9-R by the delivery arm. This can cause damage to the Fraction collector.

Sensor control

The sensor control can be switched between the two positions ***small tubes*** and ***large tubes***, indicated in the illustration below.



The position for large tubes is used for tubes of approximately 18 mm i.d. and larger. The position for small tubes is used for tubes smaller than 18 mm i.d.

Note: *The sensor control is a rough approximation. Always check that the eluent tubing is centered above the collection tube.*

5.8.5 Prepare Fraction collector F9-T

Introduction

This chapter describes how to prepare and assemble Fraction collector F9-T before a run. Fraction collector F9-T is connected to ÄKTA pure and controlled by UNICORN. Control of the fraction collector can be achieved automatically in a method run, or manually.

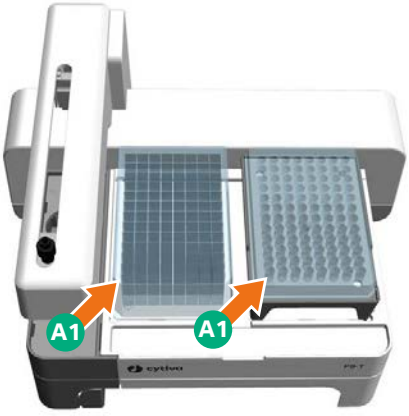
Prepare Fraction collector F9-T

Follow the steps below to prepare Fraction collector F9-T.

Step	Action
1	Make sure that Fraction collector F9-T is installed properly.
2	Check plates and tubes needed for the method run or manual run, and if cooling racks need to be prepared.

Prepare plates and small tubes

Follow the steps below to prepare plates and tubes for fractionation.

Step	Action
1	If tubes are to be used, make sure they are positioned correctly in a suitable plate or tube rack. See *Correct tube position in a 24 deep well plate, on page 150* and *Correct tube position in a rack, on page 151*.
2	Place the plates and racks on the fraction collector with the **A1** well in the front left corner as denoted on the fraction collector. 

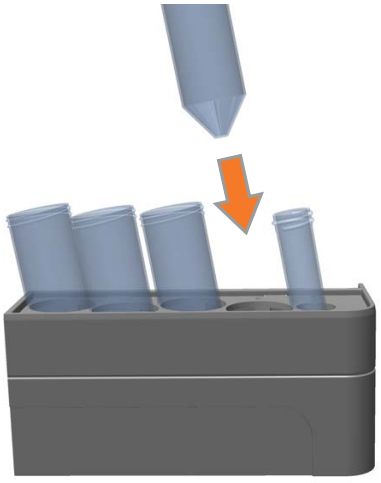
Prepare 50 mL tubes

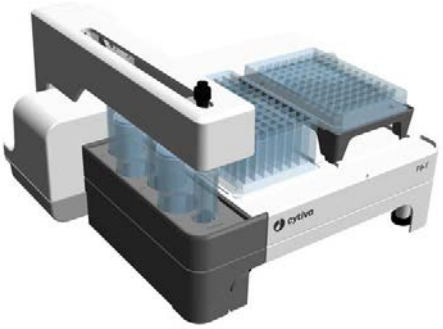
Follow the steps below to position the 50 mL tubes, and the 15 mL **Home** position tube.

Step	Action
1	Remove the tube rack for 50 mL tubes by pressing the wedge and pulling out the rack, see the illustration below.



2	Place the tubes in the tube rack. When positioned properly, the tubes lean slightly towards the back of the fraction collector.

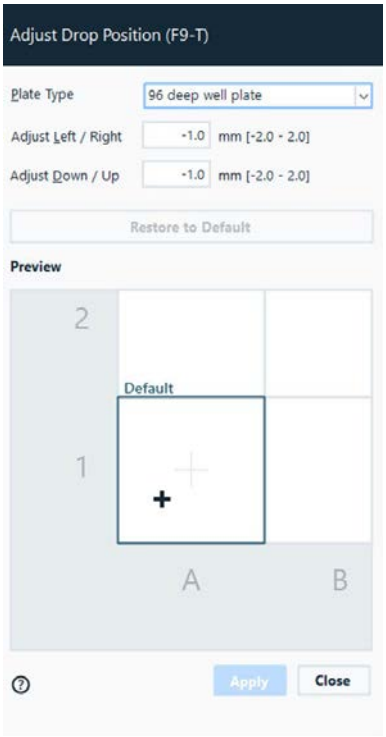


Step	Action
3	Put the tube rack back into position. 

Dispensing position and adjustment

For all plates and tubes, except for the 0.5 mL tubes, the default positioning of the nozzle is not at the center of the well/tube.

Depending on the running conditions, or if non-standard plates are used, the default position might need to be adjusted. There is an adjustment setting available in the ***System Control*** →***System*** →***Adjust drop position (F9-T)***. See the image below.



Default drop position

The default drop position is chosen for optimal liquid behavior in the wells/tubes. The values in the table below represent the nozzle distance (in mm) from the center of wells/tubes in left/right and down/up direction, respectively.

Plate type	Default offset from center of well Left/ Right [mm]	Default offset from center of well Down/Up [mm]
96 deep well plate	-1.0	-1.0
96 microplate	-0.5	-0.5
48 deep well plate	-0.8	-5.3
24 deep well plate	-5.5	-5.5
0.5 mL tubes	0.0	0.0
1.5 mL tubes	-0.8	-0.8
2 mL tubes	-0.8	-0.8
50 mL tubes	0.0	8.0

5.8.6 Fractionation settings

Fractionation modes for Fraction collector F9-C

There are three fractionation modes for Fraction collector F9-C: ***Automatic***, ***Accumulator*** and ***DropSync***. Spillage between collection vessels during a run is avoided with all three fractionation modes.

- ***Automatic:*** The fraction collector uses the Drop Sync mode for flow rates up to 2 mL/min and automatically switches to Accumulator mode for higher flow rates.
- ***Accumulator:*** Liquid is collected during movement between tubes or wells. The liquid is then dispensed in the next well or tube. Fractionation with accumulator can be used at all flow rates.
- ***DropSync:*** When using DropSync, the sensors in the Dispenser head detect when a drop is released from the nozzle. The Dispenser head moves to the next well or tube just after a drop is released. Fractionation with DropSync can be used at flow rates up to 2 mL/min. If only one Cassette is used and it is placed near the waste funnel, DropSync can be used at higher flow rates. Volatile solutions and solutions with low surface tension may require a lower flow.

Fractionation settings for Fraction collectors F9-R and F9-T

There are two fractionation settings for Fraction collectors F9-R and F9-T; ***DropSync off*** and ***DropSync on***.

- ***DropSync off***: No synchronization of collection.
- ***DropSync on***: When using ***DropSync***, the sensors in the Tube sensor detect when a drop is released. The Tube rack moves and positions the next tube under the Tube sensor just after a drop is released.

In Fraction collector F9-R, fractionation with ***DropSync*** can be used at flow rates up to 2 mL/min.

In Fraction collector F9-T, fractionation with ***DropSync*** can be used at flow rates up to 3 mL/min or 5 mL/min, depending on the plate type used.

For water and solutions with higher surface tension, a higher flow rate can be used. Volatile solutions and solutions with low surface tension may require a lower flow.

Missing tubes in Fraction collector F9-R

If a tube is missing, Fraction collector F9-R will continue the fractionation on the tube row located closer to the center of the fraction collector. The fractionation marks in the chromatogram will then not reflect the tubes in which the sample is collected.

If the fraction collector runs out of tubes, the delivery arm moves to the fraction collector center position while ÄKTA pure pauses and displays an error message.

Missing tubes or plates in Fraction collector F9-C

When automatic cassette configuration is selected in the system settings the fraction collector automatically detects which types of cassettes and plates that are present. The fraction collector will however not detect if tubes or bottles are missing in the cassettes. Make sure that the cassettes to be used are occupied by appropriate types and numbers of tubes or bottles before starting a run.

It is not possible to change the cassette configuration during a run. When the system state is set to ***Pause*** it is possible to take out cassettes or plates from the fraction collector only if they are replaced by cassettes or plates of the same type and are placed in the same positions.

The action of the system when the last tube in the fraction collector is filled is set in the instruction ***Last tube filled*** in the system settings. The flow can be directed to waste or to any of the outlets or the run can be paused. If the action is set to ***Pause***, the system automatically pauses when the last tube is filled and prompts the user to replace the filled tubes.

Peak broadening

The width of peaks at the fraction collector is influenced by the properties of the column and the dimensions of tubing connecting the components. Initial sample volume affects the peak width in size exclusion chromatography (SEC). A sample zone is broadened during passage through a SEC column so that the sample is diluted and the resolution decreases with increasing sample volume. Sample volume does not however affect the resolution in adsorption chromatography techniques such as affinity chromatography (AC), ion exchange chromatography (IEX), and hydrophobic interaction chromatography (HIC). The effect of peak broadening in the system from sample injection to peak detection (including dilution on the column) is apparent in the chromatogram from the UV monitor, but broadening from the UV monitor to fraction collection is not visible in the chromatogram. This "hidden" effect can sometimes be dramatic, especially for high-resolution columns.

To minimize peak broadening, use narrow and short tubing connections as far as possible. Remember that using narrow tubing will increase the back pressure in the system.

5.9 Create a method and perform a run

About this section

This section provides an overview of how to create a method in UNICORN and how to perform manual and method runs on ÄKTA pure.

It also contains advice on things to be considered during a run.

In this section

Section		See page
5.9.1	Create a method	285
5.9.2	Prepare and perform a run	291
5.9.3	Monitor a run	293
5.9.4	After run procedures	295

5.9.1 Create a method

Introduction

The predefined methods are built up using phases, where each phase corresponds to a step in a chromatography run with a number of properties associated with that phase.

See *UNICORN Method Manual* for more information about method structure, definitions and concepts of methods in UNICORN.

Predefined methods

There are several predefined methods to choose from. All the predefined methods are listed below.

The predefined methods available for each system are defined by the Instrument Configuration. Refer to *Section 9.7 Predefined methods and phases, on page 557* for more information about each method.

Purification methods

- Affinity Chromatography (AC)
- Affinity Chromatography (AC) - HiTrap Fibro™ PrismA
- Affinity Chromatography (AC) with Tag Removal
- Anion Exchange Chromatography (AIEX)
- Cation Exchange Chromatography (CIEX)
- Chromatofocusing (CF)
- Desalting (DS)
- Hydrophobic Interaction Chromatography (HIC)
- Manual Loop Fill
- NHS-coupling
- Reversed Phase Chromatography (RPC)
- Size Exclusion Chromatography (SEC)

Maintenance methods

- Column CIP
- Column Performance Test
- Column Preparation
- System CIP
- System Preparation

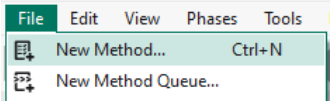
Main steps when defining a new method

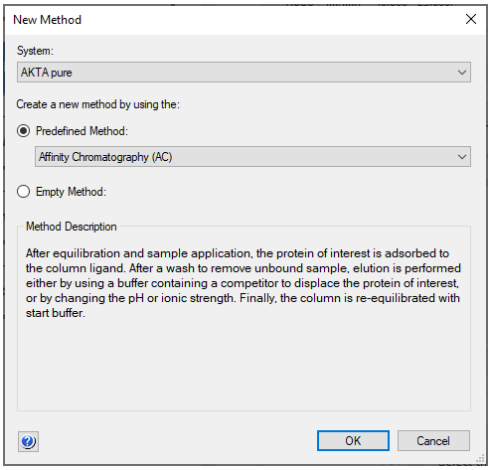
The main steps when defining a method are:

Step	Action
1	Create or open a method: • Create a ***Predefined*** method (including a set of phases that may be edited) or • Open an existing method that can be edited and saved with a new name or overwritten.
2	Build or edit the ***Method Outline*** and/or edit the ***Phase Properties*** for the appropriate phases.
3	Save the method.

Create a new method

Follow the steps below to create a new method.

Step	Action
1	In the ***Method Editor***: • Click the ***Create a new method*** icon in the ***Toolbar***  or • Select ***File*** →***New Method...***. 

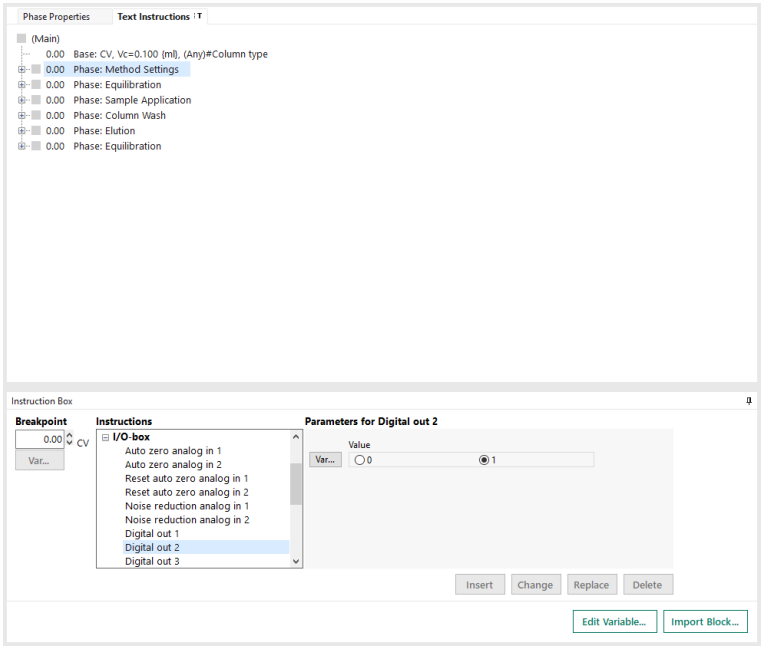
Step	Action
	Result: The ***New Method*** dialog box opens. 
2	In the ***New Method*** dialog box: **a.** Select a ***System***. **b.** Select a ***Predefined Method***. **c.** Click ***OK***. *Result:* The ***Method Outline*** pane shows the included phases for the chosen method and the ***Phase Properties*** pane shows the default settings for the currently highlighted phase.

See *UNICORN Method Manual* for more information about methods and method creation in UNICORN.

Note: *Sample loop is called capillary loop in UNICORN.*

Text Instruction mode

In most cases methods can be edited using the ***Phase Properties*** pane in the ***Method Editor*** module. However, modules without a recommended position are not supported by ***Phase Properties*** and have to be edited in the ***Text Instruction*** mode.



The following modules require that the method is created using the ***Text Instruction*** mode:

- Inlet valve IX

  Note: *When using Inlet valve IX (***V9-IX** *or* **V9H-IX***) connected to another inlet valve in a run, it is necessary to use the instructions for both Inlet valve IX and the other inlet valve, respectively.*

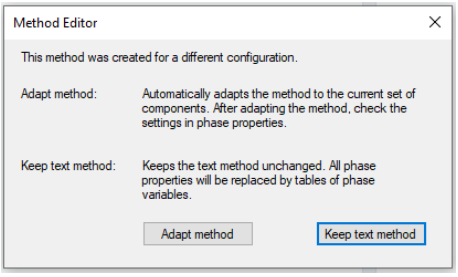
- Versatile valve
- I/O-box

Note: *If installing more than one external air sensor, it is necessary to create the methods for the additional air sensors using the* ***Text Instruction*** *mode.*

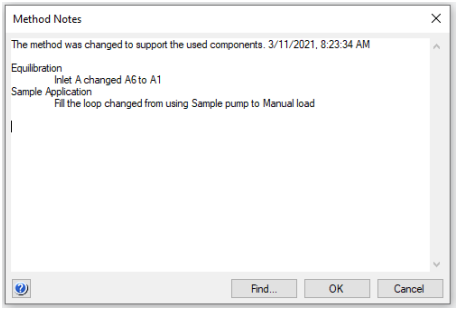
Open and save methods for different systems

New ÄKTA pure methods are always created for the set of components of the current system. Methods can be shared between systems, with the restriction that a method created for an ÄKTA pure 150 configured system cannot be used with an ÄKTA pure 25 configured system, and *vice versa*.

When a method is opened for a system that has been changed or if a method is saved for a system with a different flow configuration, a dialog box is opened where the user can choose to either adapt the method or to keep the text method unchanged.



The table below describes the result of selecting the option ***Adapt method*** or ***Keep text method***.

Option	Description
Adapt method	The method will be changed to support the used components. All functions and positions that are still available will be unchanged. For example, valve positions present on both the original and the new system will be unchanged in the adapted method. Non-compatible settings will be adjusted in order to properly adapt the method for new components. Settings that have been changed are described and saved in ***Method Notes***, which are displayed after the method has been adapted.  Change the settings in ***Phase Properties*** if required. ***Note:*** *Some predefined methods require certain components to be functional. Adapting these methods to systems that do not include the required components is not possible.*

Option	Description
Keep text method	The text method will be kept and nothing will be changed. Non-compatible settings will still be present in the method but they will not be functional. All ***Phase Properties*** of the method will be replaced by tables of ***Phase Variables***.

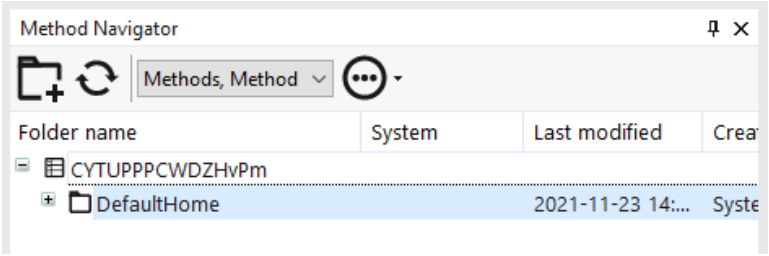
5.9.2 Prepare and perform a run

Introduction

This section describes how to start a run using a previously created method and how to perform a manual run. For further information about the capabilities of the ***System Control*** module, refer to *UNICORN System Control Manual*.

Choose and start a method

The instruction below describes how to open a method and start a run.

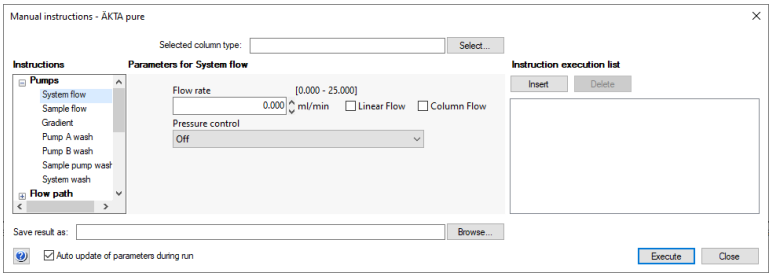
Step	Action
1	Open the ***System Control*** module and click the ***Open Method Navigator*** button.  *Result:* The ***Method Navigator*** pane opens. 
2	Select the method to run, and click the ***Run*** button.  *Result:* The ***Start Protocol*** dialog opens.
3	Step through the displayed pages in the ***Start Protocol***, add requested input and make appropriate changes if necessary. Click ***Next***.
4	Click ***Start*** on the last page of the ***Start Protocol***.

Step	Action
	Result: • If the ***Column Logbook*** option was included during installation of UNICORN and a column type was selected at method creation, the ***Select Columns*** dialog opens. For further information on column handling, please refer to *UNICORN Method Manual* and *UNICORN System Control Manual*. • If the ***Column Logbook*** option was *not* chosen during installation of UNICORN and/or *no* column type was selected at method creation, the run starts directly.

Perform a manual run

Manual runs can be convenient for procedures such as filling tubing with buffer or packing a column with chromatography resin.

Step	Action
1	On the ***Manual*** menu, click ***Execute Manual Instructions***.
2	Select instruction group and instruction.
3	Select or enter parameter values.



4	Click ***Insert*** to have several instructions performed at the same breakpoint. ***Note:*** *Manual runs are only stored temporarily. However, to save results in a chosen directory, click **Browse** before the run is started.* ***Note:*** *If a method run is started during a manual run, the results from the manual run are not stored.*
5	To perform the instructions, click ***Execute***.

5.9.3 Monitor a run

Introduction

During the run, the ***System Control*** module will display the run progress of the method being executed. This section describes how to interact with the run from the process picture or by executing manual instructions.

To find an overview of the ***System Control*** user interface, see *UNICORN System Control Manual*.

Monitor the run

To interrupt a method during a run, use the ***Hold***, ***Pause*** or ***End*** icons in ***System Control***. A held or paused method run can be resumed by using the ***Continue*** icon. See the instructions in the table below.

Intended action	Procedure
Temporarily hold the method, with current flow rate and valve positions sustained	Click the  button.
Temporarily pause the method, and stop all pumps	Click the  button.
Resume, for example, a held or paused method run.	Click the  button. ***Note:*** *An ended method cannot be resumed.*
Permanently end the run	Click the  button.

Note: *When ending a method run in advance, it is possible to save the partial result.*

More information regarding UNICORN capabilities during the method run is available in the *UNICORN System Control Manual*.

Actions in the Process Picture pane

It is possible to manually interact with an ongoing method through the ***Process Picture***, see *UNICORN System Control Manual*.



Using UNICORN to control the pressure during a run

The function ***Pressure control*** allows a method to be run with the set flow rate without the risk of a method stop due to pressure alarm. Pressure control is enabled in the instructions ***System flow*** and ***Sample flow***. Refer to *Section 9.15 Pressure control, on page 643* for more information.

The system configuration affects the pressure

Using narrow tubing between components will improve resolution but will lead to increased back pressure in the system. Narrow tubing after the column will increase the pressure in the column at a given flow rate. Make sure that the sensor limits for the pre-column pressure are set so that the maximum pressure for the column in use is not exceeded.

Note: *If Column valve* **V9-C** *or* **V9H-C** *is mounted, the integrated pressure sensor of the valve allows the system to monitor the post-column pressure. The limit for the pressure sensor in Column valve* **V9-C** *or* **V9H-C** *is automatically set so that the UV monitor and the pH monitor are protected from high pressure. If Column valve* **V9-C** *or* **V9H-C** *is not mounted, make sure to keep the pressure in the system after the column below the pressure limits for the modules in the flow path.*

5.9.4 After run procedures

Introduction

This section describes how to clean the instrument and columns after a chromatographic run, and how to prepare the system for storage.

The instrument and the columns must be cleaned between the runs. This will prevent, for example, sample contamination, protein precipitation and column clogging. If the instrument is not going to be used for a couple of days or longer, the instrument, columns and the pH flow cell should be filled with storage solution. For further information about cleaning and maintenance procedures, see *Chapter 7 Maintenance, on page 339*.



CAUTION

Hazardous chemicals and biological agents. Before maintenance, service and decommissioning, wash the ÄKTA pure instrument with a neutral solution to make sure that any hazardous solvents and biological agents have been flushed out from the system.

Tip: *To clean and fill the instrument and columns with storage solution, use **System CIP** and **Column CIP** either as separate, predefined methods or as phases included in a chromatographic method.*

System cleaning

After a method run is completed, perform the following:

- Rinse the instrument with one or several cleaning solution(s) (e.g., NaOH, buffer solution or distilled water) using ***System CIP***.
- If applicable, empty the fraction collector.
- Clean all spills on the instrument and on the bench using a moist tissue.
- Empty the waste vessel.
- Clean the manual injection port of the injection valve, see *Section 7.4.2 Perform System CIP, on page 366* for detailed instructions.
- If applicable, clean the pH electrode manually and make sure to leave it in an appropriate buffer. See *Section 7.4.9 Clean the pH electrode, on page 389* for detailed instructions.

System storage

If the instrument is not going to be used for a couple of days or longer, also perform the following:

- Fill the system and inlets with storage solution (e.g., 20% ethanol) using ***System CIP***.

- If applicable, prepare the pH electrode for storage as described in *Section 7.6.3 Storage of the pH electrode, on page 451*.

Column cleaning

After a method run is completed, perform the following:

- Clean the column with one or several cleaning solution(s) using ***Column CIP***.

Column storage

If the column is not going to be used for a couple of days or longer, also perform the following:

- Fill the column with storage solution (e.g., 20% ethanol) using ***Column CIP***.

pH electrode storage

If pH monitoring will not be used for a week or longer, perform one of the following actions:

- Inject new storage solution into the pH flow cell. Refer to *Section 7.6.3 Storage of the pH electrode, on page 451* for instructions.
- Replace the pH electrode with the dummy electrode that is installed in the pH valve on delivery.

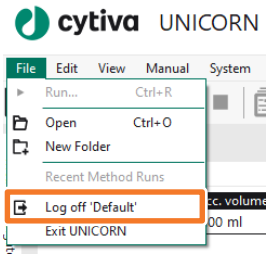
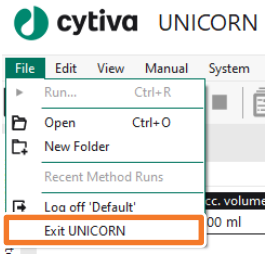
In the following situations, in order to increase the lifetime of the pH electrode, use the ***By-pass*** position and store the electrode in storage solution inside the pH flow cell:

- pH monitoring is not needed during the run.
- Organic solutions are used.
- Extremely acidic or extremely basic solutions are used.

For further information on how to prepare the pH electrode for storage, refer to *Section 7.6.3 Storage of the pH electrode, on page 451*.

Log off or exit UNICORN

Follow the instructions to log off or exit UNICORN. This can be performed from any of the UNICORN modules.

If you want to...	then...
Log off UNICORN	On the ***File*** menu, click ***Log off***.  *Result:* All open UNICORN modules close and the ***Log On*** dialog box opens.
Exit UNICORN	On the ***File*** menu, click ***Exit UNICORN***.  *Result:* All open UNICORN modules close.

Note: *If an edited method or result is open and not saved when trying to exit or log off UNICORN, a warning is shown. Click **Yes** to save, **No** to exit without saving, or **Cancel** to stay logged on.*

Shut down the instrument

Switch off the instrument by pressing the power switch to the **O** position.



5.10 ÄKTA pure micro or Micro kit operation

About this section

This section contains considerations for running micro-scale purification using the ÄKTA pure micro or Micro kit.

In this section

Section		See page
5.10.1	Columns	300
5.10.2	Injection techniques	302
5.10.3	Gradients	303
5.10.4	Fraction collection	304

5.10.1 Columns

Connect a column

To increase the resolution, it is recommended to connect the column directly to the UV monitor using a male-male connector. Both i.d. 0.25 mm and i.d. 0.13 mm are supplied. To secure the column, use the supplied Multi-directional column clamp.

Note: *With the Micro kit configuration, the gain in volume by using horizontal orientation of the column is insignificant (~1 µL).*

Select tubing kit

Use the red i.d. 0.13 mm tubing kit for columns of type 3.2/300 and 5/150. For all other Cytiva pre-packed columns the blue i.d. 0.25 mm tubing is recommended. In general, the red i.d. 0.13mm tubing kit is recommended for low flow rates up to 0.25 mL/min. With higher flow rates, the generated back pressure will be too high.

Note: *Remember that the pressure generated by tubing increases by 16x if the i.d. is halved. On the other hand, the volume is increased by 4x if the i.d. is doubled.*

Note: *The flow restrictor FR-902 is not needed with the red tubing kit. See Pressure and pressure limits, on page 300 for more information.*

Pressure and pressure limits

For a description of pressure monitoring and alarms see *Section 5.6 Pressure alarms, on page 238*.

Only the $P_{pre\text{-}column}$ pressure alarm can be set in UNICORN with the micro configuration. To protect the packed bed, the $P_{delta\text{-}column}$ pressure limit from the column list needs be used and set as $P_{pre\text{-}column}$ pressure alarm value. This is done automatically in UNICORN for predefined methods or phases for most glass columns.

Without the column valve **V9-C** with built-in pressure sensors, only the system pressure is measured.

The $P_{pre\text{-}column}$ pressure is based on:

- P_{system}
- Flow rate
- Tubing length
- Temperature

The $P_{pre\text{-}column}$ pressure value is calculated based on the system pressure and the pressure drop between the system pressure sensor and the column. Make sure to enter the value of the tubing dimensions from the injection valve to the column in the System settings, see *Adjust System Settings, on page 201*.

Flow restrictor

A flow restrictor creates a steady back pressure that keeps the air dissolved in the solution. Air bubbles formed after the column, due to pressure dropping, might disturb the detector signal.

The flow restrictor back pressure (0.2 MPa) only affects the column hardware pressure whereas the pressure on the packed bed is unaffected.

It is recommended to always keep the flow restrictor inline. However, when using the red i.d. 0.13 mm tubing kit there is enough pressure without the flow restrictor. To avoid unnecessary peak broadening it is recommended to exclude the flow restrictor when using the red tubing kit.

5.10.2 Injection techniques

Manual sample injection

For good chromatographic resolution on SEC columns in analytical or small-scale preparative work, the sample volume must not exceed 2% of the column volume. When connecting a small loop and working with complete loop filling techniques, the total sample volume is the sum of the loop volume and the loop connecting channels in the valve. The micro injection valve **V9M-J** has low internal volume to minimize extra sample load. Remember not to remove the syringe before the valve has turned.

When loading small sample volumes into injection valve, use the Fill port in the Injection kit **INV-907** which is made for syringes with needle o.d. 0.7 mm. Make sure that the needle is blunt ended.

Note: *Do not use Luer connection since that is designed for larger volumes.*

Manual complete filling

The micro injection valve **V9M-J** has minimal channel contribution to the loop volume.

Due to laminar flow, it is recommended to fill with an excess volume of sample that corresponds to 3 to 5 times the loop- and valve-volume. This recommendation is a general recommendation for all small SEC columns irrespective of the loop size chosen. Correspondingly, when injecting the sample into the column, empty the loop and the injection valve channels with an excess of buffer.

Sample injection using ALIAS autosampler

Manual partial filling requires experience with liquid chromatography systems and a more reliable method is to use an ALIAS autosampler.

The recommended volumes for manual loop filling also applies when using the Alias autosampler, both for complete and partial filling. Partial filling of a small loop, for example 1 µL in a 10 µL loop, is a way to further increase the resolution.

For more information about installing and operating the autosampler, see *Connect ALIAS Bio autosampler to lab scale ÄKTA systems Instructions (29040427)*.

5.10.3 Gradients

The size of the mixer compared to the length of the gradient is very important since a too large mixer will distort short gradients. The following table shows general recommendation for the microscale configuration. A larger mixer may be needed when creating gradients with some solvents.

Flow rate	Mixer size
0.25 – 2 mL/min	0.6 mL
0.05 – 0.25 mL/min	No mixer

Note: *If running without a mixer, it is recommended to use the online filter in the Online filter clamp on the Mixer module.*

5.10.4 Fraction collection

The fraction collector F9-R or F9-T are recommended for use with the micro-scale configuration.

The micro outlet valve **V9M-Os** has minimal volume and do not need to by-passed to achieve low band-broadening in the fraction collector. Use short tubing length between the outlet valve and the fraction collector. Pre-cut tubing of 400 mm is supplied which is appropriate for placing the fraction collector next to the ÄKTA pure system. Remember to set the appropriate delay volume, see *Set the delay volume, on page 203*.

Creating small drops

With water-based liquids at room temperature, the drop size with the micro nozzle is ~8 µL. The micro nozzle, including the use of ***Drop Sync***, is recommended when using the red tubing kit with flow rates up to 0.25 mL/min.

6 Performance tests

About this chapter

This chapter provides information about performance tests.

Performance tests must be run after installation to check the function of the ÄKTA pure system. Different tests are available and the tests to perform depend on the system configuration used.

Performance tests can also be used at any time to check the condition of the system, for example, after a prolonged stop. This chapter describes how to prepare, run, and evaluate the different performance tests available.

In this chapter

Section		See page
6.1	General performance test actions	306
6.2	Air sensor A and Inlet valve A tests	310
6.3	Air sensor B and Inlet valve B tests	312
6.4	Air sensor S and Sample inlet valve tests	314
6.5	Column valve C tests	316
6.6	Fraction collector F9-C test	318
6.7	Fraction collector F9-R test	320
6.8	Fraction collector F9-T test	322
6.9	Sample pump tests	325
6.10	System test UV U9-M	327
6.11	System test UV U9-T or U9-L	332
6.12	System test Micro kit	336

6.1 General performance test actions

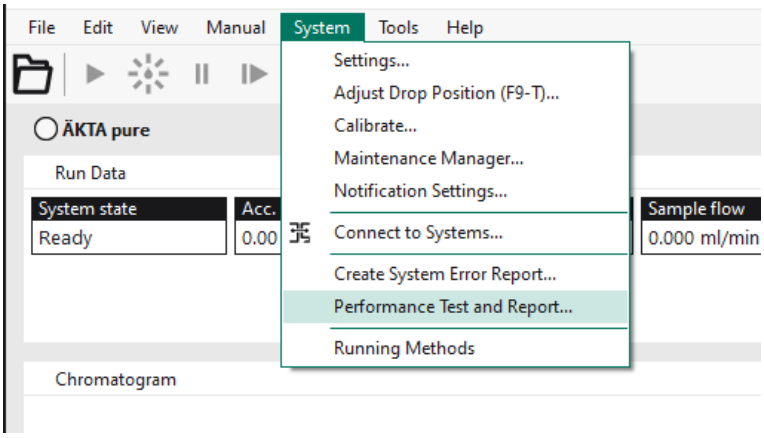
Introduction

Some actions are identical for each performance test. These actions are described in this section.

Start the performance tests

Follow the instructions to start a performance test.

Step	Action
1	In the ***System Control*** module, on the ***System*** menu, click ***Performance Test and Report***.



2	In the ***System Performance Test and Report*** dialog box, click one of the following tests:

ÄKTA pure 25	ÄKTA pure 150
Air sensor A and inlet valve V9-IA test	***Air sensor A and inlet valve V9H-IA test***
Air sensor B and inlet valve V9-IB test	***Air sensor B and inlet valve V9H-IB test***
Air sensor S and sample inlet valve V9-IS test	***Air sensor S and sample inlet valve V9H-IS test***
Column valve V9-C test	***Column valve V9H-C test***
Fraction collector F9-C test	***Fraction collector F9-C test***

Step	Action

ÄKTA pure 25	ÄKTA pure 150
Fraction collector F9-R test	***Fraction collector F9-R test***
Fraction collector F9-T test	N/A
Sample pump S9 test	***Sample pump S9H test***
System test with UV U9-M	***System test with UV U9-M***
System test with UV U9-T	***System test with UV U9-T***
System test with UV U9-L	***System test with UV U9-L***
System test Micro (blue tubing kit)	N/A
System test Micro (red tubing kit)	N/A

3 Click ***Run Performance Method***.

4 Click ***Next*** in the ***Start Protocol*** dialog box to open the next page. The pages are described in the table *Overview of the Start Protocol dialog box, on page 308*.

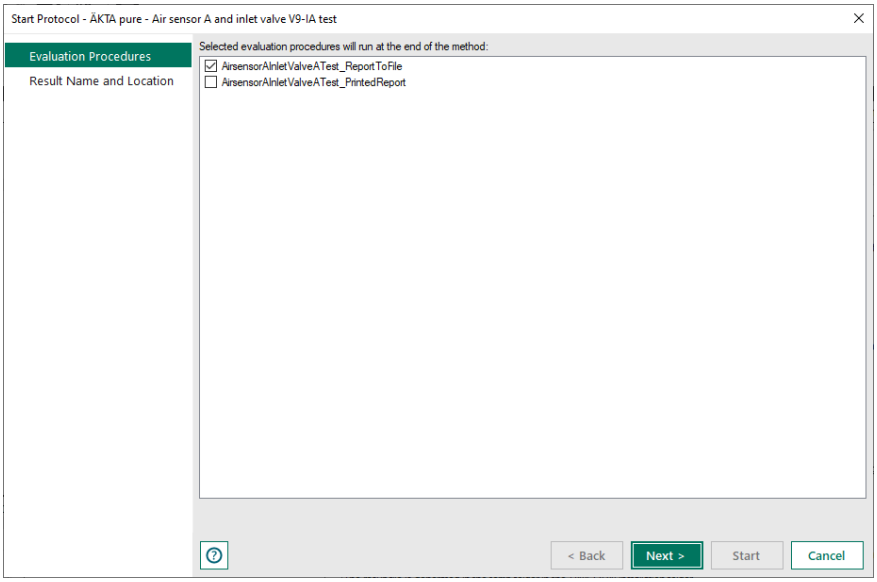
5 In the last page, ***Result name and location***, click ***Start***.

Result:

The selected performance test starts.

Overview of the Start Protocol dialog box

The following table describes the pages of the Start Protocol.



Page	Description
Evaluation Procedures	Allows the user to select to save the report to file (recommended) and/or to print the report.
Result Name and Location	Allows the user to change result name and result location.

During the run

A ***Message*** dialog box opens during the run. Read the messages in the dialog box, and make sure that necessary preparations have been performed.

- Click ***Confirm and Continue*** in the ***Message*** dialog box to change system state from ***System Pause*** to ***Run*** and proceed with the test.
- Alternatively, click ***Confirm*** in the ***Message*** dialog box and click the ***Continue*** button on the Instrument display.

Automatic evaluation

The system automatically generates a report when the test is finished. The report can be printed in two ways:

- It is recommended to select ***Save the report to file*** in the ***Evaluation Procedures*** page of the ***Start Protocol*** dialog box when starting the test. The report is saved in the folder `Temp` in your UNICORN installation folder. For example `C:\Program Files(x86)\Cytiva\UNICORN\UNICORN 7.7\Temp`.
- If the option ***Print report*** was selected in the ***Evaluation Procedures*** page of the ***Start Protocol*** dialog box when starting the test, the report is automatically printed on the system printer. Refer to *UNICORN Administration and Technical Manual* for information on how to install a printer.

Print the report and check the status of the test. For each of the tests the report states "**The test passed**" or "**The test failed**".

Note: *The fraction collector test is evaluated manually and no report is generated.*

6.2 Air sensor A and Inlet valve A tests

Air sensor A and Inlet valve A tests

The following Air sensor A and Inlet valve A tests are available, for ÄKTA pure 25 and ÄKTA pure 150, respectively:

- ***Air sensor A and inlet valve V9-IA test***
- ***Air sensor A and inlet valve V9H-IA test***

Method description

The Air sensor A and inlet valve A tests switch inlet valve A, labeled **V9-IA** or ***V9H-IA***, to an empty position and check if the air sensor detects air.

The method run takes approximately 1 minute.

Required configuration

The core components of the ÄKTA pure, and a correctly installed Inlet valve A are required to run the test.

Required material

The following materials are required:

- Syringe, 25 to 30 mL
- Distilled water

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct the outlet tubing marked **W** to a waste container.
2	Place inlet tubing **A1** into distilled water.
3	Prime the inlet tubing **A1** and purge the pump. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
4	Disconnect the inlet tubing connected to the **A3** inlet valve position. During the test method air is introduced into the inlet valve through that inlet port to test the function of the air sensor.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Possible causes of a failed test

The tables below describe possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

For a faulty air sensor A test result, see the table below.

Cause	Action
Faulty air sensor	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.
Incorrect preparation of tubing	Make sure that the tubing was correctly prepared, see *Prepare the test, on page 310*.

For a faulty inlet valve A test result, see the table below.

Cause	Action
The valve did not switch	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.

6.3 Air sensor B and Inlet valve B tests

Air sensor B and Inlet valve B tests

The following Air sensor B and Inlet valve B tests are available, for ÄKTA pure 25 and ÄKTA pure 150, respectively:

- ***Air sensor B and inlet valve V9-IB test***
- ***Air sensor B and inlet valve V9H-IB test***

Method description

The Air sensor B and inlet valve B test switches the inlet valve B, labeled **V9-IB** or ***V9H-IB***, to an empty position and checks if the air sensor detects air.

The method run takes approximately 1 minute.

Required configuration

The core components of the ÄKTA pure, and a correctly installed Inlet valve B are required to run the test.

Required material

The following materials are required:

- Syringe, 25 to 30 mL
- Distilled water

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct the outlet tubing marked **W** to a waste container.
2	Place inlet tubing **B1** into distilled water.
3	Prime the inlet tubing **B1** and purge the pump. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
4	Disconnect the inlet tubing connected to inlet valve position **B3**. During the test method air is introduced into the inlet valve through that inlet port to test the function of the air sensor.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Possible causes of a failed test

The tables below describe possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

For a faulty air sensor B test result, see the table below.

Cause	Action
Faulty air sensor	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.
Incorrect preparation of tubing	Make sure that the tubing was correctly prepared, see *Prepare the test, on page 312*.

For a faulty inlet valve B test result, see the table below.

Cause	Action
The valve did not switch	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.

6.4 Air sensor S and Sample inlet valve tests

Air sensor S and Sample inlet valve tests

The following Air sensor S and Sample inlet valve tests are available, for ÄKTA pure 25 and ÄKTA pure 150, respectively:

- ***Air sensor S and Sample inlet valve V9-IS test***
- ***Air sensor S and Sample inlet valve V9H-IS test***

Method description

The Air sensor S and Sample inlet valve test checks the functionality of the Sample inlet valve, labeled **V9-IS** or ***V9H-IS***, and the integrated air sensor in ÄKTA pure.

The method run takes approximately 1 minute.

Required configuration

The core components of the ÄKTA pure, a correctly installed Sample Inlet valve, and a Sample pump are required to run the test.

Required material

The following materials are required:

- Syringe, 25 to 30 mL
- Distilled water

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Immerse waste tubing **W** in a waste container.
2	Immerse sample inlet tubing **Buffer** in distilled water.
3	Prime sample inlet tubing **Buffer** and purge the sample pump, as described in *Section 5.4 Prime inlets and purge pump heads, on page 220*.
4	Disconnect the inlet tubing connected to the sample inlet valve position **S3**. During the test method air is introduced into the sample inlet valve through that inlet port to test the function of the air sensor.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Possible causes of a failed test

The tables below describe possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

For a faulty air sensor S test result, see the table below.

Cause	Action
Faulty air sensor	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.
Incorrect preparation of tubing	Make sure that the tubing was correctly prepared, see *Prepare the test, on page 312*.

For a faulty Sample inlet valve test result, see the table below.

Cause	Action
The valve did not switch	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.

6.5 Column valve C tests

Column valve tests

The following column valve tests are available, for ÄKTA pure 25 and ÄKTA pure 150, respectively:

- ***Column valve V9-C test***
- ***Column valve V9H-C test***

Note: *No tests are available for* **V9-Cm**, **V9-Cs**, *or* **V9H-Cs**.

Method description

The Column valve C test checks the functionality of Column valve C, labeled **V9-C** or **V9H-C**, and of the integrated pressure sensors that measure pre-column and post-column pressure. If two valves are used, the test will check the pre-column pressure sensor in the first valve and the post-column pressure sensor in the second valve.

The method run takes approximately 1 minute.

Required configuration

The core components of the ÄKTA pure, and a correctly installed Column valve C are required to run the test.

Required material

The following materials are required:

- Syringe, 25 to 30 mL
- Distilled water
- **Ref 1** tubing, see *Reference capillary, on page 544*

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Connect the **Ref 1** tubing between Column valve C ports **1A** and **1B**.
2	Make sure the Flow restrictor is inline.
3	Direct outlet tubing **W** to a waste container.
4	Place inlet tubing **A1** into distilled water.
5	Prime the inlet tubing **A1** and purge the pump. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

Cause	Action
Faulty Column valve	For further information, see *Section 8.4 Troubleshooting: Valves, on page 474*.
The integrated pressure monitors are not calibrated	Calibrate the pressure monitors, see *Section 7.3.2 Calibrate the pressure monitors, on page 351*.
Incorrect preparation of the system	Make sure that the reference tubing is connected between column ports **1A** and **1B** and that the flow restrictor is in-line.

6.6 Fraction collector F9-C test

Method description

The ***Fraction collector F9-C test*** checks the functionality of Fraction collector F9-C.

The method run takes approximately 6 minutes.

Required configuration

The core components of ÄKTA pure, an outlet valve, and a correctly installed Fraction collector F9-C are required to run the test.

Required material

The following materials are required:

- Distilled water
- Syringe, 25 to 30 mL
- Two deep well cassettes
- Two 96 deep well plates for collecting the fractions

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Immerse waste tubing **W** in a waste container
2	Immerse inlet tubing **A1** in distilled water.
3	Prime inlet tubing **A1** and purge System pump A. See *Section 5.4 Prime inlets and purge pump heads, on page 220*. No air shall be left in the pump or tubing.
4	Place two 96 deep well plates in positions 1 and 6 in the fraction collector. Make sure that no other cassettes are present in the fraction collector during the run.
5	In the ***System Control*** module, select ***System*** →***Settings***.
6	In the ***System Settings*** dialog box: **a.** Select ***Fraction collector F9-C*** →***Fractionation settings***. **b.** In the ***Fraction mode*** field, select ***Automatic***. **c.** In the ***Fractionation order*** field, select ***Row-by-Row***. **d.** Click ***OK***.
7	In the ***System Settings*** dialog box:

Step	Action
	a. Select ***Tubing and Delay volumes***.
	b. Select the instruction ***Delay volume →Monitor to frac***.
	c. Set the correct delay volume, see *Section 9.13 Delay volumes, on page 635*.
	d. Click ***OK***.

Run the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start and run the performance test.

During the run, make sure to visually check that the fraction collector wash is performed.

Several messages will be displayed on the screen during the start-up of the run. Read the messages in the dialog box and make sure that necessary preparations have been performed. Click the ***Confirm and Continue*** button in the message dialog box to proceed with the test.

Evaluate the result

To evaluate the results, do the following:

- Check that correct volumes, 2 mL/well, are collected in the three first wells in row A (A1-A3) of both plates.
- Check if the fractionation marks in the chromatogram are in accordance with the filled wells. For further information on delay volumes and fractionation marks, see *Section 5.8.2 Delay volume and pre-fractionation volume, on page 263*.

Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

Cause	Action
Incorrect volumes collected in the tubes, and disturbances of system pressure curves: • Air trapped in System pump A • Faulty System pump A	Air in pumps: Make sure to prime inlet tubing **A1** and purge System pump A before method start, see *Section 5.4 Prime inlets and purge pump heads, on page 220*. Faulty pump: See *Section 8.6 Troubleshooting: Pumps, on page 490*.
Incorrect preparation of buffer, plates, and tubing	Make sure that the system was correctly prepared, see *Prepare the test, on page 318*.

6.7 Fraction collector F9-R test

Method description

The ***Fraction collector F9-R test*** checks the functionality of Fraction collector F9-R.

The method run takes approximately 5 minutes.

Required configuration

The core components of ÄKTA pure, a correctly installed Outlet valve, and a correctly installed Fraction collector F9-R are required to run the test.

Required material

The following materials are required:

- Distilled water
- Syringe, 25 to 30 mL
- 7 tubes for collecting the fractions

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct outlet tubing **W** to a waste container.
2	Place inlet tubing **A1** into distilled water.
3	Prime inlet tubing **A1** and purge System pump A. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
4	Place 7 tubes in the Fraction collector, in positions 1 to 7.
5	In the ***System Control*** module, select ***System*** →***Settings***.
6	In the ***System Settings*** dialog box: **a.** Select ***Fraction collector F9-R*** →***Fractionation settings***. **b.** In the ***Drop sync*** field, select ***On***. **c.** Click ***OK***.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start and run the performance test.

Evaluate the result

Check that the correct volumes have been collected in the tubes. The tubes should contain the following:

- Tube **1**: the delay volume
- Tubes **2-4**: 2 mL
- Tubes **5-6**: 1 mL

Also, check that the fractionation marks in the chromatogram correspond to the filled tubes and that spillages are kept to a minimum.

For further information about delay volumes and fractionation marks, see *Section 5.8.2 Delay volume and pre-fractionation volume, on page 263*.

Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

Cause	Action
Incorrect volumes collected in the tubes, and disturbances of system pressure curves: • Air trapped in System pump A • Faulty System pump A	Air in pumps: Make sure to prime inlet tubing **A1** and purge System pump A before method start, see *Section 5.4 Prime inlets and purge pump heads, on page 220*. Faulty pump: See *Section 8.6 Troubleshooting: Pumps, on page 490*.
Liquid collected in wrong tubes	Make sure the fraction collector delivery arm is positioned above tube number 1 before starting the test.
Incorrect preparation of buffer and tubing	Make sure that the system was correctly prepared, see *Prepare the test, on page 320*.

6.8 Fraction collector F9-T test

Method description

The ***Fraction collector F9-T test*** checks that the fraction collector works as required, by testing correct liquid dispensing into wells and liquid spillage.

The method run takes approximately 6 minutes.

Required configuration

The core components of ÄKTA pure, an outlet valve and a correctly installed Fraction collector F9-T are required to run the test.

Required material

The following materials are required:

- Distilled water
- Syringe, 25 to 30 mL
- Two 96 deep well plates

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct outlet tubing **W** to a waste container.
2	Place inlet tubing **A1** into distilled water.
3	Prime inlet tubing **A1** and purge System pump A. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
4	Place two empty 96 deep well plates in the fraction collector.
5	In the ***System Control*** module, select ***System*** →***Settings***.
6	In the ***System Settings*** dialog box: **a.** Select ***Fraction collector F9-T*** →***Drop sync***. **b.** In the ***Mode*** field, select ***On***. ***Note:*** *If the micro kit is installed, make sure to turn the drop sync mode to* ***Off****.* **c.** Click ***OK***.

Note: *If the micro kit is installed with the red tubing kit, i.d. 0.13 mm, make the following alterations before running the test: Move tubing* **4** *from the injection valve position* **Pump** *to the outlet valve position* **In***.*

Run the test

Follow the instructions described in *Start the performance tests, on page 306* to start and run the performance test.

During the run

The table below shows the volumes dispensed in the corresponding wells. Two 96 deep well plates are used in the ***Fraction collector F9-T test***.

Well	Volume
1.A.1	Pre-fractionation volume
1.A.2, 1A.3, 1.A.4	2 mL
2.H.12, 2.H.11, 2.H.10	1 mL
1.A.5, 2.G.11, 2.G.12, 2.H.9	Small volume, no need to check

Evaluate the result

Check that the correct volumes have been collected in the plate wells. Make sure that:

- The pre-fractionation volume has been collected in the first position **1A1**, and that the collected volume is as the set ***Delay volume***.
- A total of 2 mL liquid has been collected in positions **1A2**, **1A3**, and **1A4**.
- A total of 1 mL liquid has been collected in positions **2H12**, **2H11**, and **2H10**.

Note: *Check that the fractionation marks at the bottom of the chromatogram correspond to the filled tubes, and that spillages are kept to a minimum.*

Possible causes of a failed test

A failed ***Fraction collector F9-T test*** indicates a malfunction of the Fraction collector. Visually inspect the system and the resulting chromatogram to understand the causes for test failure. Check the table below for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the ***Fraction collector F9-T test***.

Cause	Action
Unstable pressure and incorrect volumes dispensed in the wells: • Air trapped in System pump A • Faulty System pump A	Air in pumps: Make sure to prime inlet tubing **A1** and purge System pump A before method start, see *Section 5.4 Prime inlets and purge pump heads, on page 220*. Faulty pump: See *Section 8.6 Trouble-shooting: Pumps, on page 490*.

Cause	Action
Incorrect volume dispensed in 1.A.1: • ***Delay volume*** incorrectly set	Set correct ***Delay volume*** in ***System settings***.
Liquid dispersed in the wrong well or outside of the well: • Calibrate Fraction collector • Deep well plate incorrectly positioned in the fraction collector	Calibration: Contact Service personnel for help with calibration. Plate position: Repeat the test with a properly positioned deep well plate.
Spillage between wells: • Drop sync function is inactive	Set the drop sync function to ***Auto*** or ***On*** in ***System Settings***.

6.9 Sample pump tests

Sample pumps tests

The following Sample pump tests are available, for ÄKTA pure 25 and ÄKTA pure 150, respectively:

- ***Sample pump S9 test***
- ***Sample pump S9H test***

Method description

The Sample pump test checks the functionality of the sample pump, labeled **S9** or ***S9H***.

The method run takes approximately 12 minutes.

Required configuration

The core components of ÄKTA pure and a correctly installed Sample pump are required to run the test.

Required material

The following materials are required:

- Distilled water
- 1% acetone in 1.00 M NaCl
- Syringe, 25 to 30 mL
- **Ref 1** tubing, see *Reference capillary, on page 544*

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Connect a tubing between Injection valve port **Col** and the UV monitor, preferably the **Ref 1** tubing included with the system at delivery.
2	Immerse the waste tubing **W**, **W1** and **W2** in a waste container.
3	Immerse the inlet tubing **A1** in distilled water.
4	Immerse the sample inlet tubing **Buffer** in the acetone solution.
5	Prime the inlet tubing **A1** and the sample inlet **Buffer** and purge the pumps. See *Section 5.4.2 Sample pump, on page 228*. Make sure that no air is left in the pumps or tubing.

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

Cause	Action
The two injected peaks of liquid are not delivered as proportional peaks: • Air trapped in Sample pump • Faulty Sample pump	Air in pump: Make sure to prime inlet tubing **Buffer** and purge sample pump before method start, see *Section 5.4.2 Sample pump, on page 228*. Faulty pump: See *Section 8.6 Troubleshooting: Pumps, on page 490*.
The delivery of liquid is pulsating (unstable pump pressure)	Air in pump: Make sure to prime inlet tubing **Buffer** and purge sample pump before method start, see *Section 5.4.2 Sample pump, on page 228*. Faulty pump: See *Section 8.6 Troubleshooting: Pumps, on page 490*.
The pump pressure is too high (at a flow rate of 25 mL/min or 150 mL/min for ÄKTA pure 25 and ÄKTA pure 150, respectively)	Folded, twisted or blocked tubing: Check the tubing. The Sample pump pressure monitor is not calibrated: Calibrate the pressure monitor, see *Section 7.3.2 Calibrate the pressure monitors, on page 351*. Faulty pump: See *Section 8.6 Troubleshooting: Pumps, on page 490*.
Incorrect preparation of buffer and tubing	Make sure that the system was correctly prepared, see *Prepare the test, on page 325*.

6.10 System test UV U9-M

Method description

The System test with UV monitor **U9-M** checks the functionality of the solvent delivery, the pumps, the System pump pressure sensor, UV monitor **U9-M** and the Conductivity monitor.

The method run takes approximately 36 minutes.

Required configuration

The core components of ÄKTA pure, a correctly installed UV monitor **U9-M**, and a correctly installed Conductivity monitor are required to run the test.

Required material

The following materials are required:

- Distilled water
- Acetone in 1.00 M NaCl

Cell length (mm)	Acetone (%)
< 10	1
10	0.8

- **Ref 1** tubing, see *Reference capillary, on page 544*
- Syringe, 25 to 30 mL
- Mixer, 1.4 mL

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct outlet tubing **W** and **W1** to a waste container.
2	Place inlet tubing **A1** into distilled water.
3	Place inlet tubing **B1** into the acetone solution.
4	Connect the **Ref 1** tubing between Injection valve port **Col** and the UV monitor **U9-M**, to generate a back pressure.
5	Make sure that the Mixer with a chamber volume of 1.4 mL is installed. For further information, see *Section 7.5.4 Replace the mixer, on page 408*.

Step	Action
6	Prime the buffer inlets and purge System pump A and System pump B. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.

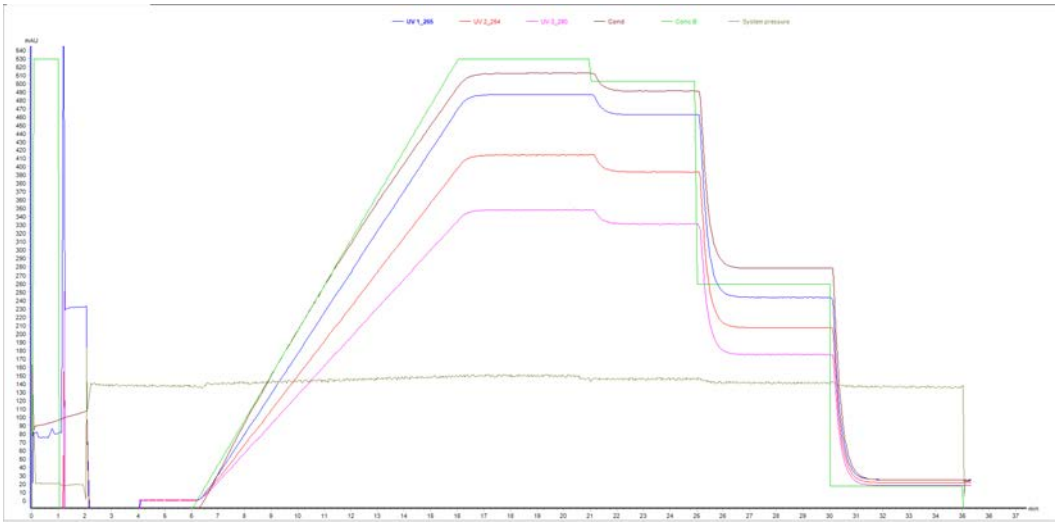
Note: *If the test is performed at cold room temperature, select* ***Conductivity →Cond temp compensation*** *in the* ***System Settings*** *dialog, and set the* ***Compensation factor*** *to 2.1.*

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Illustration of chromatogram

The illustration below shows a chromatogram from a System test with UV monitor **U9-M**.



Possible causes of a failed test

The following tables describe possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

For a faulty gradient test result, see the table below.

Cause	Action
Disturbances caused by air trapped in any of the pumps	Make sure to prime the buffer inlets and to purge the System pumps before method start. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.

Cause	Action
Disturbances caused by damaged pump piston seals	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.
Unstable or incorrect UV signal, or drifting base line: • Faulty UV monitor	See *Section 8.3 Troubleshooting: Monitors, on page 458*.
Wrong Mixer chamber size or faulty Mixer	Replace the Mixer. See *Chapter 7 Maintenance, on page 339*.

For a faulty step response test result, see the table below.

Cause	Action
If all values are faulty: • Air in the pump or a faulty pump	Air in pumps: Make sure to prime the buffer inlets and to purge the System pumps before method start. See *Section 5.4 Prime inlets and purge pump heads, on page 220*. Faulty pump: See *Chapter 7 Maintenance, on page 339*
Faulty values at 5%: • Damaged pump piston seal in System pump B	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.
Faulty values at 50%: • Using a 10 mm UV flow cell	Wrong acetone concentration. Use 0.8% for 10 mm UV flow cell, see *Required material, on page 327*.
Faulty values at 95%: • Damaged pump piston seal in System pump A	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.

For a faulty UV response test result, see the table below.

Cause	Action
Faulty UV monitor	Restart the instrument to calibrate the UV monitor. See *Section 8.3 Troubleshooting: Monitors, on page 458*.

For a faulty UV absorbance test result, see the table below.

Cause	Action
Incorrectly prepared acetone solution	Make sure that the acetone concentration is correct, for example make sure that acetone has not evaporated from the solution. See *Required material, on page 327*.

For a faulty pulsation test result, see the table below.

Cause	Action
Air trapped in the pumps	Make sure to prime and purge the system pumps before starting the test, see *Section 5.4 Prime inlets and purge pump heads, on page 220*.

For a faulty conductivity test result, see the table below.

Cause	Action
Faulty Conductivity monitor	See *Section 8.3.3 Conductivity monitor, on page 467*.
Incorrectly prepared NaCl solution	Make sure that the NaCl solution is 1.00 M.
The value set for the ***Cond temp compensation*** factor is not optimal	If the test is performed at cold room temperature, open the ***System Settings*** dialog box, select ***Conductivity*** →***Cond temp compensation*** and set the ***Compensation factor*** to 2.1.

For a faulty UV noise test result, see the table below.

Cause	Action
Air or dirt in the UV flow cell	Flush or clean the UV cell, see *Section 7.4.10 Clean the UV flow cell, on page 392*.
Impure buffer	Check buffers for impurities.

For a faulty pressure check test result, see the table below.

Cause	Action
Folded, twisted or blocked tubing	Check the tubing.
Dirt in online filter	Replace the online filter, see *Section 7.5.9 Replace the online filter, on page 417*.

Cause	Action
System pressure monitor not calibrated	Calibrate the pressure monitor, see *Section 7.3.2 Calibrate the pressure monitors, on page 351*.

6.11 System test UV U9-T or U9-L

Method description

The System test with UV monitor **U9-T** or **U9-L** checks the functionality of the solvent delivery, the pumps, the System pump pressure sensor, UV monitor (**U9-T** or **U9-L**) and the conductivity monitor.

The method run takes approximately 36 minutes.

Required configuration

The core components of ÄKTA pure, a correctly installed UV monitor **U9-T** or **U9-L**, and a correctly installed conductivity monitor are required to run the test.

Required material

The following materials are required:

- Distilled water
- 1% acetone in 1.00 M NaCl
- **Ref 1** tubing, see *Reference capillary, on page 544*
- Syringe, 25 to 30 mL
- Mixer, 1.4 mL

Prepare the test

Follow the steps below to prepare the system before method start.

Step	Action
1	Direct outlet tubing **W** and **W1** to a waste container.
2	Place inlet tubing **A1** into distilled water.
3	Place inlet tubing **B1** into the acetone solution.
4	Connect the **Ref 1** tubing between Injection valve port **Col** and the UV monitor, to generate a back pressure.
5	Make sure that the Mixer with a chamber volume of 1.4 mL is installed. For further information, see *Section 7.5.4 Replace the mixer, on page 408*.
6	Prime the buffer inlets and purge System pump A and System pump B. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.

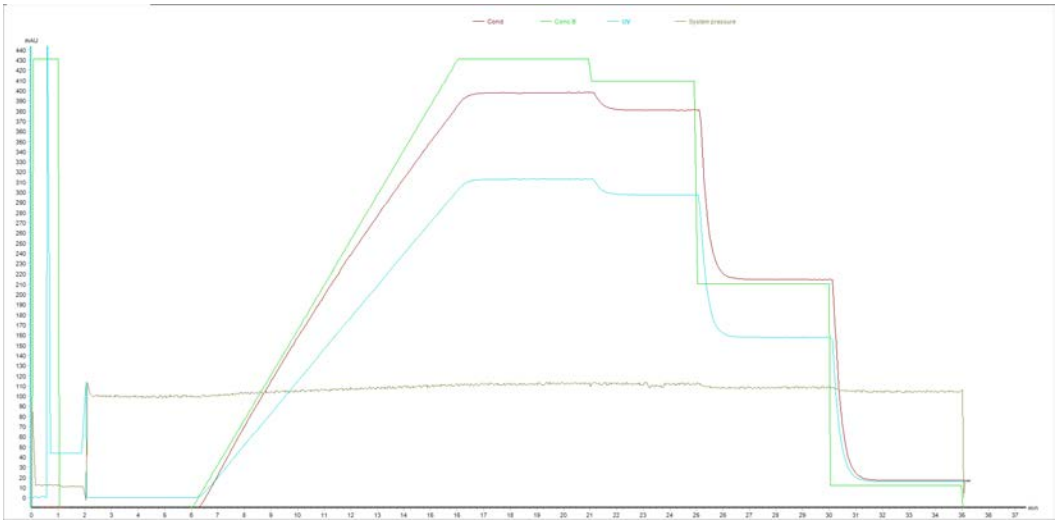
Note: *If the test is performed at cold room temperature, select **Conductivity** →**Cond temp compensation** in the **System Settings** dialog, and set the **Compensation factor** to 2.1.*

Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test.

Illustration of chromatogram

The illustration below shows an example of a chromatogram from a System test with UV monitor **U9-L**. The chromatogram from a System test with UV monitor **U9-T** is similar.



Possible causes of a failed test

The following tables describe possible causes of a failed test. When possible sources of error have been checked and corrected, repeat the test.

For a faulty gradient test result, see the table below.

Cause	Action
Disturbances caused by air trapped in any of the pumps	Make sure to prime the buffer inlets and to purge the System pumps before method start. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
Disturbances caused by damaged pump piston seals	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.
Unstable or incorrect UV signal, or drifting base line: • Faulty UV monitor	See *Section 8.3 Troubleshooting: Monitors, on page 458*.

Cause	Action
Wrong Mixer chamber size or faulty Mixer	Replace the Mixer. See *Chapter 7 Maintenance, on page 339*.

For a faulty step response test result, see the table below.

Cause	Action
If all values are faulty: • Air in the pump or a faulty pump	Air in pumps: Make sure to prime the buffer inlets and to purge the System pumps before method start. See *Section 5.4 Prime inlets and purge pump heads, on page 220*. Faulty pump: See *Chapter 7 Maintenance, on page 339*.
Faulty values at 5%: • Damaged pump piston seal in System pump B	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.
Faulty values at 95%: • Damaged pump piston seal in System pump A	Replace piston seals. See *Chapter 7 Maintenance, on page 339*.

For a faulty UV response test result for UV monitor **U9-T**, see the table below.

Cause	Action
Faulty UV monitor	See *Section 8.3 Troubleshooting: Monitors, on page 458*.

For a faulty UV absorbance test result, see the table below.

Cause	Action
Incorrectly prepared acetone solution	Make sure that the acetone concentration is correct. For example, make sure that acetone has not evaporated from the solution. See *Required material, on page 332*.
Wrong UV cell path length set in UNICORN	See *Calibration of the UV monitor U9-T and U9-L flow cell length, on page 360*.

For a faulty pulsation test result, see the table below.

Cause	Action
Air trapped in the pumps	Make sure to prime and purge the system pumps before starting the test, see *Section 5.4 Prime inlets and purge pump heads, on page 220*.

For a faulty conductivity test result, see the table below.

Cause	Action
Faulty Conductivity monitor	See *Section 8.3.3 Conductivity monitor, on page 467*.
Incorrectly prepared NaCl solution	Make sure that the NaCl solution is 1.00 M.
The value set for the ***Cond temp compensation*** factor is not optimal	If the test is performed at cold room temperature, open the ***System Settings*** dialog box, select ***Conductivity*** →***Cond temp compensation*** and set the ***Compensation factor*** to 2.1.

For a faulty UV noise test result, see the table below.

Cause	Action
Air or dirt in the UV flow cell	Flush or clean the UV cell, see *Section 7.4.10 Clean the UV flow cell, on page 392*.
Impure buffers	Check the buffers.

For a faulty pressure check test result, see the table below.

Cause	Action
Folded, twisted or blocked tubing	Check the tubing.
The online filter is dirty	Replace the online filter, see *Section 7.5.9 Replace the online filter, on page 417*.
The System pressure monitor is not calibrated	Calibrate the pressure monitor, see *Section 7.3.2 Calibrate the pressure monitors, on page 351*.

6.12 System test Micro kit

This section describes the system tests for blue tubing kit, and red tubing kit. The information is valid for both tests unless otherwise specified.

Method description

The System test with the Micro kit checks the functionality of the solvent delivery, the pumps, the system pump pressure sensor, UV monitor, and the Conductivity monitor. The method run takes approximately 35 minutes for blue tubing kit, and 70 minutes for red tubing kit.

Required configuration

Both kits require the following configuration:

- The core components of ÄKTA pure
- UV monitor **U9-M** with flow cell **U9-2M**
- Conductivity monitor
- Tubing **5** connected directly to the UV flow cell, see *Install the tubing kit, on page 112*

The blue tubing kit also requires the following:

- 0.6 mL mixer, see *Section 7.5.4 Replace the mixer, on page 408*
- Flow restrictor **FR-902**
- Blue tubing kit i.d. 0.25 mm

The red tubing kit also requires the following:

- Online filter in the online filter clamp (no mixer)
- Red tubing kit i.d. 0.13 mm

Required material

The following materials are required:

- Distilled water
- 1% acetone in 1.00 M NaCl
- Syringe, 25 to 30 mL

Prepare the test

Follow the steps below to prepare the system before method start.

Note: *If the test is performed at cold room temperature, select* ***Conductivity →Cond temp compensation*** *in the* ***System Settings*** *dialog box, and set the* ***Compensation factor*** *to 2.1.*

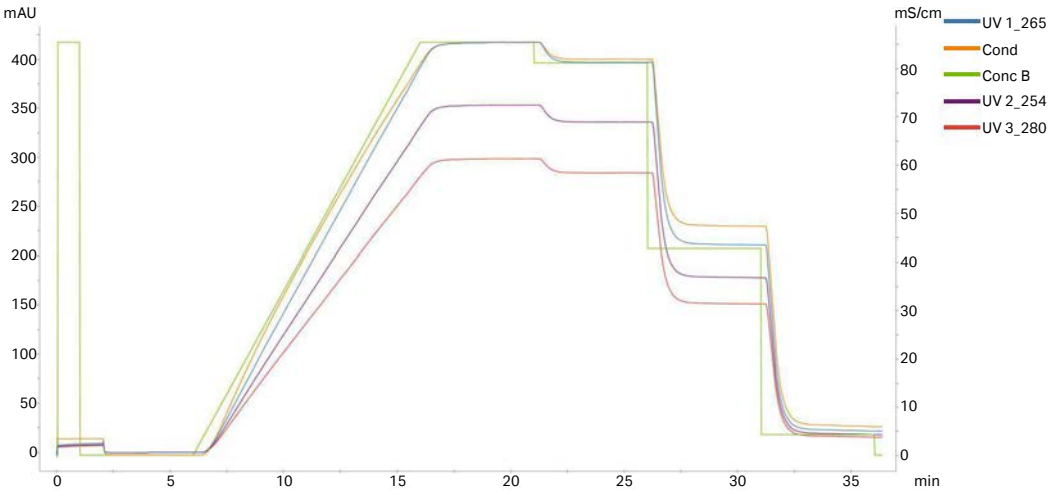
Step	Action
1	Direct the outlet tubing **W** and **W1** to a waste container.
2	Immerse the inlet tubing **A/A1** in distilled water.
3	Immerse the inlet tubing **B/B1** in the acetone solution.
4	Prime the buffer inlets, and purge System pump A and System pump B. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
5	Start a flow of 0.25 mL/min.
6	Check for leakage. The pressure must be stable and above 0.2 MPa.
7	Change to 100% **B** and repeat step 6.

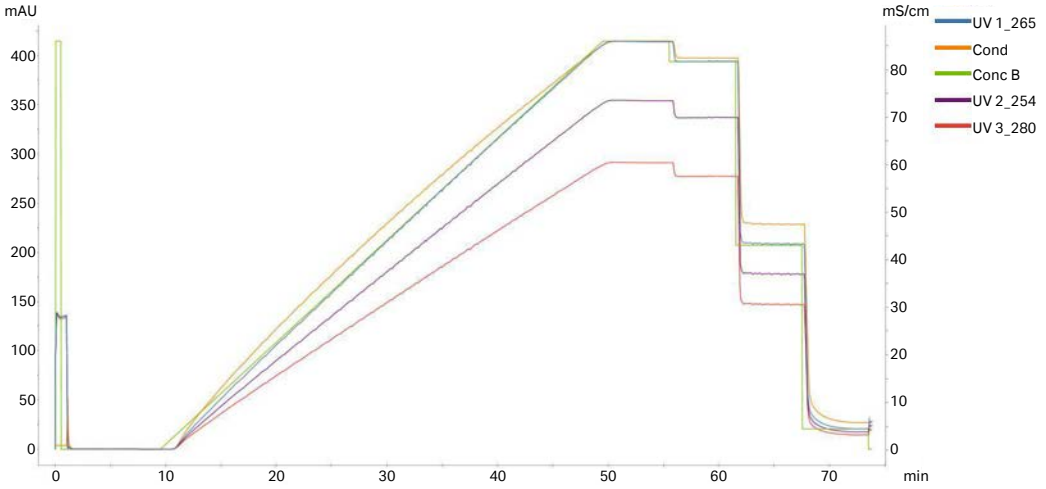
Run and evaluate the test

Follow the instructions described in *Section 6.1 General performance test actions, on page 306* to start, run and automatically evaluate the performance test. Select ***System test Micro (red tubing kit)*** or ***System test Micro (blue tubing kit)*** depending on the installed tubing kit.

Illustration of chromatogram

The illustrations below show chromatograms from System test Micro for **Blue tubing kit** (top), and **Red tubing kit** (bottom).





Possible causes of a failed test

See the tables in *Possible causes of a failed test, on page 328* for possible causes of a failed test.

7 Maintenance

About this chapter

This chapter describes the maintenance program for ÄKTA pure and provides instructions for maintenance and replacement of spare parts.

In this chapter

Section		See page
7.1	Maintenance Manager	340
7.2	Maintenance program	345
7.3	Calibration procedures	348
7.4	Cleaning procedures	364
7.5	Replacement procedures	401
7.6	Other maintenance procedures	443

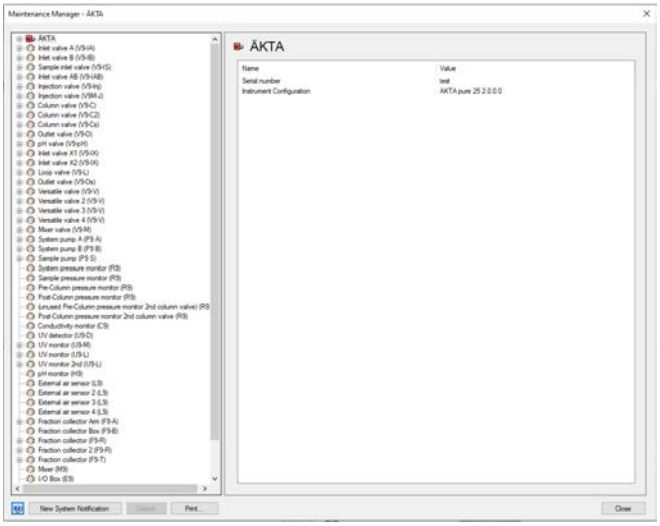
7.1 Maintenance Manager

Introduction

Maintenance Manager allows the user to display general information about the system and its modules, and also operational statistics of the modules. Notifications for maintenance actions of the system and its modules are predefined. The user can add automated maintenance notifications for the system. Maintenance notifications are based on calender periods of system use, and for some systems also on operational statistics for the modules.

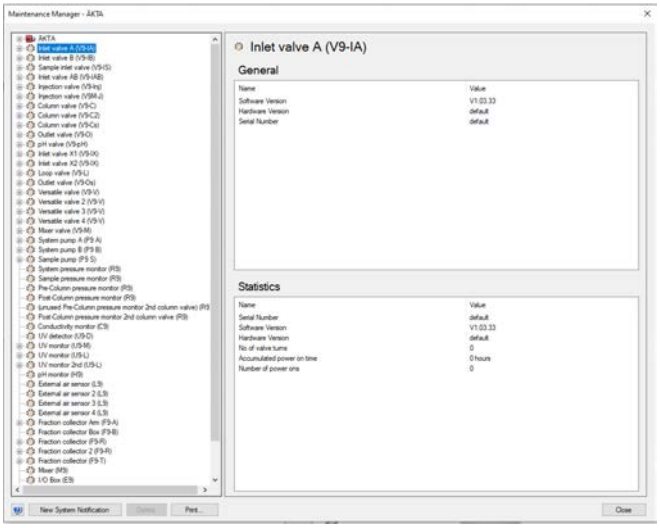
Open Maintenance Manager

In the ***System Control*** module, on the ***System*** menu, click ***Maintenance Manager*** to open the ***Maintenance Manager*** dialog box.



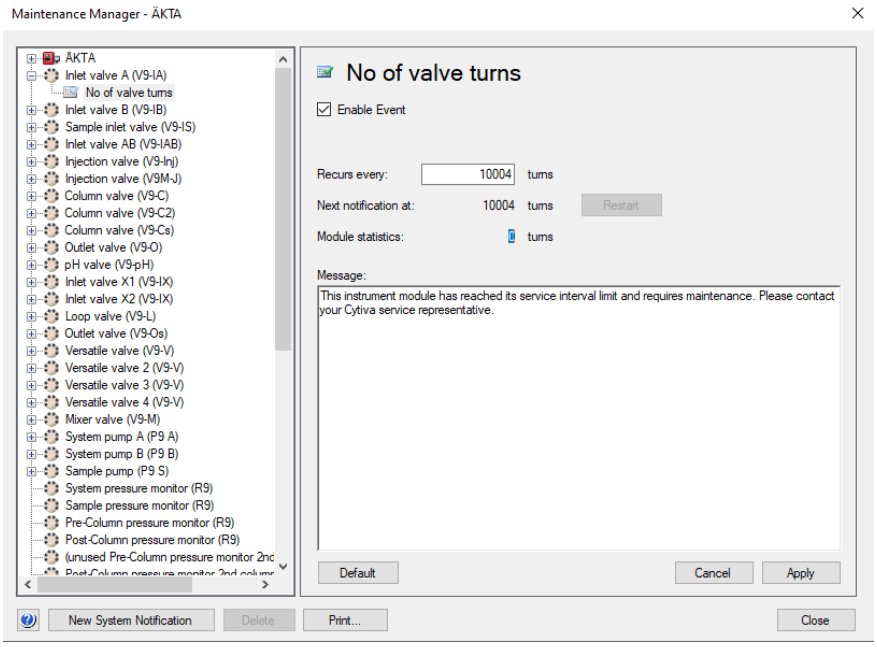
View general information and statistics

In the left pane of the ***Maintenance Manager*** dialog box, select the system of interest to view general information of the selected system. For some systems it is possible to view information for a module of interest. When modules are selected, operational statistics are also displayed.



View maintenance notifications

Click the plus symbol (**+**) of the system of interest to expand the list of related maintenance notifications. For some systems it is possible to view maintenance notifications for a module of interest. Select a notification to view notification details.

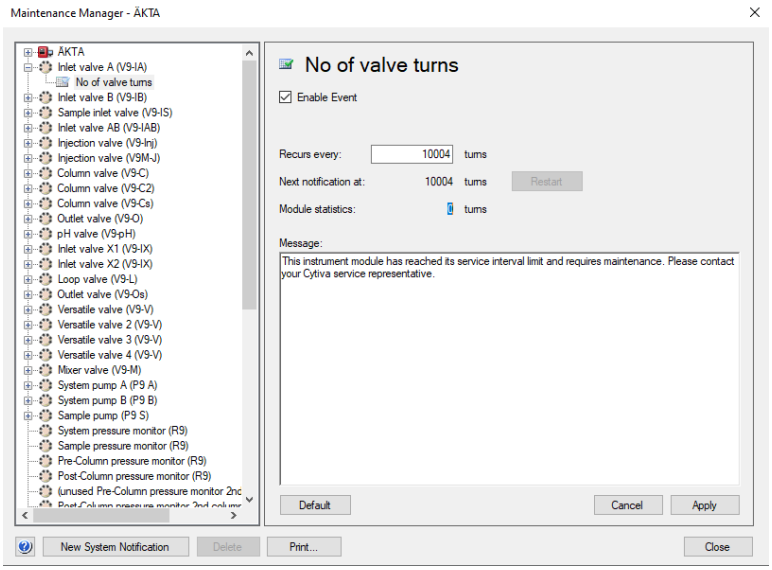


Note: *Modules with no plus symbol (**+**) have no related maintenance notifications.*

Edit a maintenance notification

Follow the instruction to edit a maintenance notification.

Step	Action
1	In the left pane of the ***Maintenance Manager*** dialog box, select a maintenance notification.

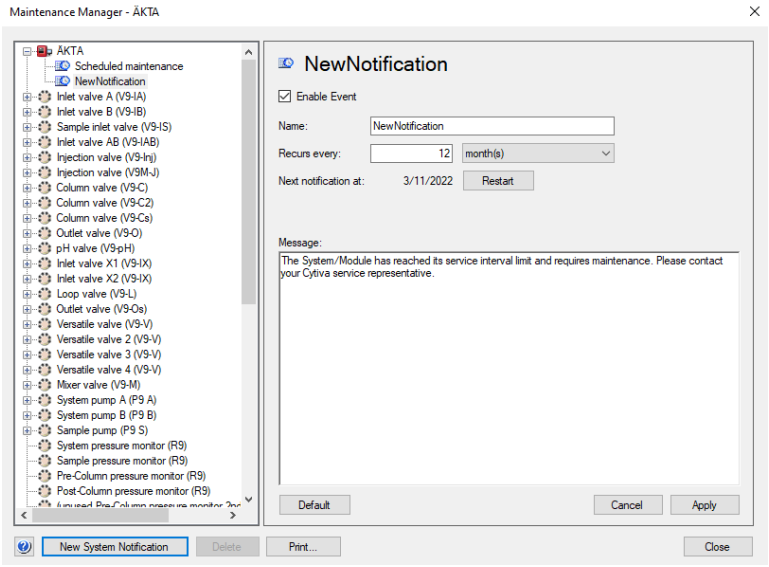


2	Edit the maintenance notification as desired: • Select the ***Enable Event*** check box to activate the notification. If the box is unchecked, the notification is not issued. • Enter a new interval after which the new notification is issued. • Click ***Restart*** to reset the counter and add a complete interval before the next notification. • Edit the message that is shown in the maintenance notification. • Click ***Default*** to restore the default settings for maintenance notifications.
3	Click ***Apply*** to save the changes.

Add a new system notification

Follow the instructions to add a new system notification to the list of system events.

Step	Action
1	In the ***Maintenance Manager*** dialog box, click ***New System Notification***.



2	In the ***NewNotification*** field: • Enter a name for the new notification. • Select a time interval after which the new notification is issued. • If desired, write a message that is shown for the maintenance notification.
3	Click ***Apply*** to save the changes and apply the notification settings.

Delete a user defined system notification

To delete a user defined system notification, select the notification in the ***Maintenance Manager*** dialog box and press the ***Delete*** button.

Note: *Module notifications are predefined and cannot be deleted. If desired, they can be disabled.*

Handle a maintenance notification

Each maintenance notification has a time interval after which the notification is issued. When this time interval has been reached, a ***Maintenance Notification*** dialog box appears.

Note: *The possibility to add maintenance notifications for modules is only available for some systems, but all systems can add maintenance notifications for the complete system.*

Follow the instruction to handle the notification. Click the applicable options in the ***Maintenance Notification*** dialog box, as described in the table below.

Option	Description
Acknowl-edge	Reset the counter for a new maintenance notification period. ***Note:*** *Make sure that the maintenance action is performed as instructed after the notification is acknowledged, otherwise the system performance can deteriorate.*
Ignore	Close the dialog box without action. ***Note:*** *The **Maintenance Notification** is displayed each time the **System Control** module is opened until the notification is acknowledged.*

Note: *The predefined maintenance notifcation periods use average values. The actual service interval for a specific module can be shorter or longer.*

7.2 Maintenance program

Introduction

This section lists the periodic maintenance activities that must be performed by the user of ÄKTA pure, as well as maintenance activities that must be performed when required.

Maintenance is divided into:

- Daily maintenance
- Weekly maintenance
- Monthly maintenance
- Semiannual maintenance
- Maintenance when required



WARNING

Hazardous biological agents during run. When using hazardous biological agents, perform a system cleaning to flush the entire system tubing with storage solution (e.g. NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.



CAUTION

Hazardous chemicals during run. When using hazardous chemicals, run ***System CIP*** and ***Column CIP*** to flush the entire system tubing with distilled water, before service and maintenance.



CAUTION

Always use appropriate personal protective equipment when decommissioning the equipment.

Periodic maintenance program

The following periodic maintenance must be performed by the user of ÄKTA pure.

Interval	Maintenance action	See section
Daily	Calibrate the pH monitor	*Section 7.3.1 Calibrate the pH monitor, on page 349*

Interval	Maintenance action	See section
Weekly	Calibrate pressure monitors	*Section 7.3.2 Calibrate the pressure monitors, on page 351*
Weekly	Change pump rinsing solution	*Section 7.6.1 Change pump rinsing solution, on page 444*
Weekly	Clean the Fraction collector F9-C diodes	*Section 7.4.5 Clean the Fraction collector F9-C sensors, on page 383*
Weekly	Clean the Fraction collector F9-T drop sync sensor	*Section 7.4.8 Clean the Fraction collector F9-T drop sync sensor, on page 388*
Monthly	Check the Flow restrictor	*Section 7.6.2 Check the flow restrictor, on page 449*
Semiannually	Clean the UV flow cell	*Section 7.4.10 Clean the UV flow cell, on page 392*
Semiannually	Replace pH electrode	*Section 7.5.3 Replace the pH electrode, on page 407*

Maintenance when required

The following maintenance must be performed by the user of ÄKTA pure when required.

Maintenance action	See section
Clean the instrument externally	*Section 7.4.1 Clean the instrument externally, on page 365*
Perform System CIP	*Section 7.4.2 Perform System CIP, on page 366*
Perform Column CIP	*Section 7.4.3 Perform Column CIP, on page 377*
Clean Fraction collector F9-C	*Section 7.4.4 Clean Fraction collector F9-C, on page 381*
Clean Fraction collector F9-R	*Section 7.4.6 Clean Fraction collector F9-R, on page 385*
Clean Fraction collector F9-T	*Section 7.4.7 Clean Fraction collector F9-T, on page 386*
Replace tubing and connectors	*Section 7.5.1 Replace tubing and connectors, on page 402*
Storage of pH electrode	*Section 7.6.3 Storage of the pH electrode, on page 451*
Clean the pH electrode	*Section 7.4.9 Clean the pH electrode, on page 389*

Maintenance action	See section
Clean the Conductivity flow cell	*Section 7.4.11 Clean the conductivity flow cell, on page 395*
Calibrate the Conductivity monitor	*Section 7.3.3 Calibrate the conductivity monitor, on page 355*
Calibrate the UV monitor	*Section 7.3.4 Calibrate the UV monitors, on page 360*
Replace Mixer	*Section 7.5.4 Replace the mixer, on page 408*
Replace online filter	*Section 7.5.9 Replace the online filter, on page 417*
Replace O-ring in Mixer	*Section 7.5.5 Replace the O-ring inside the mixer, on page 409*
Replace UV flow cell	*Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411* and *Section 7.5.7 Replace the UV monitor U9-T and U9-L flow cell, on page 414*
Replace the Flow restrictor	*Section 7.5.8 Replace flow restrictor, on page 416*
Replace inlet filters	*Section 7.5.10 Replace the inlet filters, on page 418*
Wipe off excess oil from the pump head	*Section 7.4.13 Wipe off excess oil from the pump head, on page 400*
Clean the check valves	*Section 7.4.12 Clean the pump head check valves, on page 397*
Replace check valves	*Section 7.5.11 Replace the pump head check valves, on page 419*
Replace pump piston seals, pump P9 and P9H	*Section 7.5.12 Replace pump piston seals of Pump P9 or P9H, on page 422*
Replace pump piston seals, pump P9-S	*Section 7.5.13 Replace pump piston seals of Pump P9-S, on page 431*
Replace pump pistons	*Section 7.5.14 Replace pump pistons, on page 439*
Replace pump rinsing system tubing	*Section 7.5.15 Replace pump rinsing system tubing, on page 440*

7.3 Calibration procedures

About this section

This section provides instructions for calibration procedures.

In this section

Section		See page
7.3.1	Calibrate the pH monitor	349
7.3.2	Calibrate the pressure monitors	351
7.3.3	Calibrate the conductivity monitor	355
7.3.4	Calibrate the UV monitors	360

7.3.1 Calibrate the pH monitor

Maintenance interval

Calibrate the pH monitor once a day, when the pH electrode has been replaced, or if the ambient temperature has changed by more than ± 5°C.

Required material

Use two pH calibration buffers with a difference of at least one pH unit. Preferably use a pH standard buffer, pH 4 or pH 7, as the first calibration point, and a pH standard buffer close to the lowest or highest pH as second calibration point. Allow the buffers to equilibrate to ambient temperature before use.

Calibrating the pH monitor



CAUTION

pH electrode. Handle the pH electrode with care. The glass tip may break and cause injury.

Follow the steps below to calibrate the pH monitor.

Step	Action
1	Open the ***System Control*** module and select ***System*** →***Calibrate***.
2	In the ***Calibration*** dialog box, select ***pH*** from the ***Monitor to calibrate*** drop-down list.
3	Click the ***Prepare for calibration*** button. *Result:* The pH valve switches to the calibration position.
4	Enter the pH of the first pH standard buffer in the ***pH for buffer 1*** field.

Step	Action
5	Fill a syringe with approximately 10 mL of the first pH standard buffer. Connect the syringe to the Luer connector of pH valve port **Cal**, and inject the buffer. 
6	When the ***Current value*** is stable, click the ***Calibrate*** button.
7	Wash the pH flow cell by injecting water into pH valve port **Cal** using a new syringe.
8	Enter the pH of the second pH standard buffer in the ***pH for buffer 2*** field.
9	Repeat steps 5-7 using the second pH standard buffer. *Result:* The calibration date and time are displayed in the dialog box, along with values for ***Calibrated electrode slope*** and ***Asymmetry potential at pH 7***.
10	• If the ***Calibrated electrode slope*** ≥ 80% and the ***Asymmetry potential at pH 7*** is inside the interval ± 60 mV, click the ***Close*** button to switch the pH valve back to the default position and to close the ***Calibration*** dialog box. • If not, clean the pH electrode and repeat the calibration procedure. If this does not help, replace the electrode.

7.3.2 Calibrate the pressure monitors

Maintenance interval

ÄKTA pure can have up to four pressure monitors:

- System pump pressure monitor
- Sample pump pressure monitor
- Pre-column pressure monitor
- Post-column pressure monitor

Check the pressure monitors every week, or when the ambient temperature has changed by more than ± 5°C. Calibrate the pressure monitor if the zero pressure reading is outside the range ± 0.02 MPa.

Check the monitors

Follow the steps below to check the pressure monitors. The procedure is the same for each monitor.

Step	Action
1	Disconnect the relevant tubing from the pressure monitor to obtain zero pressure, see table *Tubing and pressures, on page 352*.
2	Click the ***Customize*** icon to open the ***Customize*** dialog box. In the ***Customize*** dialog box, under the ***Run Data Groups*** tab, select the relevant pressure to display, see table *Tubing and pressures, on page 352*. Click ***OK*** to close the ***Customize*** dialog box.
3	In the ***Run Data*** pane in the ***System Control*** module, check what pressure is displayed.
4	If the zero pressure reading is outside the range ± 0.02 MPa, calibrate the pressure monitor according to the instruction below.

Calibrating the pressure sensors

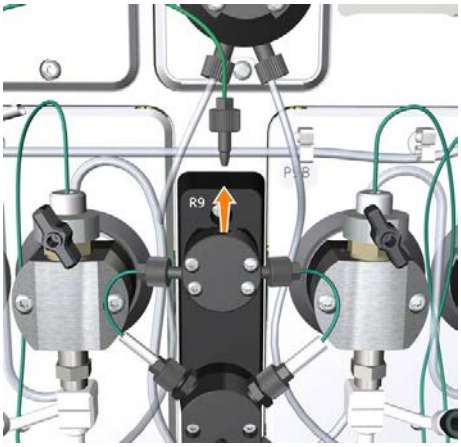
Follow the steps below to calibrate any of the pressure sensors.

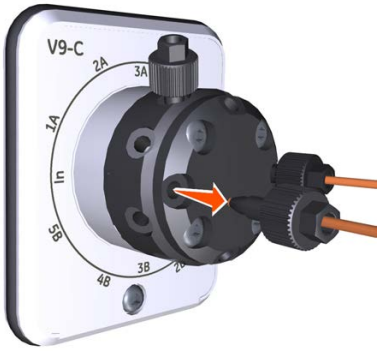
Step	Action
1	Disconnect the relevant tubing from the pressure monitor, see table *Tubing and pressures, on page 352*.
2	Open the ***System Control*** module and select ***System*** →***Calibrate***.
3	In the ***Calibration*** dialog box, select the pressure monitor to calibrate from the ***Monitor to calibrate*** drop-down list.

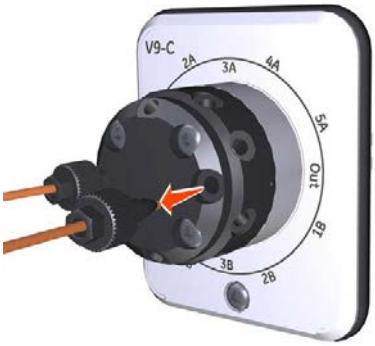
Step	Action
4	Click the ***Reset pressure*** button. *Result:* The atmospheric pressure is defined as zero. The date and time of the most recent calibration, and the current pressure value are displayed.
5	Reconnect the tubing to the pressure monitor.

Tubing and pressures

The table below shows the tubing to disconnect when checking and calibrating the pressure monitors. The UNICORN names of the pressures measured by the monitors are also shown.

Pressure monitor	Tubing to disconnect	Pressure in UNICORN
System pump pressure monitor	Tubing from the System pump pressure monitor 	***System pressure***

Pressure monitor	Tubing to disconnect	Pressure in UNICORN
Sample pump pressure monitor	Tubing from the Sample pump pressure monitor 	***Sample pressure***
Pre-column pressure monitor	Tubing from the Column valve port **In** (**V9-C**, **V9H-C** or **V9-C2**, **V9H-C2**) 	***PreC pressure***

Pressure monitor	Tubing to disconnect	Pressure in UNICORN
Post-column pressure monitor	Tubing from the Column valve port **Out** (**V9-C**, **V9H-C** or **V9-C2**, **V9H-C2**) 	***PostC pressure***

7.3.3 Calibrate the conductivity monitor

Introduction

Two types of calibrations can be performed:

Calibration type	Description
Factory calibration	Restores the conductivity cell constant to the factory default value.
User calibration	Calibrates the conductivity cell constant.

Both calibration types can be performed on primary and secondary conductivity monitors.

Maintenance interval

Recommended maintenance intervals for the two types of calibrations:

Calibration type	Maintenance interval
Factory calibration	Perform calibration to override an incorrect user calibration.
User calibration	The conductivity cell is factory calibrated, and should not require recalibration with normal use. Perform calibration when the signal is unstable or when the signal is suspected to be incorrect. It is also recommended to recalibrate the Conductivity monitor after cleaning.

Restoring factory default value

Follow the steps below to restore the conductivity cell constant to the factory default value.

Step	Action
1	Open the ***System Control*** module and select ***System*** →***Calibrate***.
2	In the ***Calibration*** dialog box, select the following from the ***Monitor to calibrate*** drop-down list:

Conductivity monitor	Select
Primary	***Conductivity monitor - factory calibration***
Secondary	***Conductivity monitor - factory calibration, 2nd***

Step	Action
	Result: The time for the new calibration and the current value are displayed.
3	Click ***Restore***. *Result:* The conductivity cell constant is restored to the factory default value. The conductivity cell constant is written on the packaging of the conductivity monitor.

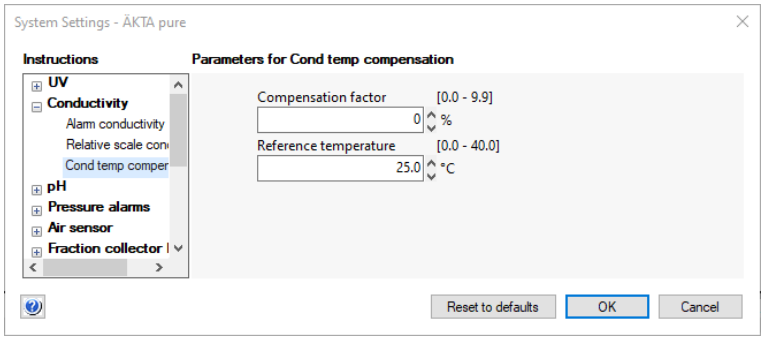
Calibrating the conductivity flow cell

Follow the steps below to calibrate the conductivity flow cell constant.

Step	Action
1	Make sure that the instrument has been switched on for at least one hour.
2	In the ***System Control*** module, select ***System*** →***Settings***.
3	**a.** In the ***System Settings*** dialog box, select the following:

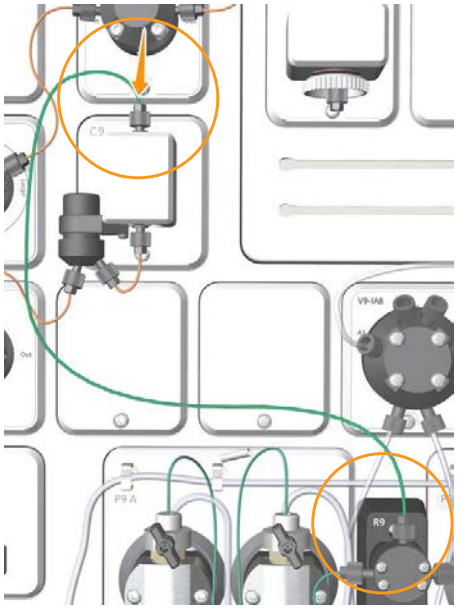
Conductivity monitor	Select
Primary	***Conductivity*** →***Cond temp compensation***
Secondary	***Conductivity*** →***Cond temp compensation, 2nd***

b. Set the ***Compensation factor*** to 0%, and click ***OK***.



4	Wash the whole flow path and fill it with deionized water using a suitable inlet and the System pump, until the conductivity value reaches 0.00 mS/cm.

Step	Action
5	Connect the tubing (labelled 3) from the system pump pressure monitor directly to the conductivity cell inlet, by-passing the mixer, injection valve, column valve and UV monitor, see image below. Make sure that the flow restrictor is inline.



6	Prime and purge the inlet used in step 4 with the 1.00 M NaCl calibration solution. Fill the conductivity cell with the calibration solution at 1 mL/min. Pump in at least 15 mL of the calibration solution and wait until the conductivity signal and the temperature have stabilized before continuing the calibration. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
7	Under continuous flow, read the current conductivity temperature from ***Run Data*** with an accuracy of one decimal place.
8	In the ***Calibration*** dialog box, select the following from the ***Monitor to calibrate*** drop-down list:

Conductivity monitor	Select
Primary	***Conductivity monitor - user calibration***
Secondary	***Conductivity monitor - user calibration, 2nd***

Step	Action

9 In the ***Run Data*** pane of ***System Control***, read the current value for:

Conductivity monitor	Read
Primary	***Cond temp***
Secondary	***Cond temp, 2nd***

Tip:

*If **Cond temp** and/or **Cond temp, 2nd** is not showing, click the **Customize** icon. In the **Customize** dialog box, under the **Run Data Groups** tab, select to view **Cond temp** and/or **Cond temp, 2nd**.*

10 In the ***Calibration*** dialog box, enter the theoretical conductivity value at the current conductivity temperature in the ***Enter theoretical conductivity value*** input field.

- If a certified conductivity standard solution is used, use the supplied theoretical conductivity value.
- If a manually prepared 1.00 M NaCl calibration solution is used, see the graph for conductivity value at the current temperature *Graph for conductivity value, on page 359*.

11 In the ***Calibration*** dialog box, click ***Calibrate***.

Result:

The new conductivity cell constant is displayed in the ***Conductivity cell 1 constant/cm*** box. The new constant is normally 40 ± 10 cm^{-1}. The date and time for the calibration are displayed.

12 In the ***System Control*** toolbar, click the ***End*** icon to end the run.

13 **a.** In the ***System Settings*** dialog box, select the following:

Conductivity monitor	Select
Primary	***Conductivity →Cond temp compensation***.
Secondary	***Conductivity →Cond temp compensation, 2nd***.

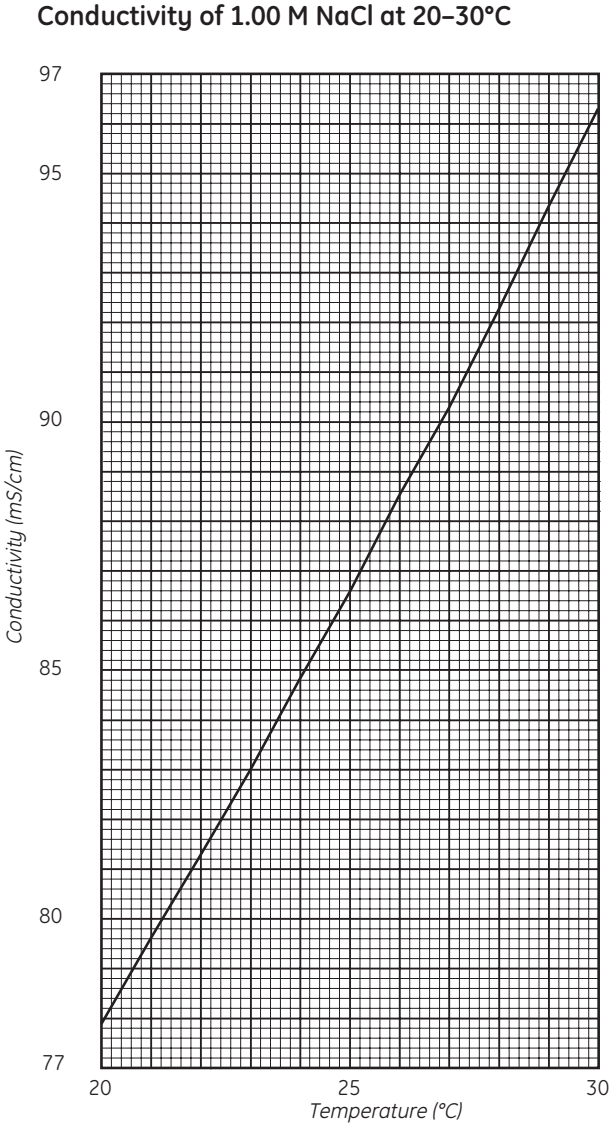
b. Set the ***Compensation factor*** to the desired value, default 2%, and click ***OK***.

Note:

When using NaCl, the optimal compensation factor is 2.1%.

Graph for conductivity value

The graph below shows the conductivity value at the current temperature when 1.00 M NaCl calibration solution is used.



7.3.4 Calibrate the UV monitors

Automatic calibration of the UV monitor U9-M

The wavelength is automatically calibrated every time the instrument is switched on. If the instrument has been switched on for a couple of days, and the ambient temperature and/or the humidity has changed, restart the instrument using the power switch to calibrate the UV monitor.

The cell length is factory calibrated and the value is stored in the cell. This value is automatically read and transferred to UNICORN.

Calibration of the UV monitor U9-T and U9-L flow cell length

The path length in the UV flow cell of the primary or the secondary UV monitor might differ from the nominal length, which leads to incorrect results in the calculation of protein concentration in the eluate. To achieve a normalized absorbance, the path length in the UV flow cell must be calibrated. The calibration procedure that is described below is the same for both the UV flow cell of the primary and the secondary UV monitor, unless otherwise stated.

Note: *The flow cell path length must be registered or updated in UNICORN, when the flow cell is replaced.*

Required material

- UV Test accessories (29293950)
- Waste beaker
- UV Test Kit, 1 and 2 mm (29276997) or UV Test Kit, 5 and 10 mm (29276998)
- UV Test Calculation protocol (29032336)
- UV Test Kit Instructions (29304919)

Calibration test kit and calculation protocol

To perform the calibration, a calibration kit containing test solutions, syringes and accessories is needed.

Theoretical path length of UV flow cell	Calibration kit
0.4, 1 and 2 mm	UV Test Kit, 1 and 2 mm (product code 29276997)
5 and 10 mm	UV Test Kit, 5 and 10 mm (product code 29276998)

The *UV Test Kit Calculation Protocol (29032336)* and *UV test kit instructions (29304919)* can be downloaded from *cytiva.com*. Search for the product code of the suitable calibration kit as indicated above, and click on ***Related documents*** to access the documents.

Preparing for calibration

Follow the steps below to prepare for the calibration of the UV monitor **U9-T** and **U9-L**.

Step	Action
1	Make sure that the flow restrictor is inline in the flow path after the UV flow cell.
2	Mount the union Luer female/1/16" male, included in the test kit, in the upper inlet of the UV flow cell.
3	Open the *UV Test Kit Calculation Protocol (29032336)* file.
4	The solution bottles are labelled with the concentration value and the reference absorbance value for each solution. Enter the concentrations of the solutions in ascending order into the column ***UV Test Kit, Concentration Fe(III)SULF (mg/L)***. Enter the corresponding absorbance values into the column ***UV Test Kit, Certificate Absorbance (AU/cm)***.

Performing the calibration

Follow the steps below to calibrate the UV monitor **U9-T** and **U9-L**.

Step	Action
1	In the ***System Control*** module, select ***Manual*** →***Execute Manual Instructions***.
2	In the ***Manual Instructions*** dialog box, select ***Pumps*** →***System flow*** and set the ***Flow rate*** to 0.0 mL/min.
3	Click ***Execute***. *Result:* The absorbance can now be monitored.
4	Fill one of the supplied syringes with 1.5 to 2 mL of the first solution (0 mg/L). Make sure that there are no air bubbles in the syringe.

Step	Action
5	Fit the syringe in the union Luer connector and inject the solution. Do not remove the syringe.



Note:

Air trapped in the UV cell causes inaccurate measurements. To avoid introducing air into the UV cell, gently fill the union Luer up to the edge with test solution that is to be introduced, using the syringe. Then insert the syringe into the union Luer.

6	Wait until the monitored absorbance value has stabilized.
7	**a.** In the ***Manual Instructions*** dialog box: • For the primary UV monitor: Select ***Monitors →Auto zero UV***. • For the secondary UV monitor: Select ***Monitors →Auto zero UV 2nd***. **b.** Click ***Execute***. *Result:* The UV absorbance is set to zero.
8	Remove the syringe.
9	Repeat the injections with the remaining four test solutions in increasing concentration order. Use a new syringe for each solution.
10	After each injection, wait for a stable absorbance value. Write down the measured absorbance values for each solution.
11	Enter the measured absorbance values into the table in the column ***UV Monitor, Measured Absorbance (AU)***. ***Note:*** *The values must be converted from mAU to AU.*

Step	Action
12	Read the ***Real cell length in mm*** and ***Linearity Deviation*** when all absorbance values have been entered in the table. The values are shown at the bottom of the table. ***Note:*** *The **Coefficient of regression (R^2)** should be larger than 0.999. If this is not the case, one or more measured values are faulty.*

Updating the cell path length

Follow the steps below to define the ***UV cell path length*** or the ***UV 2nd cell path length***. The flow cell path length must be updated when the flow cell has been replaced or calibrated.

Step	Action
1	In the ***System Control*** module, select ***System*** →***Calibration***.
2	In the ***Calibration*** dialog box, select one of the following options from the ***Monitor to calibrate*** drop-down list: • ***UV cell path length*** or • ***UV 2nd cell path length***
3	Enter the nominal flow cell path length in the ***Nominal length*** input field and click ***Set***.
4	<table><tr><th>Calibration performed?</th><th>Action</th></tr><tr><td>Yes</td><td>Enter the calculated flow cell path length, obtained in the calibration procedure, in the ***Real length*** input field and click ***Set***.</td></tr><tr><td>No</td><td>Enter the nominal flow cell path length in the ***Real length*** input field and click ***Set***.</td></tr></table> *Result:* The UV flow cell path length is updated.

7.4 Cleaning procedures

Introduction

This section gives instructions for cleaning procedures.

In this section

Section		See page
7.4.1	Clean the instrument externally	365
7.4.2	Perform System CIP	366
7.4.3	Perform Column CIP	377
7.4.4	Clean Fraction collector F9-C	381
7.4.5	Clean the Fraction collector F9-C sensors	383
7.4.6	Clean Fraction collector F9-R	385
7.4.7	Clean Fraction collector F9-T	386
7.4.8	Clean the Fraction collector F9-T drop sync sensor	388
7.4.9	Clean the pH electrode	389
7.4.10	Clean the UV flow cell	392
7.4.11	Clean the conductivity flow cell	395
7.4.12	Clean the pump head check valves	397
7.4.13	Wipe off excess oil from the pump head	400

7.4.1 Clean the instrument externally

Maintenance interval

Clean the the instrument externally when required. Do not allow spilled liquid to dry on the instrument.

Required material

The following materials are required:

- Cloth
- Mild cleaning agent or 20% ethanol

Cleaning the instrument

Follow the steps below to clean the instrument externally.

Step	Action
1	Check that no run is in progress.
2	Switch off the instrument.
3	Wipe the surface with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol. Wipe off any excess.
4	Let the instrument dry completely before using it.

7.4.2 Perform System CIP

Introduction

The ***System CIP*** method is used to fill the instrument and the selected inlets and outlets with cleaning solution.



WARNING

Hazardous biological agents during run. When using hazardous biological agents, perform a system cleaning to flush the entire system tubing with storage solution (e.g. NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.



CAUTION

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation, maintenance and decommissioning of the equipment.



CAUTION

Explosion hazard if flammable liquid leaks during cleaning of the flow path. When cleaning the flow path of Fraction collector F9-C with a flammable liquid like ethanol, carefully inspect the flow path, including the waste tubing, to make sure there will be no leakage.

Tip: *If hazardous chemicals are used for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.*

Maintenance interval

Perform a System cleaning in place (System CIP) when required, for example between runs where different samples and buffers are used. This is important to prevent cross-contamination and bacterial growth in the instrument.

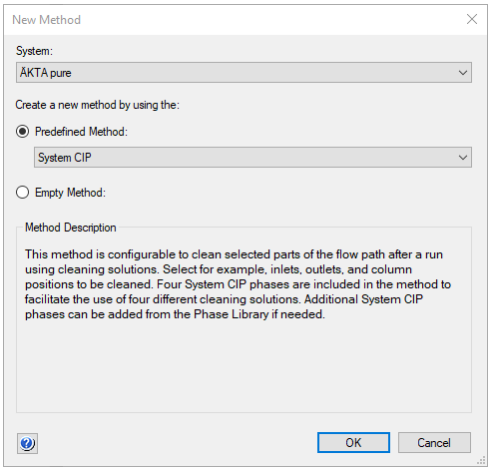
Required material

The following materials are required:

- Appropriate cleaning solutions (such as NaOH, buffer solution, or distilled water)
- Syringe, 25 to 30 mL

Creating a System CIP method

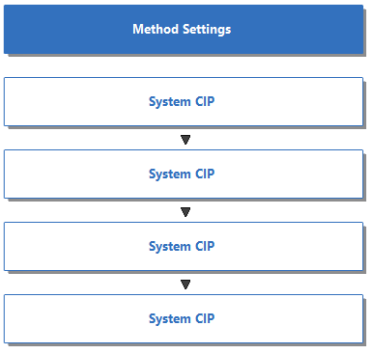
Follow the steps below to create a ***System CIP*** method.

Step	Action
1	In the ***Method Editor*** module, • Click the ***New Method*** icon  or • Select ***File*** →***New Method***.
2	In the ***New Method*** dialog box, select ***System*** and then ***System CIP*** in the ***Predefined Method*** drop-down list. Click ***OK***. 

Step	Action

Result:

One ***Method Settings*** phase and four ***System CIP*** phases show in the ***Method Outline*** pane. Each ***System CIP*** phase uses one cleaning solution.



3 If desired, add additional ***System CIP*** phases to the method using the ***Phase Library***.

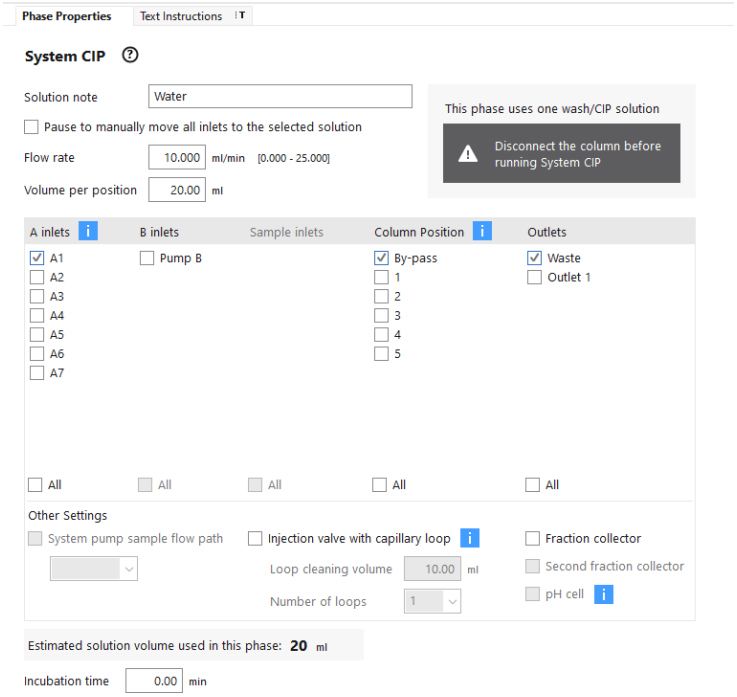
4 In the ***Phase Properties*** tab of each of the ***System CIP*** phases:

a. Type a comment in the ***Solution note*** field (optional).

b. Select values for ***Flow rate***, ***Volume per position*** and ***Incubation time***.

Step	Action

c. Define the extent of cleaning by checking the check boxes.



Note:
For complete cleaning of the Injection valve, see Cleaning Injection valve V9-Inj or V9H-Inj, on page 371 or Cleaning Injection valve V9M-J, on page 372).

Note:
Replace the pH electrode with a dummy electrode when including pH cleaning in the System CIP. Continued contact with 1 M NaOH shortens the life time of the pH electrode.

Note:
If the fraction collector is included, a lower flow rate might need to be set in the instruction ***Fraction collector wash settings****.*

Note:
Make sure not to exceed the pressure limits for any of the modules that are part of the flow path during the CIP.

5 In the ***Method Editor*** module,

Step	Action
	• Click the ***Save the method*** icon  or • Select ***File*** →***Save As***
6	In the ***Save As*** dialog box: **a.** Select a target folder to enable the ***Save*** button. **b.** Type a ***Name*** for the method. **c.** Select a ***System*** from the list. **d.** Click ***Save***.

Performing a System CIP

Follow the steps below to run a ***System CIP*** method.

Step	Action
1	In the ***Method Editor*** module, create a ***System CIP*** method according to the instructions above.
2	Connect bypass tubing to all selected column positions and loop positions, if a loop valve is used.
3	Prepare cleaning solutions and immerse the selected inlet tubing in the solutions. ***Note:*** *Each phase uses one solution. All inlets selected in one phase must be immersed in the same cleaning solution.*
4	In the ***System Control*** module, select the created method and start the run.
5	For complete cleaning of the flow path, clean the manual injection port of the Injection valve and the pH valve manually, see the instructions below.

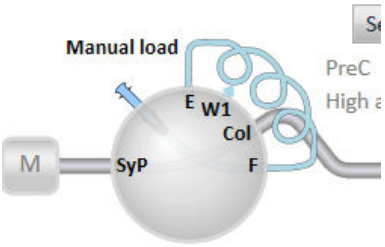
Cleaning Injection valve V9-Inj or V9H-Inj

Follow the steps below to clean Injection valve **V9-Inj** or **V9H-Inj**, and the sample loop.

Step	Action
1	Fill a syringe with wash solution, such as the buffer solution used during the run.
2	Connect the syringe to the Injection valve port **Syr**.

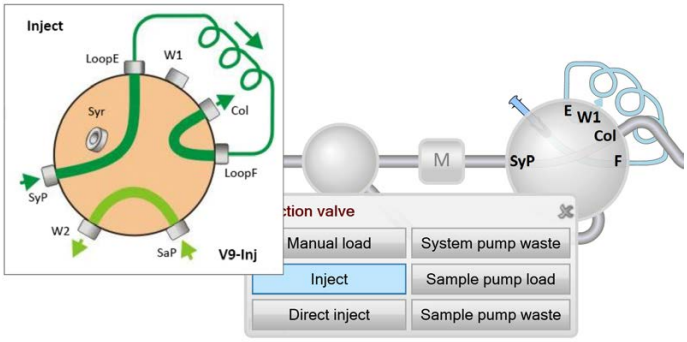


3	In the ***Process Picture***, check that ***Manual load*** is selected for the injection valve.



4	Inject the wash solution into the sample loop. *Result:* The injection valve and sample loop are filled with wash solution, and any excess will leave the injection valve through port **W1**.

Step	Action
5	In the ***Process Picture***: • Click on the ***Injection valve*** and select ***Inject***.



6	Make sure that the used inlet is placed in wash solution.
7	Set a suitable flow rate.
8	When the UV signal and conductivity signal stabilizes, click ***End***.

The injection valve **V9-Inj** or **V9H-Inj** and the sample loop are ready for the next sample application. See *Section 7.4.2 Perform System CIP, on page 366* and *Section 7.4.3 Perform Column CIP, on page 377* for cleaning the system and column between runs.

Cleaning Injection valve V9M-J

Follow the steps below to clean Injection valve **V9M-J**, and the sample loop.

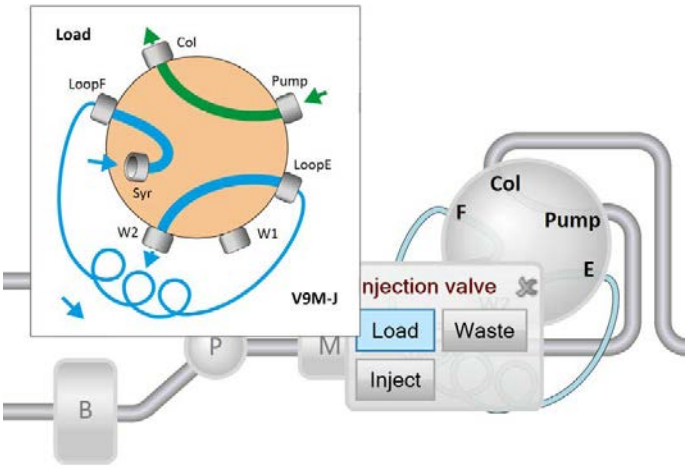
Step	Action
1	Fill a syringe with wash solution, such as the buffer solution used during the run.

Step	Action
2	Connect the syringe to the Injection valve port **Syr**.



3 In the ***Process Picture***:

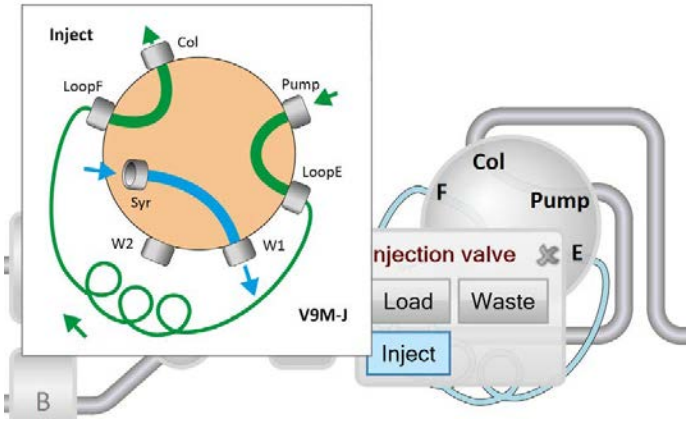
- Click on the ***Injection valve*** and check that ***Load*** is selected.



4 Inject wash solution into the sample loop.

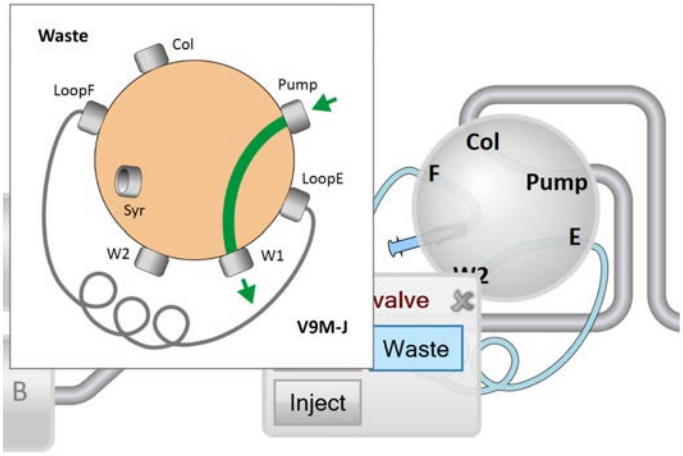
Result:
The injection valve and sample loop are filled with wash solution, and any excess will leave the injection valve through port **W2**.

Step	Action
5	In the ***Process Picture***: • Click on the ***Injection valve*** and select ***Inject***.



6	Inject wash solution into the injection valve. *Result:* The injection valve is filled with wash solution, and any excess will leave the valve through port **W1**.
7	Make sure that the used inlet is placed in wash solution.
8	Set a suitable flow rate.
9	When the UV signal and conductivity signal stabilizes, click ***End***.
10	In the ***Process Picture***: • Click on the ***Injection valve*** and select ***Waste***.

Step	Action



11	Set a suitable flow rate.
12	When the UV signal and conductivity signal stabilizes, click ***End***.

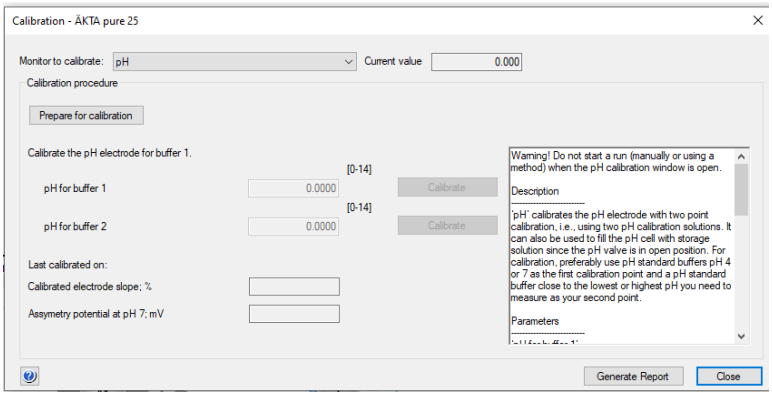
Injection valve **V9M-J** and the sample loop are ready for the next sample application. See *Section 7.4.2 Perform System CIP, on page 366* and *Section 7.4.3 Perform Column CIP, on page 377* for cleaning the system and column between runs.

Cleaning the pH valve

Follow the steps below to clean the pH valve. The calibration function is used to switch the valve position. However, no calibration is performed.

Step	Action
1	Connect tubing to pH valve port **W3**, and direct the other end of this tubing to a waste container.
2	Unscrew the pH electrode from the pH valve, and replace it with the dummy electrode.

Step	Action
3	Open the ***System Control*** module and select ***System*** →***Calibrate***.



4	In the ***Calibration*** dialog box, select ***pH*** from the ***Monitor to calibrate*** drop-down list.
5	Press the ***Prepare for calibration*** button. *Result:* The pH valve switches to the calibration position.
6	Fill a syringe with approximately 10 mL of 1 M NaOH. Connect the syringe to the pH valve port **Cal**, and inject the NaOH solution.
7	Disconnect the syringe.
8	Fill a syringe with distilled water. Connect the syringe to the pH valve port **Cal**, and inject the distilled water.
9	Press the ***Close*** button. *Result:* The pH valve switches back to the default position and the ***Calibration*** dialog box closes. No calibration is performed.
10	Disconnect the syringe.

For short-term storage, keep the pH electrode in the pH valve. Make sure that the pH electrode is hydrated in storage solution (1:1 mixture of standard buffer, pH 4, and 1 M KNO_3).

For long-term storage, disconnect the pH electrode from the pH valve and put it in the plastic end-cover. Fill the plastic end-cover with storage solution.

See *Section 7.6.3 Storage of the pH electrode, on page 451*.

7.4.3 Perform Column CIP

Maintenance interval

Perform a Column cleaning in place (Column CIP) when required, for example between runs where different samples are used.

Required material

The following materials are required:

- Appropriate cleaning solutions. Please refer to the instructions for use of the column.

Introduction

The ***Column CIP*** method is used to clean the column after purification runs, to remove non-specifically bound proteins and to minimize the risk for carry-over between different purification runs.



WARNING

Hazardous biological agents during run. When using hazardous biological agents, perform a system cleaning to flush the entire system tubing with storage solution (e.g. NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.



CAUTION

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation, maintenance and decommissioning of the equipment.

Tip: *If hazardous chemicals are used for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.*

Creating a Column CIP method

Follow the steps below to create a ***Column CIP*** method.

Step	Action
1	In the ***Method Editor*** module,

Step	Action

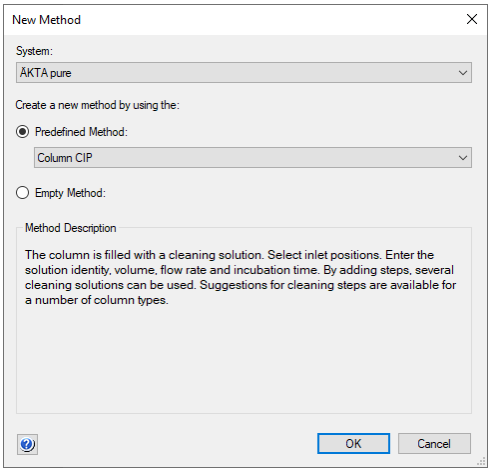
- Click the ***New Method*** icon



or

- Select ***File*** →***New Method***.

2 In the ***New Method*** dialog box, select ***System*** and then ***Predefined Method*** and ***Column CIP***. Click ***OK***.



Result:

One ***Method Settings*** phase and one ***Column CIP*** phase will be displayed in the ***Method Outline*** pane.

3 In the ***Phase Properties*** tab of the ***Method Settings*** phase, select ***Column type*** and ***Column position***.

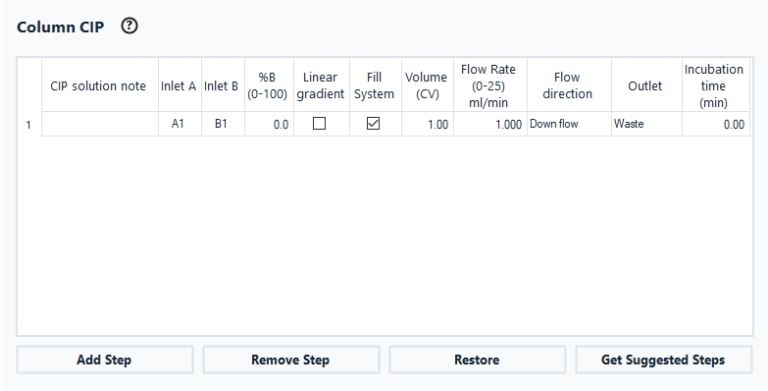
4 In the ***Phase Properties*** tab of the ***Column CIP*** phase:

a. Click ***Add Step*** to add a step.

b. Select the step and click ***Remove Step*** to remove a step.

Step	Action

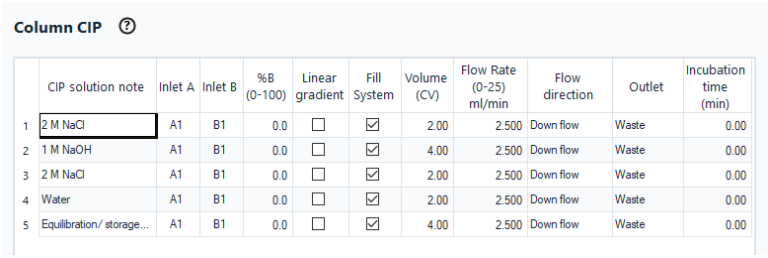
c. To enter a value, select the cell and enter or select a new value.



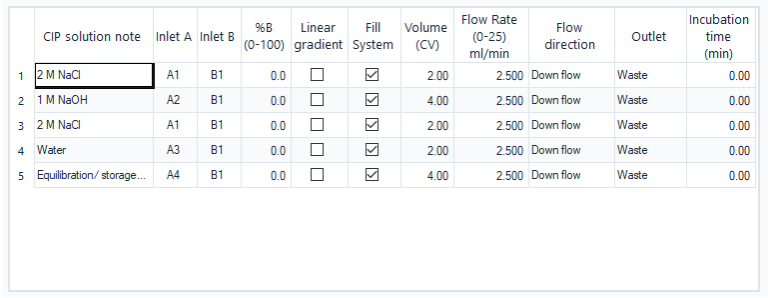
5 In the ***Phase Properties*** tab of the ***Column CIP*** phase, click the ***Get Suggested Steps*** button to get a suggested procedure for the selected column type. Note that this function is not available for all column types.

Result:

Suggested cleaning steps for the selected column type are displayed.



6 If several cleaning solutions are used, change settings for ***Inlet A*** and/or ***Inlet B*** . Select one inlet for each solution. If Inlet B is used, remember to edit the values in the **%B** column.



Step	Action
7	In the ***Method Editor*** module, • Click the ***Save the method*** icon  or • Select ***File*** →***Save As***
8	In the ***Save As*** dialog box: **a.** Select a target folder to enable the ***Save*** button. **b.** Type a ***Name*** for the method. **c.** Select a ***System*** from the list. **d.** Click the ***Save*** button.

Performing a Column CIP

Follow the steps below to run a ***Column CIP*** method.

Step	Action
1	In the ***Method Editor*** module, create a ***Column CIP*** method according to the instruction above.
2	Prepare cleaning solutions and immerse the selected inlets in the solutions.
3	Connect the column to the selected column position.
4	In the ***System Control*** module, select the created method and start the run.

7.4.4 Clean Fraction collector F9-C

Maintenance interval

Clean the Fraction collector F9-C when required, for example if liquid has been spilled in the Fraction collector chamber. The internal tubing of the fraction collector may need to be replaced for maintenance or for process purposes. For information on how and when to replace the internal tubing, see *Section 7.5.2 Replace internal tubing in Fraction collector F9-C, on page 404*.

Clean the ***Accumulator*** of the Fraction collector F9-C weekly or monthly, to prevent salt precipitation in the fraction collector. Add a wash of the ***Accumulator*** in the ***Equilibration*** phase of a method. For more information, see *Section 9.7.3 Predefined phases, on page 565*.

Required material

The following materials are required:

- Wash bottle
- Water or 20% ethanol
- Cloth

Cleaning the Fraction collector F9-C

Follow the steps below to clean the interior of the Fraction collector F9-C. The locations of the components of the fraction collector are shown in *Section 3.2.2 Fraction collector F9-C illustrations, on page 125*.

Step	Action
1	Perform a fraction collector wash: In ***System Control***, select ***Manual*** →***Execute Manual Instructions*** →***Fraction collector F9-C*** →***Fraction collector wash***. Click ***Execute***.

NOTICE

If no column valve is used, make sure to replace any columns in the flow path with tubing before a fraction collector wash is performed.

NOTICE

Lower the flow for the fraction collector wash in the instruction ***Fraction collector wash settings*** if the system back pressure is elevated during the wash.

Step	Action
	NOTICE Make sure not to exceed the pressure limits for any of the modules that are part of the flow path.
2	In ***System Control***, select ***Manual*** →***Execute Manual Instructions*** →***Fraction collector F9-C*** →***Frac cleaning position***. Click ***Execute***. *Result:* The Dispenser head moves to cleaning position, and the Instrument display states ***System pause***.
3	Open the door of the fraction collector and remove the rack.
4	Wash the Cassette tray or rack and the Cassettes (if applicable), with water and a mild cleaning agent.
5	Check if there is liquid in the Waste funnel. If this is the case, follow the steps below: **a.** Check if the waste tubing is twisted or bent, if so, replace the waste tubing. **b.** Check if the waste tubing is below the liquid surface. If so, empty the waste vessel and make sure the tubing is at the top of the waste vessel.
6	Lift off the Waste funnel and wash it with water and a mild cleaning agent. Refit the Waste funnel.
7	Wipe off the interior of the fraction collector using a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol.
8	Wipe off the Dispenser head and its diode windows (the Drop sync sensor and the Type code reader) using a wash bottle with water or 20% ethanol and a cloth.
9	Let the fraction collector dry completely before starting a run.
10	Close the door of the fraction collector. *Result:* Automatic scanning is performed.
11	In the System Control module, press the ***End*** icon in the toolbar. *Result:* The Dispenser head moves to home position.

7.4.5 Clean the Fraction collector F9-C sensors

Maintenance interval

Clean the sensors of the fraction collector F9-C every week, or when required, for example if the fraction collector fails to read the tray ID or do not collect fractions correctly.

Required material

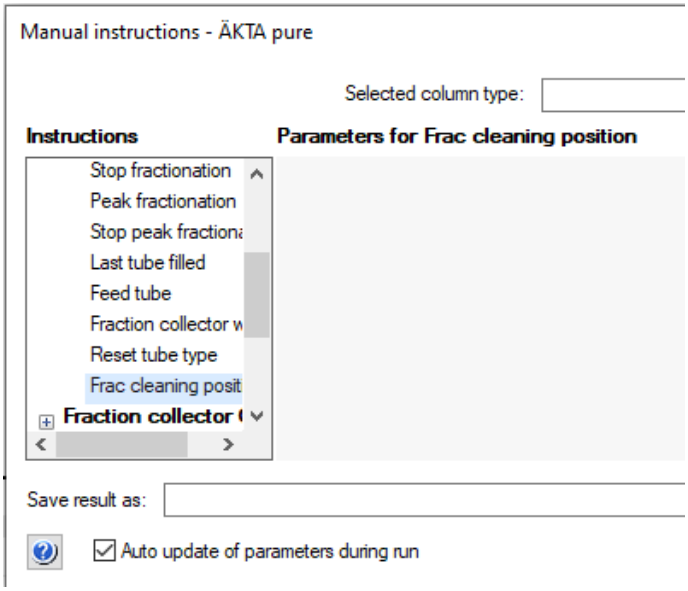
The following material is required:

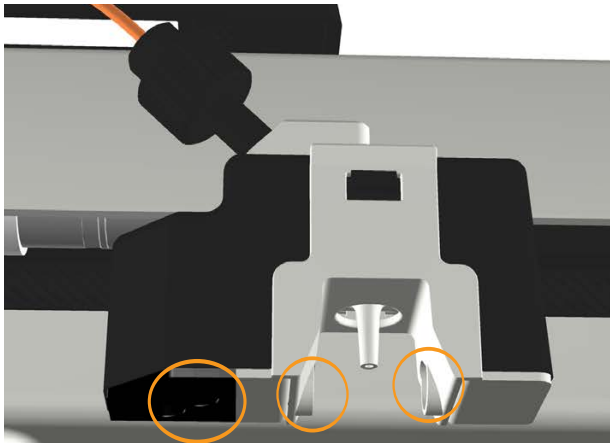
- Wash bottle
- Water, mild cleaning agent or 20% ethanol.
- Cloth

Instructions

Follow the steps below to clean the fraction collector diodes. See *Section 3.2.2 Fraction collector F9-C illustrations, on page 125* for the locations of the components of the fraction collector.

Step	Action
1	In ***System Control***, select ***Manual*** →***Execute Manual Instructions*** →***Fraction collector F9-C*** →***Frac cleaning position***. Click ***Execute***.



Step	Action
	Result: The Dispenser head moves to cleaning position, and the Instrument display states ***System pause***.
2	Wipe off the Dispenser head, the Drop sync, and the Type code reader sensor windows using a wash bottle with water or 20% ethanol, and a cloth. 
3	Let the Dispenser head dry completely before starting a run.
4	Close the fraction collector door. *Result:* Automatic scanning is performed.
5	In the System Control module, press the ***End*** icon in the toolbar.  *Result:* The Dispenser head moves to home position, and the Instrument display states ***Ready***.

7.4.6 Clean Fraction collector F9-R

Maintenance interval

Clean the Fraction collector when required, for example in case of liquid spill.

Required material

The following materials are required:

- Water or 20% ethanol
- Cloth

Cleaning the Fraction collector F9-R

Follow the steps below to clean the Fraction collector F9-R externally.

Step	Action
1	Check that no run is in progress.
2	Switch off the instrument.
3	Wipe the surface with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol. Wipe off any excess.
4	Let the Fraction collector F9-R dry completely before restart.

Cleaning the DropSync sensor

Clean the drop sensor photocell located above the tube sensor (see *Front view illustration, on page 137*) with a damp cloth.

7.4.7 Clean Fraction collector F9-T

Maintenance interval

Clean the sensors weekly, and the exterior of Fraction collector F9-T when necessary. Do not allow spilled liquid to dry on the Fraction collector.

Required material

The following materials are required:

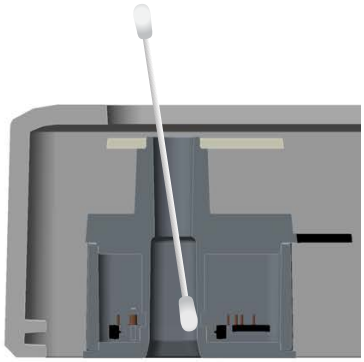
- Cloth
- Water, mild cleaning agent or 20% ethanol
- Wash bottle

Cleaning the Fraction collector F9-T

Follow the steps below to clean the fraction collector F9-T externally.

Step	Action
1	Check that no run is in progress.
2	Turn off the ÄKTA pure instrument.
3	Remove the tube rack by pressing the wedge and pulling the rack out. Discard any remaining tubes and wash the tube rack with mild cleaning agent or 20% ethanol.
	
4	Wipe the surface of the Fraction collector with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol. Remember to wipe all sides, including the underside of the fractionation arm. Wipe off any excess of liquid.
5	Lift out the nozzle.

Step	Action
6	Wet a cotton swab lightly with water and clean the hole through the fractionation arm. Make sure to clean the area around the Drop sync sensor thoroughly.



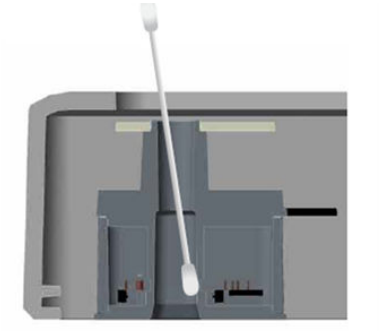
7	Insert the nozzle into the fractionation arm.
8	When the tube rack is dry, add a 15 mL tube and put it back into position.
9	Let the Fraction collector dry completely before restart.

7.4.8 Clean the Fraction collector F9-T drop sync sensor

Clean the fraction collector sensors every week. In addition, clean them when required, for example if the fraction collector fails to detect drops or do not collect fractions correctly. For instructions on how to clean the Fraction collector F9-T, see *Section 7.4.7 Clean Fraction collector F9-T, on page 386*.

Instructions

Follow the steps below to clean the drop sync sensor.

Step	Action
1	Make sure that no run is in progress.
2	Turn off the instrument.
3	Remove the tube rack by pressing the wedge and pulling the rack out.
4	Lift out the nozzle.
5	Wet a cotton swab lightly with water.
6	Clean the hole through the fraction collector arm with the damp cotton swab. Be extra thorough around the Drop sync sensor. 
7	Insert the nozzle into the fraction collector arm.
8	Put the tube rack back into position.

7.4.9 Clean the pH electrode

Maintenance interval

Clean the pH electrode when required. The pH electrode can be cleaned either when it is installed in the pH valve or when it has been removed. The pH electrode has a limited longevity and must be replaced every six months or when the response time is slow, see *Section 7.5.3 Replace the pH electrode, on page 407*. After cleaning has been performed, re-calibrate the pH monitor, see *Section 7.3.1 Calibrate the pH monitor, on page 349*.

Required material

The following materials are required:

- Syringe, 25 to 30 mL
- Distilled water
- 0.1 M HCl and 0.1 M NaOH

  or
- Liquid detergent

  or
- 1% pepsin solution in 0.1 M HCl

  or
- 1 M KNO_3

Cleaning salt precipitate

Dissolve the precipitated salt by immersing the electrode for a five minute period in each of the following solutions:

- 0.1 M HCl
- 0.1 M NaOH
- 0.1 M HCl

Rinse the electrode tip in distilled water between each solution.

Cleaning oil or grease layers

Wash the electrode tip in liquid detergent and water. If the oil or grease layers are known to be soluble in a particular organic solvent, wash with this solvent. Rinse the electrode tip in distilled water.

Cleaning protein precipitate

Dissolve the precipitated protein by immersing the electrode in a solution of 1% pepsin in 0.1 M HCl for five minutes, followed by thorough rinsing with distilled water.

If these procedures fail to rejuvenate the electrode, try the following procedure.

NOTICE

This procedure can only be performed when the pH electrode is not installed in the pH valve.

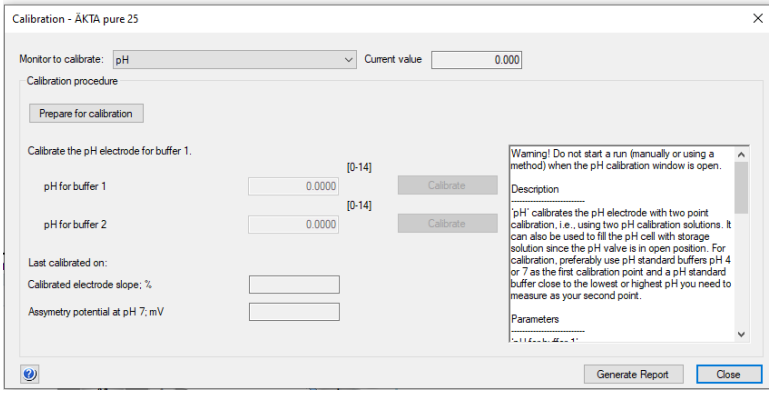
Step	Action
1	Heat a 1 M KNO_3 solution to 60°C to 80°C.
2	Place the electrode tip in the heated KNO_3 solution.
3	Allow the electrode to cool while immersed in the KNO_3 solution before re-testing.

If these steps fail to improve the electrode, replace the electrode, see *Section 7.5.3 Replace the pH electrode, on page 407*.

Cleaning the pH electrode

Follow the steps below to clean the pH electrode installed in the pH valve. The calibration function is used to switch the position of the pH valve. However, no calibration is performed.

Step	Action
1	Open the ***System Control*** module and select ***System*** →***Calibrate***.



2	Set ***Monitor to calibrate*** by selecting ***pH*** from the list.
3	Press the ***Prepare for calibration*** button. *Result:* The pH valve switches to the calibration position.

Step	Action
4	Fill a syringe with approximately 10 mL of chosen cleaning solution. Connect the syringe to the pH valve port **Cal**. Inject the liquid and wait for 5 minutes. Disconnect the syringe.
	
5	If several cleaning solutions are to be used, repeat step 4 with distilled water and then with the next solution.
6	As the last step in the cleaning procedure: **a.** Fill a syringe with distilled water. **b.** Connect the syringe to the pH valve port **Cal**. **c.** Inject the water. **d.** Disconnect the syringe from the pH valve.
7	Press the ***Close*** button. *Result:* The pH valve switches back to the default position and the ***Calibration*** dialog box closes. No calibration is performed.

For long-term storage, disconnect the pH electrode from the pH valve and put it in the plastic end-cover. Fill the plastic end-cover with storage solution (a 1:1 mixture of pH 4 buffer and 1 M KNO_3 solution). For detailed instructions, see *Section 7.6.3 Storage of the pH electrode, on page 451*.

7.4.10 Clean the UV flow cell

Maintenance interval

Clean the UV flow cell every six months, or when required.

NOTICE

Keep UV flow cell clean. Do not allow solutions containing dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell, as damage to the flow cell may occur.

Required material

The following materials are required:

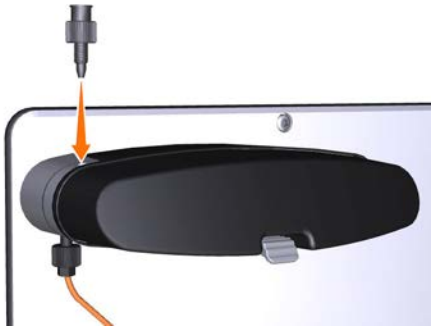
- Luer connector
- Waste container
- Syringe, 25 to 30 mL
- 10% surfactant detergent solution (e.g., Decon 90, Deconex 11, or RBS 25)
- Distilled water

Cleaning the UV flow cell

Follow the steps below to clean the UV flow cell for UV monitors **U9-M**, **U9-T**, or **U9-L**. The UV flow cell can be mounted or not mounted on the instrument during the cleaning procedure.

Note: *The illustrations in the instructions below show UV monitor* **U9-M***. UV monitor* **U9-T** *and* **U9-L** *are cleaned in a similar way.*

Step	Action
1	Disconnect the tubing from the top of the UV flow cell, and replace the fingertight connector with a Luer connector.



2	Disconnect the tubing from the bottom of the UV flow cell, and connect a piece of waste tubing to the UV flow cell. Insert the waste tubing into a waste container.
3	Fill a syringe with distilled water, and connect the syringe to the Luer connector.



4	Inject the distilled water through the UV flow cell in small amounts. Disconnect the syringe.
5	Fill a syringe with a 10% surfactant detergent solution, such as Decon™ 90, Deconex 11, RBS 25 or equivalent, and connect the syringe to the Luer connector.

Tip:

Heat the 10% surfactant detergent solution to 40°C to increase the cleaning effect.

Step	Action
6	Inject the detergent solution through the UV flow cell about five times.
7	Leave the detergent solution in the flow cell for at least 20 minutes.
8	Disconnect the syringe.
9	Fill a syringe with distilled water. Connect the syringe to the Luer connector.
10	Inject the distilled water into the UV flow cell to rinse the flow cell. Disconnect the syringe.
11	Disconnect the Luer connector from the top of the UV flow cell. Reconnect the piece of tubing from the Column valve to the top of the UV flow cell.
12	Disconnect the waste tubing from the bottom of the UV flow cell. Reconnect the piece of tubing from the Conductivity monitor to the bottom of the UV flow cell.

7.4.11 Clean the conductivity flow cell

Maintenance interval

Clean the conductivity flow cell when required.

Required material

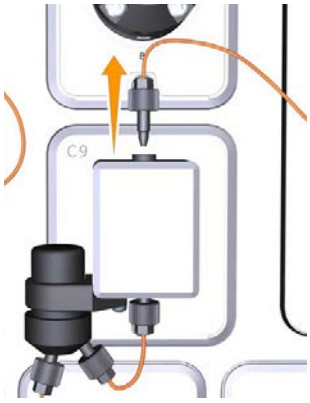
The following materials are required:

- Luer connector
- Waste container
- Syringe, 25 to 30 mL
- 1 M NaOH
- Distilled water

Cleaning the conductivity flow cell

Follow the steps below to clean the flow cell of the conductivity monitor.

Step	Action
1	Disconnect the fingertight connector and the piece of tubing from the top of the conductivity monitor, and attach a Luer connector.



Step	Action
2	Fill a syringe with distilled water, and connect the syringe to the Luer connector.



3	Disconnect the piece of tubing from the bottom of the conductivity monitor, and connect a piece of waste tubing to the conductivity monitor. Place the waste tubing in a waste container.
4	Inject distilled water through the conductivity flow cell in small amounts. Disconnect the syringe.
5	Fill a syringe with 1 M NaOH, and connect the syringe to the Luer connector.
6	Inject 1 M NaOH through the conductivity flow cell about five times.
7	Leave the liquid in the flow cell for at least 15 minutes.
8	Fill a syringe with distilled water. Connect the syringe to the Luer connector.
9	Inject the distilled water into the conductivity flow cell to rinse the flow cell. Disconnect the syringe.
10	Disconnect the Luer connector from the top of the conductivity flow cell, and reconnect the fingertight connectors with tubing.

7.4.12 Clean the pump head check valves

Maintenance interval

Clean the check valves when required, for example if solids in the check valve cause irregular or low flow. The cleaning procedure is the same for the system pumps and the sample pump.

Required material

The following materials are required:

- Adjustable wrench
- Methanol
- Distilled water
- Ultrasonic bath

Cleaning the pump head check valves



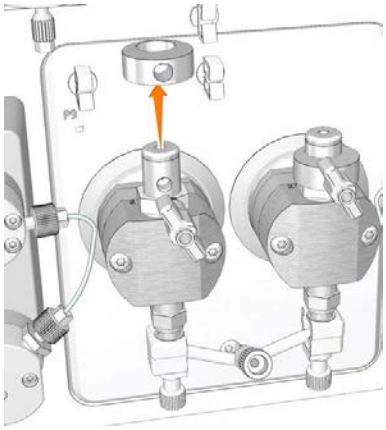
CAUTION

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation, maintenance and decommissioning of the equipment.

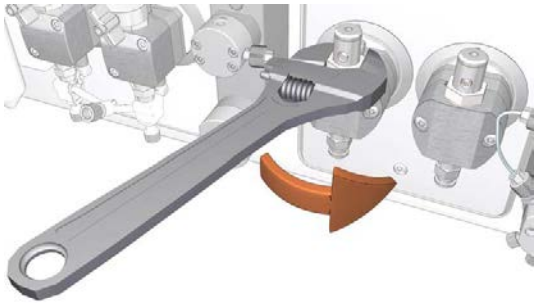
Follow the steps below to remove and clean the pump head check valves.

Step	Action
1	Switch off the instrument.
2	Disconnect the tubing from the pump head and disconnect the pump inlet tubing. Disconnect the tubing of the pump rinsing system.

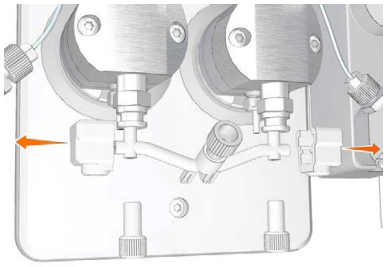
Step	Action
3	Unscrew the purge valve by turning it counter-clockwise, and lift off the metal ring.

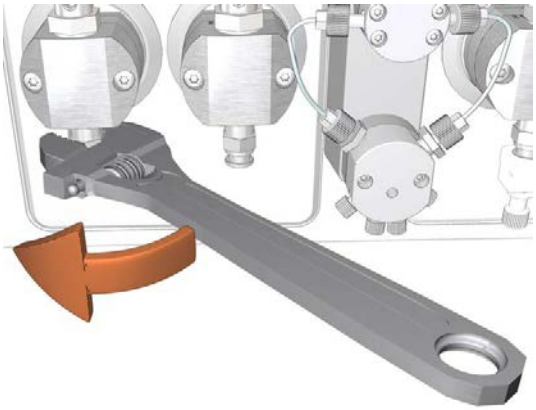


4	Unscrew the plastic nut of the upper check valve using an adjustable wrench, and gently lift off the upper check valve.



5	Unscrew the two white plastic screws located below each pump head. Pull the plastic connectors to the sides to release the inlet manifold.



Step	Action
6	Unscrew the lower check valve using an adjustable wrench. 
7	Immerse the valves completely in methanol and place them in an ultrasonic bath for a few minutes. Repeat the ultrasonic bath with deionized water.
8	Refit the check valves.
9	Tighten the nut until fully finger-tight and then use the adjustable wrench to tighten a further 90 degrees.
10	Refit the inlet manifold and reconnect the tubing to the pump head.

7.4.13 Wipe off excess oil from the pump head

Maintenance interval

During the first months of use it is normal that excess oil leaks out of the drain hole below the System pump. The function of the pump is not in any way affected by this.

Required material

The following materials are required:

- Cloth
- Mild cleaning agent or 20% ethanol

Cleaning the pump head

Follow the steps below to clean the System pumps externally.

Step	Action
1	Check that no run is in progress.
2	Switch off the instrument.
3	Wipe off the excess oil from the pump head with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol.
4	Let the pump dry completely before using the instrument.

7.5 Replacement procedures

Introduction

This section gives instructions for the replacement procedures to be performed by the user of ÄKTA pure.



WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

In this section

Section		See page
7.5.1	Replace tubing and connectors	402
7.5.2	Replace internal tubing in Fraction collector F9-C	404
7.5.3	Replace the pH electrode	407
7.5.4	Replace the mixer	408
7.5.5	Replace the O-ring inside the mixer	409
7.5.6	Replace the UV monitor U9-M flow cell	411
7.5.7	Replace the UV monitor U9-T and U9-L flow cell	414
7.5.8	Replace flow restrictor	416
7.5.9	Replace the online filter	417
7.5.10	Replace the inlet filters	418
7.5.11	Replace the pump head check valves	419
7.5.12	Replace pump piston seals of Pump P9 or P9H	422
7.5.13	Replace pump piston seals of Pump P9-S	431
7.5.14	Replace pump pistons	439
7.5.15	Replace pump rinsing system tubing	440

7.5.1 Replace tubing and connectors

Maintenance interval

Replace tubing and connectors when required, for example when a tubing has clogged or has been bent so that the flow is stopped.

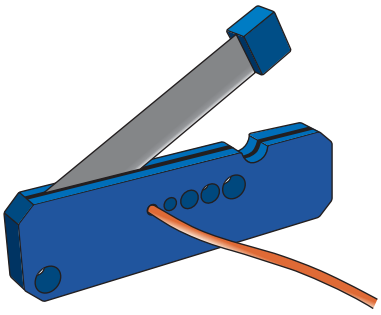
Required material

The following material are required:

- Tubing and connectors
- Tubing cutter
- Fingertight wrench

Instruction

Follow the steps below to replace tubing and connectors.

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Unscrew the connectors, and disconnect the tubing.
3	If the tubing has labels, remove the labels to be used with the new tubing later. Discard the tubing and connectors.
4	**CAUTION** **Cut injuries.** The tubing cutter is very sharp and must be handled with care to avoid injuries. Cut the new tubing to the same length as the old tubing. Use a tubing cutter to get a straight angle cut. 

Step	Action
	Note: *When replacing system tubing, use the original inner diameter and length to make sure that the correct delay volumes are maintained. Inlet and outlet tubing may be shortened if required.*
5	Put the old labels on the new tubing.
6	Mount the connectors on the tubing. For fingertight connectors: • Slide the connector onto the tubing. For tubing connectors 1/8": • Slide the connector onto the tubing. • Slide the ferrule onto the tubing with the thick end towards the end of the tubing.
7	Insert the tubing with connector into the port. Make sure to insert the tubing all the way into the bottom of the port.
8	Tighten the connector fully. For areas difficult to access, use the fingertight wrench included in the accessory kit.

7.5.2 Replace internal tubing in Fraction collector F9-C

Maintenance interval

Replace tubing and connectors when required, for example when the tubing has clogged or has been bent so that the flow is stopped.

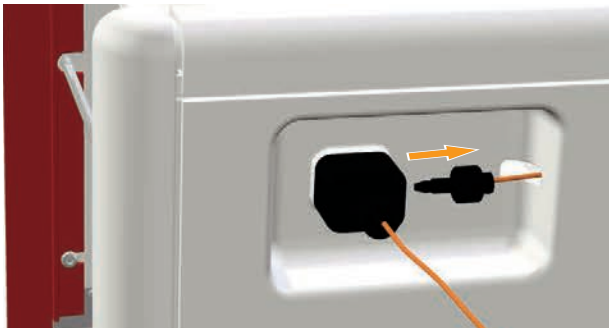
Required material

The following material are required:

- Tubing and connectors
- Tubing cutter
- Fingertight wrench

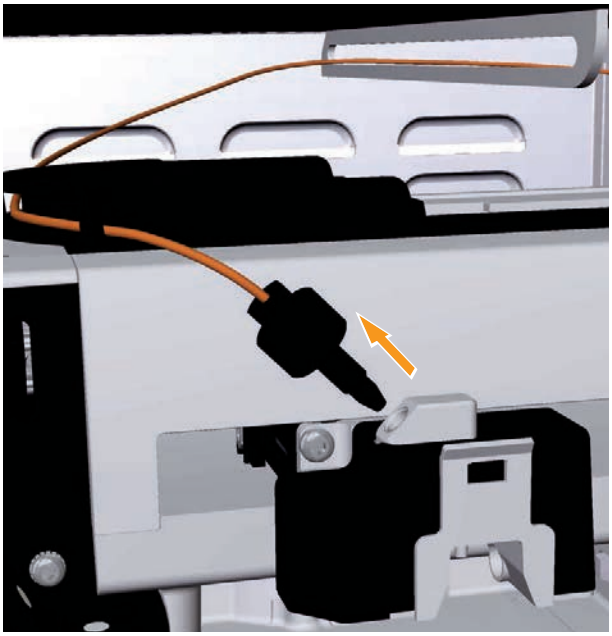
Instructions

Follow the steps below to replace tubing and connectors. See *Section 3.2.2 Fraction collector F9-C illustrations, on page 125* for the locations of the components of the fraction collector.

Step	Action
1	Switch off the power to the fraction collector by switching off power to the ÄKTA pure instrument.
2	Remove the connector for the internal tubing that is attached to the inlet port: **a.** Unscrew the connector from the inlet port.  **b.** Remove the connector from the tubing.
3	Remove the tubing connector from the dispenser head: **a.** Open the fraction collector and gently move the fractionation arm forward.

Step	Action

b. Unscrew the connector from the dispenser head and remove the tubing.

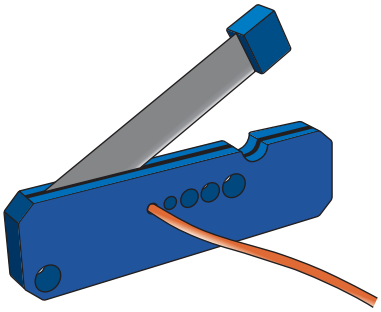


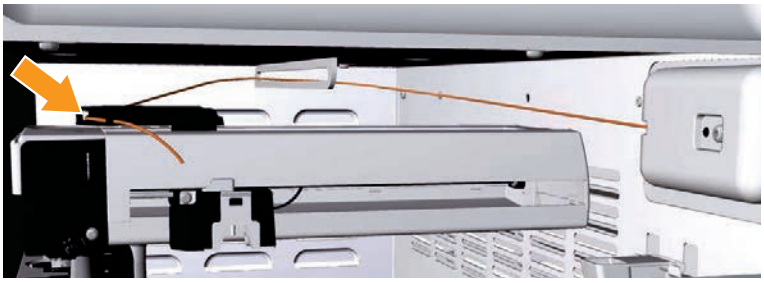
c. Gently move the fractionation arm all the way back.

4

> **CAUTION**
>
> **Cut injuries.** The tubing cutter is very sharp and must be handled with care to avoid injuries.

Cut the new tubing to the same length as the old tubing. Use a tubing cutter to get a straight angle cut.



Step	Action
	Note: *Use tubing with the original inner diameter and length to make sure that the correct delay volume is maintained.*
5	Attach the new tubing to the inlet port: **a.** Thread the tubing from the interior of the fraction collector to the inlet port. **b.** Slide the fingertight connector onto the tubing. **c.** Insert the tubing with connector into the port. Make sure to insert the tubing all the way into the bottom of the port. **d.** Tighten the connector fully.
6	Thread the tubing into the fraction collector and through the tubing guide in the ceiling of the fractionation chamber.
7	Position the new tubing: **a.** Gently move the fractionation arm forward while holding the tubing. **b.** Thread the tubing through the second tubing guide. 
8	Attach the new tubing to the dispenser head: **a.** Slide the fingertight connector onto the tubing. **b.** Insert the tubing with connector into the port. Make sure to insert the tubing all the way into the bottom of the port. **c.** Tighten the connector fully.
9	Make sure that the tubing not is twisted or bent and that it does not dip downwards.

7.5.3 Replace the pH electrode

Maintenance interval

Replace the pH electrode every six months, or when required.

Required material

The following materials are required:

- pH electrode
- Deionized water
- Standard buffer pH 4

Replacing the pH electrode



CAUTION

pH electrode. Handle the pH electrode with care. The glass tip may break and cause injury.

Follow the steps below to replace the pH electrode.

Step	Action
1	Disconnect the pH electrode cable of the used pH electrode from the connection on the front of the pH valve.
2	Unscrew the nut of the pH electrode by hand, and pull the used electrode away.
3	Unpack the new pH electrode. Remove the cover from the tip of the new pH electrode. Make sure that the electrode is not broken or dry.
4	Prior to first use of the electrode, immerse the glass bulb in deionized water for 30 minutes and then in a standard buffer, pH 4, for 30 minutes.
5	Carefully insert the new pH electrode into the pH flow cell. Tighten the nut by hand to secure the electrode.
6	Connect the pH electrode cable of the new electrode to the connection on the front of the pH valve.
7	Calibrate the new pH electrode, see *Section 7.3.1 Calibrate the pH monitor, on page 349*.

7.5.4 Replace the mixer

Maintenance interval

Replace the mixer when a different mixer chamber is desired, or when the mixer is damaged.

Required material

The following is required:

- Mixer

Instruction

Follow the steps below to change the mixer.

Step	Action
1	Disconnect the tubing from the top and bottom of the mixer. 
2	Pull the mixer away from the instrument. 
3	Attach the new mixer.
4	Reconnect the tubing to the top and bottom of the new mixer.

7.5.5 Replace the O-ring inside the mixer

Maintenance interval

Replace the O-ring inside the mixer if it is damaged.

Required material

One of the following O-rings are required:

- Standard O-ring 13.1 × 1.6 mm (for mixer chambers 0.6, 1.4, and 5 mL)
- Highly resistant O-ring 13.1 × 1.6 mm (for mixer chambers 0.6, 1.4, and 5 mL)

  Tip: *Use the highly resistant O-ring when the system is exposed to organic solvents or high concentrations of organic acids such as acetic acid or formic acid for longer periods of time.*
- O-ring 22.1 × 1.6 mm (for mixer chamber 15 mL)

Instruction

Follow the steps below to replace the O-ring inside the mixer.

Tip: *Use a forceps and gloves during the replacement procedure to avoid contaminating the mixer components.*

Step	Action
1	Loosen the top section of the mixer.
2	Unscrew the top section of the mixer and pull apart the mixer in two halves.



Step	Action
3	Remove the outer locking O-ring from the top section. 
4	Lift up the top section of the mixer and pull away the old O-ring inside. 
5	Wet the new O-ring with 20% ethanol and fit it in position. Make sure that the online filter is still in position.
6	Reassemble the mixer components and, while holding the mixer upright, screw the top section back onto the Mixer.

7.5.6 Replace the UV monitor U9-M flow cell

Maintenance interval

Replace the UV flow cell when it is desired to use a flow cell with a different path length, or if the cell is damaged. Clean the optical fiber connectors if they have accidentally been touched.

Required materials

The following items are required for replacing the UV flow cell, and for cleaning the optical fiber connectors:

- UV flow cell
- Lens paper
- Isopropanol

Replace the flow cell

CAUTION

Hazardous chemicals or biological agents in UV flow cell. Make sure that the entire flow cell has been flushed thoroughly with storage solution (e.g., NaOH) and distilled water, before service and maintenance.

Follow the steps to replace the UV flow cell.

Step	Action
1	Switch off the instrument.
2	Disconnect the tubing from the UV flow cell.
3	Push the latch on the UV detector to disconnect the detector.



Step	Action
	Note: *While the UV detector is disconnected, the UV lamp becomes inoperable so no UV light can be emitted from the instrument.*
4	Pull off the detector and the flow cell from the monochromator. Be careful not to damage the UV flow cell. 
	Note: *Make sure that the flow cell does not come into contact with any liquid, and that no liquid enters the UV detector or monochromator.*
	Note: *While the UV detector is disconnected, protect the fiber connectors from dust or other impurities by mounting the rubber protective caps onto them.*
	Note: *Do not touch the optical fiber connectors as this will result in poor monitor performance. If you accidentally touch the optical fiber connectors, clean them according to* *Clean the optical fiber connectors, on page 413**.*
5	Pull off the UV flow cell from the detector.
6	Pull off the black protective caps from the new UV flow cell, and connect the new UV flow cell to the detector.
7	Connect the detector, with the new flow cell connected, to the monochromator. Pull the latch upwards to fasten the detector.
8	Connect the tubing to the new flow cell.
9	Switch on the instrument. *Result:* The flow cell path length is automatically recognized by the monitor when a new flow cell is connected.

Clean the optical fiber connectors

Follow the steps to clean the optical fiber connectors.



WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.

Step	Action
1	Wrap a lint-free lens paper around a thin wood splinter (e.g a match or tooth-pick).
2	Wipe the optical fiber connectors with iso-propanol on lens paper through the small hole of the metal plate which covers the sensors.
3	Wipe the optical fiber connectors dry with lens paper.

7.5.7 Replace the UV monitor U9-T and U9-L flow cell

Maintenance interval

Replace the UV flow cell when it is desired to use a flow cell with a different path length, or if the cell is damaged.

Required material

- UV flow cell

Replace the flow cell

CAUTION

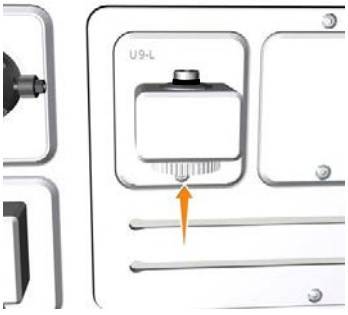
Hazardous chemicals or biological agents in UV flow cell. Make sure that the entire flow cell has been flushed thoroughly with storage solution (e.g., NaOH) and distilled water, before service and maintenance.

Follow the steps below to replace the UV flow cell.

Step	Action
1	Switch off the instrument.
2	Disconnect the tubing from the UV flow cell.
3	Unscrew the wheel at the bottom of the UV monitor (1). Press the wheel upwards to release the flow cell (2).



1



2

Step	Action
4	Pull the flow cell upwards out of the monitor. Hold the flow cell by the top part with the O-ring. Do not touch the optical surfaces of the flow cell.  ***Note:*** *Make sure that the flow cell does not come into contact with any liquid, and that no liquid enters the monitor.*
5	Insert a new flow cell into the monitor.
6	Tighten the wheel at the bottom of the UV monitor firmly.
7	Connect the tubing to the new flow cell.
8	Switch on the instrument and log on to UNICORN.
9	Update the UV flow cell path length in the ***Calibrate*** dialog box, in ***System Control***. ***Note:*** *The flow path cell length can be:* • *Updated with the nominal value, see Updating the cell path length, on page 363 to set the nominal flow cell path length.* • *Calibrated and updated with the calculated value, see Calibration of the UV monitor U9-T and U9-L flow cell length, on page 360 to calibrate the flow cell path length and to update the software with the calculated value.*

7.5.8 Replace flow restrictor

Maintenance interval

Replace the flow restrictor when required, for example when the back pressure of the flow restrictor is outside the range 0.2 ± 0.05 MPa.

Required material

The following material is required:

- Flow restrictor **FR-902**

Instruction

Follow the steps to replace the flow restrictor.

Step	Action
1	Disconnect the tubing connected from the used Flow restrictor, and discard the used flow restrictor.
2	Connect the tubing to the new flow restrictor. **a.** Connect tubing to the flow restrictor port **IN**, and connected the other end of the tubing to the pH valve port **ToR** (To Restrictor). **b.** Connect tubing to the unnamed flow restrictor port, and connect the other end of the tubing to the pH valve port **FrR** (From Restrictor). ***Note:*** *If no pH valve is used, connect the tubing from the* **IN** *port to the Conductivity monitor and tubing from the unnamed port to the outlet valve.*
3	Check the back-pressure of the new flow restrictor, see *Section 7.6.2 Check the flow restrictor, on page 449*.

7.5.9 Replace the online filter

Maintenance interval

Replace the online filter when required, for example when the filter becomes clogged. The online filter is located in the top section of the Mixer.

Required material

The following materials are required:

- Online filter kit
- Forceps
- Gloves

Instruction

Follow the steps below to replace the online filter that is located in the top of the mixer.

Tip: *Use forceps and gloves during the replacement procedure to avoid contaminating the mixer components.*

Step	Action
1	Unscrew the top section of the mixer. 
2	Remove the old online filter using a forceps. Replace the support net if it is damaged. Fit the new online filter.
3	Check the O-ring of the mixer. If the O-ring is damaged, replace it according to *Section 7.5.5 Replace the O-ring inside the mixer, on page 409*.
4	While holding the mixer upright, screw the top section back onto the mixer.

7.5.10 Replace the inlet filters

Maintenance interval

Replace the inlet filter when required, for example when the filters are clogged.

Required materials

The following material is required:

- Inlet filter set

Instruction

Follow the steps to replace an inlet filter and a support net.

Step	Action
1	Pull off the inlet filter and the support net from the inlet filter holder. 
2	Fit the new support net and inlet filter, and press the filter into position.

7.5.11 Replace the pump head check valves

Maintenance interval

Replace a check valve when required, for example if the check valve is damaged or clogged. The following instructions are valid for the system pumps and the sample pump.

Required materials

The following materials are required:

- Check valve kit
- Adjustable wrench

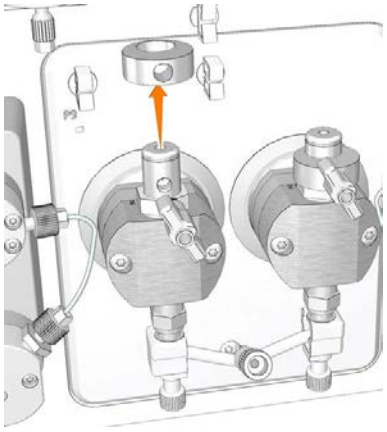
Instruction

NOTICE

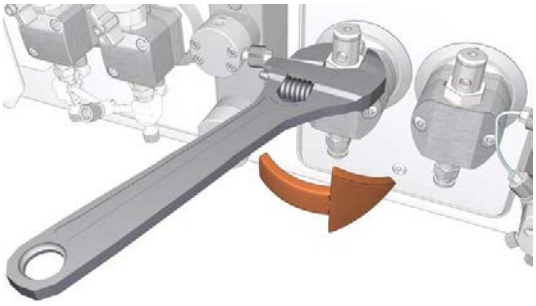
Handle the check valves with care when they have been removed from the pump heads, to prevent loss of any internal components.

Follow the steps to replace the check valves of a pump.

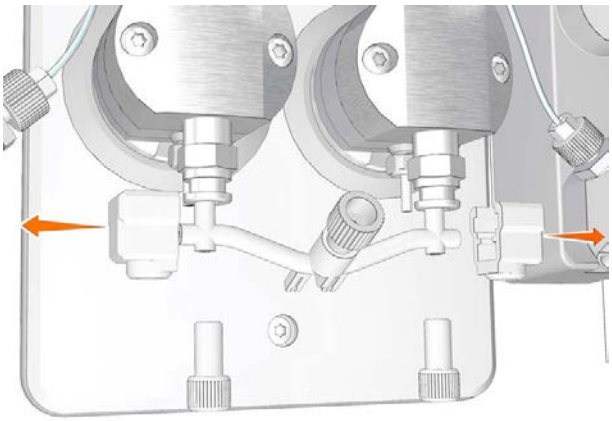
Step	Action
1	Disconnect the tubing from the pump head and disconnect the pump inlet tubing.
2	Unscrew the purge valve by turning it counter-clockwise, and lift off the metal ring.

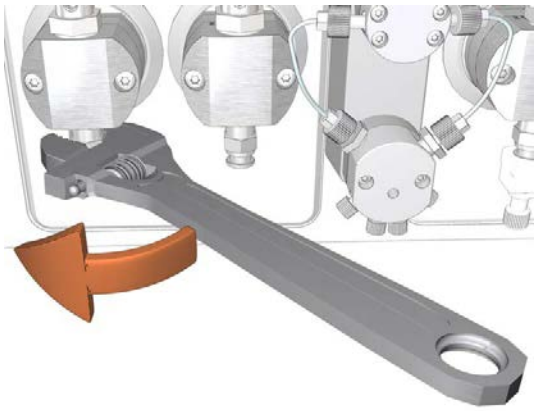


Step	Action
3	Unscrew the plastic nut of the upper check valve using an adjustable wrench, and gently lift off the upper check valve.



4	Replace the upper check valve with a new one.
5	Tighten the nut until fully finger-tight and then use the adjustable wrench to tighten a further 180 degrees.
6	Place the new metal ring onto the new upper check valve, and screw the new purge valve.
7	Unscrew the two white plastic screws located below each pump head. Pull the plastic connectors to the sides to release the inlet manifold.



Step	Action
8	Unscrew the lower check valve using an adjustable wrench. 
9	Replace the lower check valve with a new one.
10	Tighten the nut until fully finger-tight and then use the adjustable wrench to tighten a further 180 degrees.
11	Refit the inlet manifold and reconnect the tubing to the pump head.

7.5.12 Replace pump piston seals of Pump P9 or P9H

Maintenance interval

Replace the O-rings and piston seals and rinse the membranes of the pumps if they are damaged. After replacement, perform a run to break in the new piston seals.

The instructions in this section apply to the following pumps.

Configuration	Label	Pump type
System pump A, ÄKTA pure 25	**P9 A**	P9
System pump B, ÄKTA pure 25	**P9 B**	P9
System pump A, ÄKTA pure 150	**P9H A**	P9H
System pump B, ÄKTA pure 150	**P9H B**	P9H
Sample pump **S9H**, ÄKTA pure 150	**P9H S**	P9H

NOTICE

Advanced maintenance. Read the instruction carefully before disassembly of the pump head.

Required material

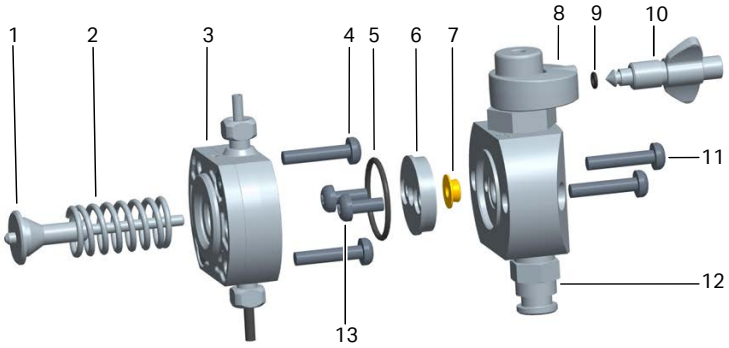
The following materials are required:

Pump P9	Pump P9H
• Adjustable wrench • Ethanol, 20% • P9 Seal kit, 25 mL • Star screwdriver, T20 • Tubing giving a back pressure of 6 to 8 MPa (60 to 80 bar) • Lubricant 56686700 • Ultrasonic bath	• Adjustable wrench • Ethanol, 20% • P9H Seal kit, 150 mL • Star screwdriver, T10 and T20 • Tubing giving a back pressure of 2 to 3 MPa (20 to 30 bar) • Lubricant 56686700 • Ultrasonic bath

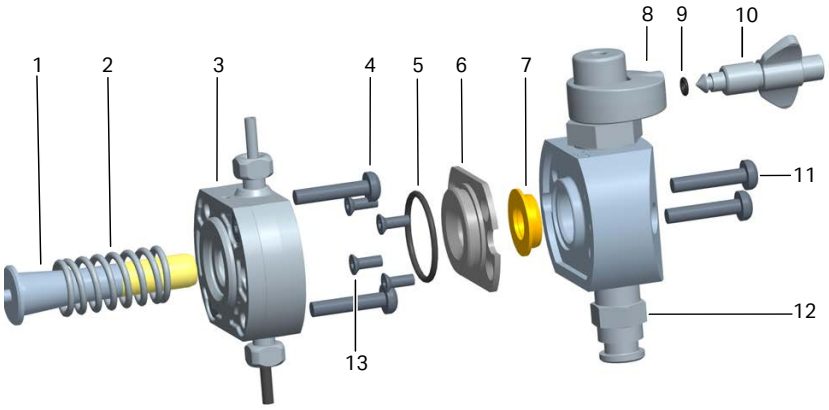
Illustrations

The illustrations below show the parts of the pump heads of the pumps **P9** and **P9H**.

Pump P9



Pump P9H



Part	Description	Part	Description
1	Piston	8	Outlet check valve
2	Return spring	9	O-ring
3	Pump membrane housing	10	Purge valve
4	Star screws	11	Star screws
5	O-ring	12	Inlet check valve
6	Support washer	13	Star screws
7	Piston seal		

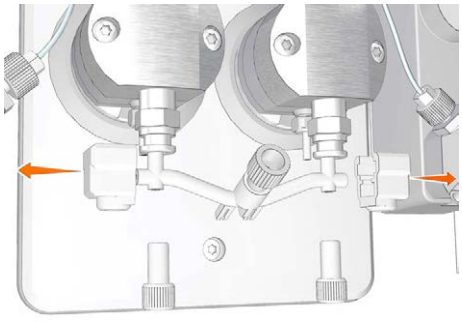
Introduction

Follow the instructions below to replace the O-rings, piston seal, and pump membrane housing of pumps **P9** and **P9H**.

Note: *Always replace the O-rings, piston seals, and pump membrane housing of both pump heads of a pump at the same time.*

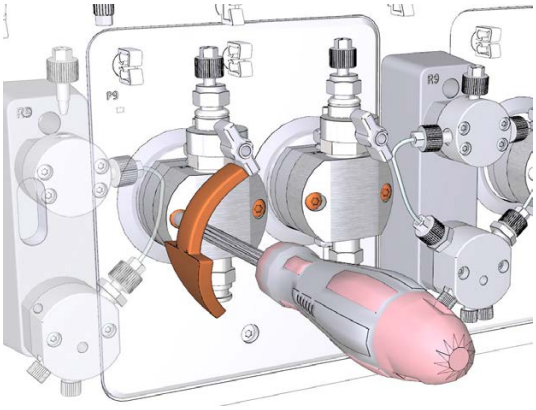
Disassemble the pump head

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Disconnect the tubing from the pump head, and disconnect the pump inlet tubing.
3	Unscrew the two white plastic screws located below each pump head by hand. Pull the plastic connectors to the sides to release the inlet manifold.



4	Disconnect the tubing of the pump piston rinsing system.

Step	Action
5	Unscrew the two screws of the front section of the pump head using a star screwdriver T20, and pull off the front section.



6

a. Place the front section of the pump head face down on the bench.

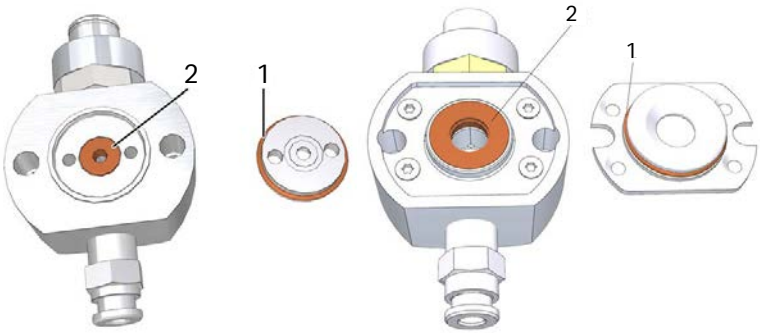
b.
- For Pump **P9** , unscrew the two screws of the support washer using a star screwdriver, T20.
- For Pump **P9H** , unscrew the four screws of the support washer using a star screwdriver, T10.

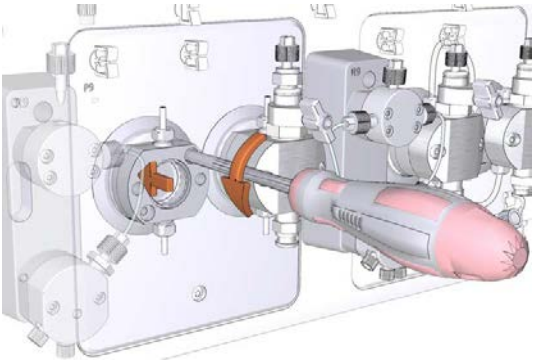
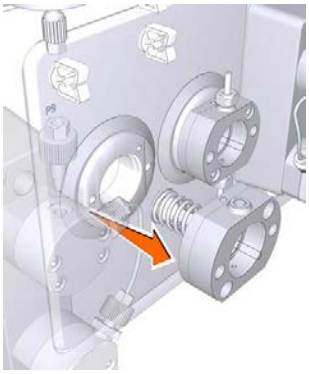
c. Discard the O-ring (1) on the support washer, and discard the piston seal (2) located in the front section of the pump head.

Note:

Be careful not to scratch the metal surfaces.

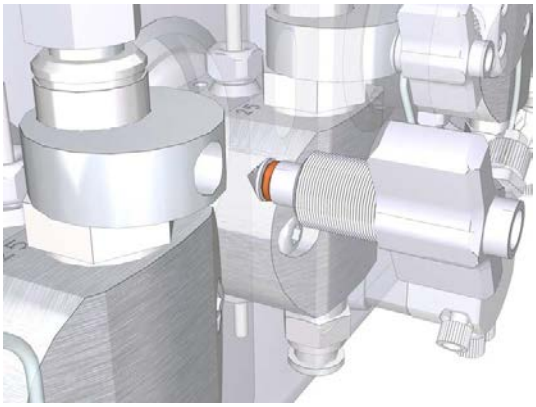
Pump **P9** Pump **P9H**



Step	Action
7	**a.** Unscrew one of the two screws securing the pump membrane housing using a star screwdriver, T20. **b.** Unscrew the second screw, and at the same time push firmly on the front of the pump membrane housing to compensate for the pressure of the piston return spring. 
8	Carefully pull off the pump membrane housing together with the piston and return spring. 
9	Inspect the piston and return spring for signs of damage. If damaged, discard the piston and return spring and use a new piston and return spring when assembling the pump head.
10	**a.** Clean the pump head and pump membrane housing in an ultrasonic bath. **b.** If there are particles on any surfaces, the check valves must be removed and cleaned separately, see *Section 7.4.12 Clean the pump head check valves, on page 397*.

Replace O-rings, piston seal and pump membrane housing

Step	Action
1	Unscrew the purge valve of the pump head. Replace the O-ring on the purge valve with a new O-ring, 3 × 1 mm, and screw the purge valve back into the pump head.



Note:

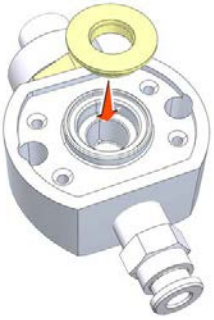
Always use Lubricant 56686700 when exchanging the O-ring 3 × 1 mm.

2	Wet a new seal with 20% ethanol. Place the new seal in the hole in the front section of the pump head and press it into position.

Pump **P9**



Pump **P9H**

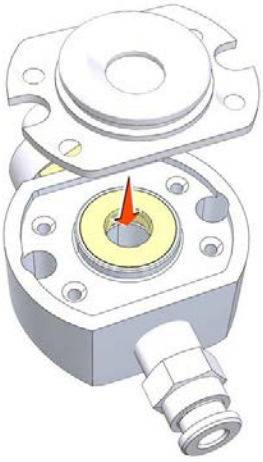


Step	Action
3	Place the support washer on top of the new seal in the front section of the pump head. Screw the two or four screws of the support washer. Make sure to tighten the screws fully.

Pump **P9**



Pump **P9H**

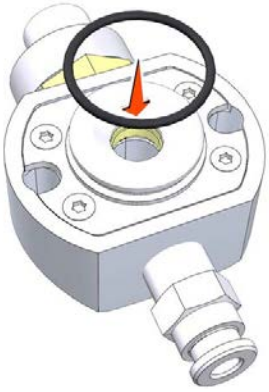


4	Wet a new O-ring, 21.4 x 1.6 mm, with 20% ethanol. Fit the O-ring around the support washer.

Pump **P9**



Pump **P9**



Assemble the pump head

Make sure to assemble the pump head correctly. Refer to *Illustrations, on page 423*.

Step	Action
1	Insert the piston into the return spring. Insert piston and return spring into hole in the pump module. ***Note:*** *Do no touch the ceramic or glass part of the pump piston.*
2	Wet the membrane in the hole with 20% ethanol before mounting.
3	Place the pump membrane housing onto the locating pins on the front of the pump module.
4	Screw one of the two screws securing the pump membrane housing using a star screwdriver, T20. Push firmly on the front of the pump membrane housing to compensate for the pressure of the piston and then screw the second screw.
5	Make sure that the new seal is wetted with 20% ethanol and then tighten both screws fully.
6	Reconnect the tubing of the pump piston rinsing system.
7	Reconnect the inlet manifold.
8	Reconnect the tubing to the pump head, and reconnect the pump inlet tubing.

Break in the new pump piston seal

Follow the steps below to run in the new pump piston seal of pumps P9 and P9H.

Step	Action
1	Fill a buffer vessel with 20% ethanol in water. Immerse the inlet tubing, for example **A1** for System pump A, or **B1** for System pump B, in the buffer vessel. Place the buffer vessel on the Buffer tray.
2	Prime the inlets and purge the pump, see *Section 5.4 Prime inlets and purge pump heads, on page 220*.
3	• For pump P9: Connect tubing that gives a back pressure of 6 to 8 MPa to one of the column positions of the Column valve or between the Injection valve and the UV monitor. • For pump P9H: Connect tubing that gives a back pressure of 2 to 3 MPa to one of the column positions of the Column valve or between the Injection valve and the UV monitor.

Step	Action
4	Fully put the waste tubing in the buffer vessel to recirculate the liquid.
5	For a system pump, in the ***Manual instructions*** dialog box: **a.** Select ***Flow path*** →***Column valve***, and select the position of the tubing connected to the Column valve, in this example ***Position 1***. Click ***Insert***. **b.** Select ***Flow path*** →***Inlet A*** (for System pump A) or ***Flow path*** →***Inlet B*** (for System pump B) and select a ***Position***, in this example ***A1*** or ***B1***. Click ***Insert***. **c.** Select ***Pumps*** →***Gradient*** and set ***Target*** to 0% B (for System pump A) or 100% B (for System pump B). **d.** • For pump P9: Select ***Pumps*** →***System flow*** and set the ***Flow rate*** to 5.0 mL/min. Click ***Insert***. • For pump P9H: Select ***Pumps*** →***System flow*** and set the ***Flow rate*** to 25.0 mL/min. Click ***Insert***. **e.** Click ***Execute***.
6	For a sample pump, in the ***Manual instructions*** dialog box: **a.** Select ***Flow path*** →***Column valve***, and select the position of the tubing connected to the Column valve, in this example ***Position 1***. Click ***Insert***. **b.** Select ***Flow path*** →***Sample inlet*** and select a ***Position***, in this example ***S1***. Click ***Insert***. **c.** Select ***Flow path*** →***Injection valve*** and select ***Direct inject*** from the ***Position*** drop-down list. Click ***Insert***. **d.** Select ***Pumps and Pressures*** →***Sample flow*** and set the ***Flow rate*** to 25.0 mL/min. Click ***Insert***. **e.** Click ***Execute***.
7	Run the flow for 2 hours.
8	Discard the used buffer.

7.5.13 Replace pump piston seals of Pump P9-S

Maintenance interval

Replace the O-ring and the piston seal, and rinse the membrane of the pump if they are damaged. After replacement, perform a run to break in the new piston seals.

The instructions in this section apply to the following pump.

Configuration	Label	Pump type
Sample pump **S9**	**P9-S**	P9-S

NOTICE

Advanced maintenance. Read the instruction carefully before disassembly of the pump head.

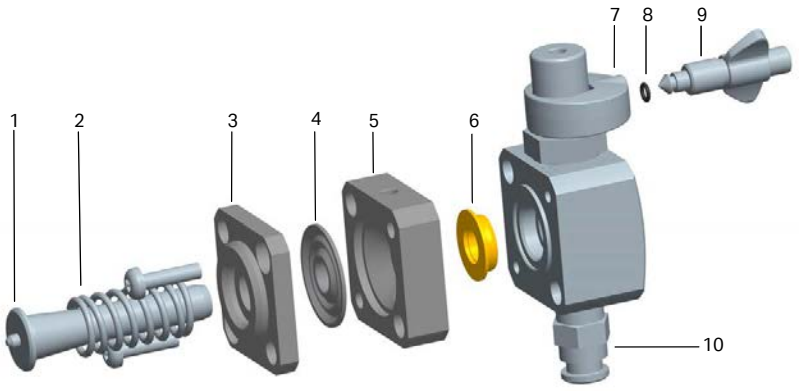
Required material

The following materials are required:

- Adjustable wrench
- Cross-headed screwdriver
- Ethanol, 20%
- Hex wrench
- Lubricant 56686700
- P9-S Seal kit, 65 mL
- Reference capillary **Ref 1**
- Star screwdriver, T20
- Ultrasonic bath

Illustration

The illustration below shows the parts of the pump heads of Pump **P9-S**.



Part	Description	Part	Description
1	Piston	6	Piston seal
2	Return spring	7	Outlet check valve
3	Drain plate	8	O-ring
4	Rinse membrane	9	Purge valve
5	Rinse chamber	10	Inlet check valve

Introduction

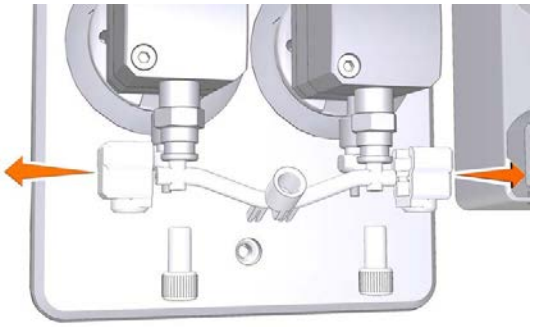
Follow the instructions below to replace the O-ring, piston seal, and rinse membrane of pump P9-S.

Note: *Always replace the O-rings, piston seals, and rinse membranes of both pump heads of a pump at the same time.*

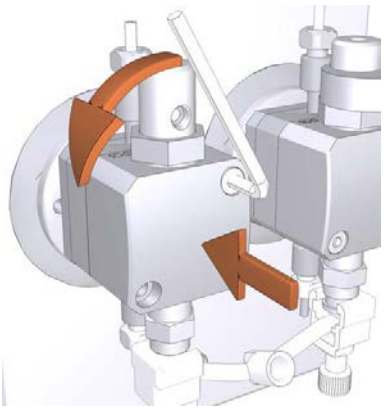
Disassemble the pump head

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Disconnect the tubing from the pump head, and disconnect the pump inlet tubing.

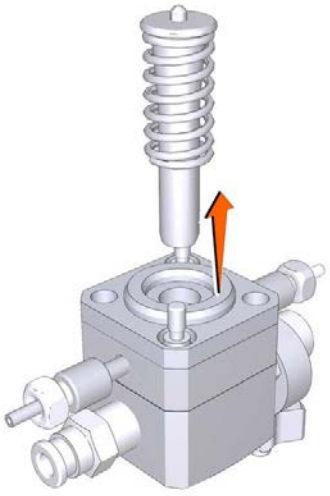
Step	Action
3	Unscrew the two white plastic screws located below each pump head by hand. Pull the plastic connectors to the sides to release the inlet manifold.



4	Disconnect the tubing of the pump piston rinsing system.
5	**a.** Unscrew one of the two screws of the pump head using a hex wrench. **b.** Unscrew the second screw, and at the same time push firmly on the front of the rinse chamber to compensate for the pressure of the piston return spring.

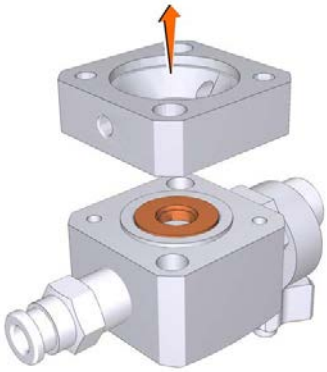


Step	Action
6	Place the pump head face down on the bench. Pull out the piston together with the return spring.

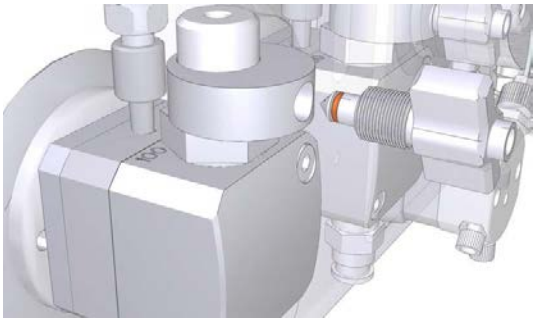


7	Inspect the piston and return spring for sign of damage. If damaged, discard the piston and return spring and use a new piston and return spring when assembling the pump head.
8	**a.** Unscrew the two screws securing the drain plate and the rinse chamber. **b.** Lift off the drain plate, and discard the membrane located between the drain plate and the rinse chamber.



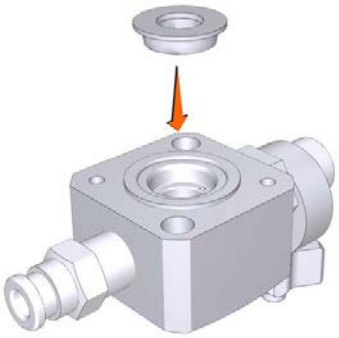
Step	Action
9	Lift off the rinse chamber. Gently pull off the piston seal. Discard the used seal. 
10	**a.** Clean the pump head, rinse chamber and drain plate in an ultrasonic bath. **b.** If there are particles on any surfaces, the check valves must be removed and cleaned separately, see *Section 7.4.12 Clean the pump head check valves, on page 397*.

Replace O-ring, piston seal, and rinse membrane

Step	Action
1	Unscrew the purge valve of the pump head. Replace the O-ring on the purge valve with a new O-ring, 3 × 1 mm, and screw the purge valve back into the pump head. 

Note:

Always use Lubricant 56686700 when exchanging the O-ring 3 × 1 mm.

Step	Action
2	Wet a new seal with 20% ethanol. Place the new seal in the hole in the front section of the pump head and press it into position. 
3	**a.** With the pump head facing downwards on the bench, place the rinse chamber onto the front section of the pump head with the rinse ports in line with the check valves. The conical depression in the rinse chamber must be facing upwards. **b.** Wet a new membrane with 20% ethanol, and place the membrane into the rinse chamber with the conical face upwards. 

Assemble the pump head

Make sure to assemble the pump head correctly. Refer to *Illustrations, on page 423*.

Step	Action
1	Place the drain plate on top of the assembly. Screw the two screws through the drain plate and the rinse chamber using a cross-headed screwdriver.
2	**a.** Wipe clean the piston and remove all finger prints. **b.** Wet the piston with 20% ethanol, and insert the piston into the return spring.

Step	Action
	c. With the pump head facing downwards on the bench, insert the piston into the pump head by pushing it gently but firmly vertically downwards into the seal.
3	**a.** Place the complete pump head over the locating pins on the front panel of the sample pump module. **b.** Turn the pump head so that the text **UP** on the drain plate is facing upwards. **c.** Push firmly on the front of the pump head, and at the same time screw one of the screws to fasten the pump head onto the front of the module using a hex wrench. **d.** Screw the second screw of the pump head. Make sure to tighten both screws fully.
4	Reconnect the tubing of the pump piston rinsing system.
5	Reconnect the inlet manifold.
6	Reconnect the tubing to the pump head, and reconnect the pump inlet tubing.
7	Break in the new pump piston seal, see instruction below.

Break in the new pump piston seal

Follow the steps below to break in the new pump piston seal of pump P9-S.

Step	Action
1	**a.** Fill a buffer vessel with 20% ethanol in water. **b.** Immerse a piece of sample inlet tubing, for example **S1**, in the buffer vessel. **c.** Place the buffer vessel on the Buffer tray.
2	Prime the inlets and purge the pump, see *Section 5.4.2 Sample pump, on page 228*.
3	Connect the reference capillary **Ref 1** (or equivalent tubing that gives a backpressure of 2 to 3 MPa) to one of the column positions of the Column valve or between the Injection valve and the UV monitor.
4	Immerse the waste tubing in the buffer vessel to recirculate the liquid.
5	In the ***Manual instructions*** dialog box: **a.** Select ***Flow path*** →***Column position***, and select the ***Position*** of the tubing connected to the Column valve, in this example ***1***. Click ***Insert***.

Step	Action
	b. Select ***Flow path*** →***Sample inlet*** and select a ***Position***, in this example ***S1***. Click ***Insert***.
	c. Select ***Flow path*** →***Injection valve*** and select ***Direct inject*** from the ***Position*** drop-down list. Click ***Insert***.
	d. Select ***Pumps*** →***Sample flow*** and set the ***Flow rate*** to 25.0 mL/min. Click ***Insert***.
	e. Click ***Execute***.
6	Run the flow for 2 hours.
7	Discard the used buffer.

7.5.14 Replace pump pistons

Maintenance interval

Replace the pump pistons if they are damaged.

Required material

The following materials are required:

- Adjustable wrench
- Star screwdriver, T20
- Piston kit

Replace pump pistons of Pump P9 and P9H

If a damaged piston has been in operation, the piston seal will be destroyed and must also be replaced. To replace the piston and the seal of a system pump, see *Section 7.5.12 Replace pump piston seals of Pump P9 or P9H, on page 422*.

Replace pump pistons of Pump P9-S

If a damaged piston has been in operation, the piston seal will be destroyed and must also be replaced. To replace the piston and the seal of Pump **P9-S** , see *Section 7.5.13 Replace pump piston seals of Pump P9-S, on page 431*.

7.5.15 Replace pump rinsing system tubing

Maintenance interval

Replace the pump rinsing system tubing when required, for example if the tubing is clogged or damaged. Replacement instructions for the system pumps and the sample pump (external module) are given below.

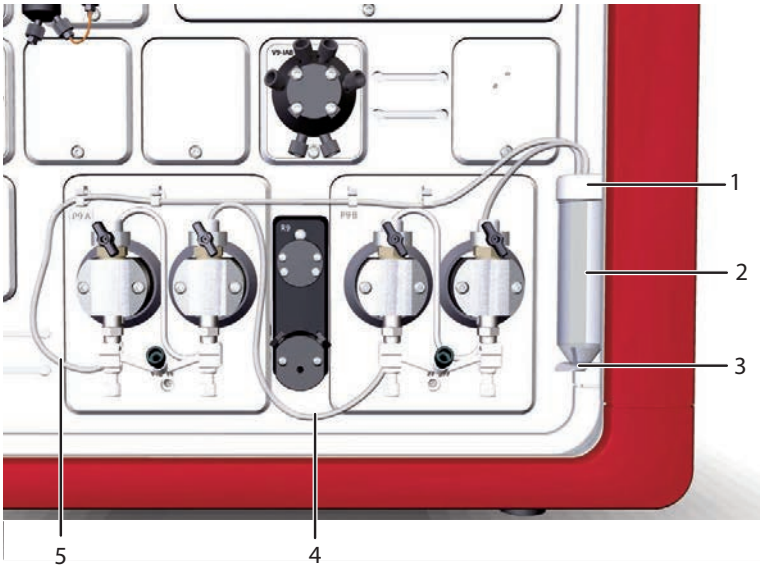
Required material

The following material is required:

- Rinsing system tubing

System pump piston rinsing system

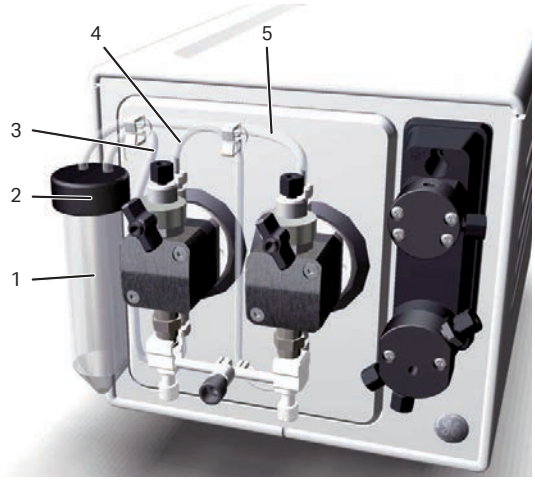
The illustration below show the parts and tubing of the system pump piston rinsing system.



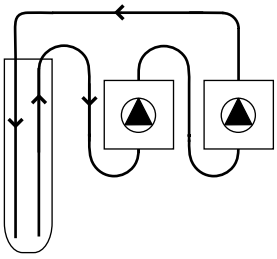
Part	Description
1	Rinsing system tube holder, top
2	Rinsing system tube
3	Rinsing system tube holder, bottom
4	Outlet tubing
5	Inlet tubing

Sample pump piston rinsing system

The illustrations below show the parts, tubing and flow path of the sample pump piston rinsing system.



Parts and tubing



Rinsing system flow path

Part	Description
1	Pump rinsing liquid tube
2	Pump rinsing liquid tube holder
3	Inlet tubing, from the rinsing liquid tube to the left pump head (lower rinsing system connection).
4	Tubing between the left pump head (upper rinsing system connection) and the right pump head (lower rinsing system connection).
5	Outlet tubing, from the right pump head (upper rinsing system connection) to the rinsing liquid tube.

Connect new tubing

Step	Action
1	Disconnect the used tubing.
2	Cut the new tubing to desired length.
3	Connect the new tubing according to the illustrations above.
4	Fit all pieces of tubing into the tubing holders on the pump modules.

Prime the rinsing systems

Before usage, prime the pump rinsing system tubing. Refer to *Section 7.6.1 Change pump rinsing solution, on page 444* for detailed instructions.

7.6 Other maintenance procedures

Introduction

This section provides instructions for other maintenance activities, such as changing the pump rinsing solution, and checking the flow restrictor.

In this section

Section		See page
7.6.1	Change pump rinsing solution	444
7.6.2	Check the flow restrictor	449
7.6.3	Storage of the pH electrode	451

7.6.1 Change pump rinsing solution

Maintenance interval

Replace the pump rinsing solution in the system pumps and the sample pump (optional module) every week to prevent bacterial growth.

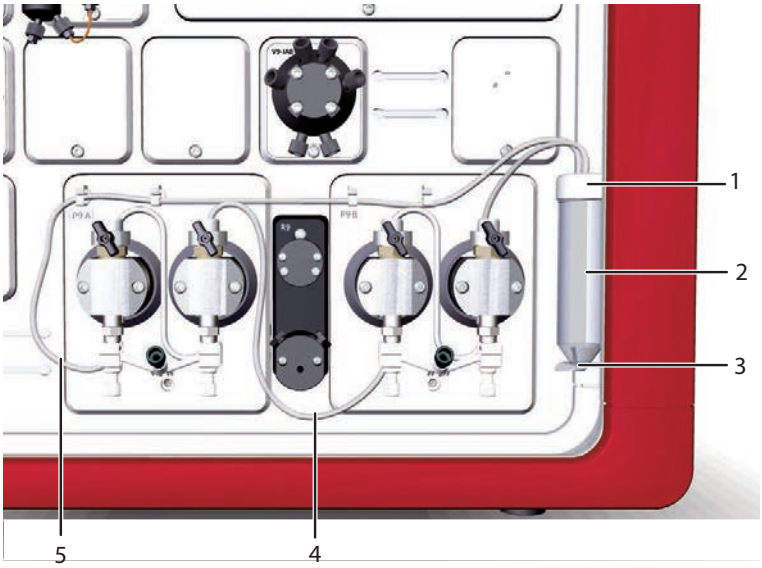
Required material

The following material are required:

- 20% ethanol
- Syringe, 25 to 30 mL

Illustration of the system pump piston rinsing system

The illustrations below show the parts and tubing of the system pump piston rinsing system.



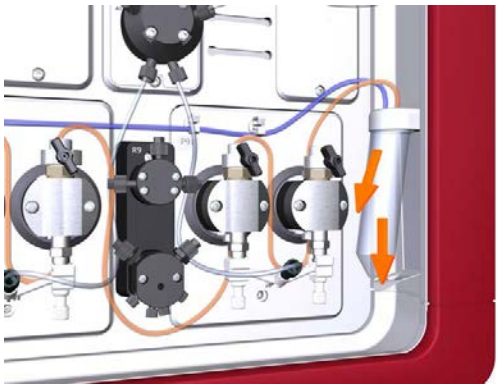
Part	Description
1	Rinsing system tube holder, top
2	Rinsing system tube
3	Rinsing system tube holder, bottom
4	Outlet tubing

Part	Description
5	Inlet tubing

Prime the system pump piston rinsing system

Follow the steps below to fill the pump piston rinsing system with rinsing solution. See the tubing configuration of the rinsing system in the illustration above.

Step	Action
1	Remove the rinsing system tube from the holder.

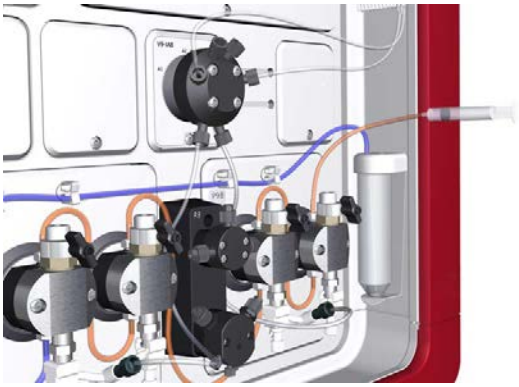


2	Fill the rinsing system tube with 50 mL of 20% ethanol.
3	Place the rinsing solution tube back in the holder.
4	Insert the inlet tubing to the System pump piston rinsing system into the fluid in the rinsing solution tube.

Note:

Make sure that the inlet tubing reaches close to the bottom of the rinsing solution tube.

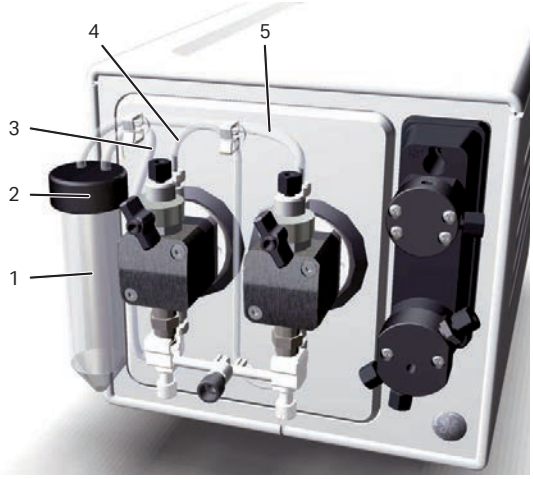
Step	Action
5	Connect a 25 to 30 mL syringe to the outlet tubing of the System pump piston rinsing system. Draw liquid slowly into the syringe.



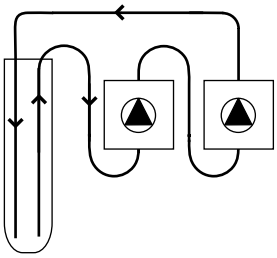
6	Disconnect the syringe and discard its contents.
7	Insert the outlet tubing into the fluid in the rinsing solution tube.
8	Fill the rinsing solution tube so that the tube contains 50 mL of 20% ethanol.

Illustrations of the sample pump piston rinsing system

The illustrations below show the parts, tubing and flow path of the sample pump piston rinsing system.



Parts and tubing



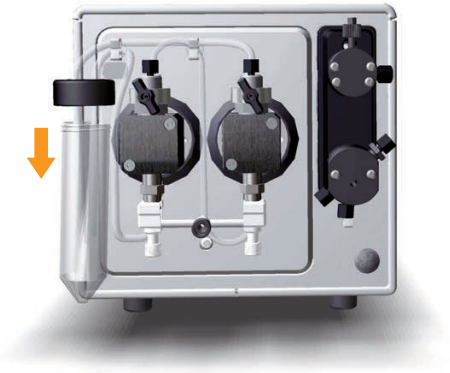
Rinsing system flow path

Part	Description
1	Pump rinsing liquid tube
2	Pump rinsing liquid tube holder
3	Inlet tubing, from the rinsing liquid tube to the left pump head (lower rinsing system connection).
4	Tubing between the left pump head (upper rinsing system connection) and the right pump head (lower rinsing system connection).
5	Outlet tubing, from the right pump head (upper rinsing system connection) to the rinsing liquid tube.

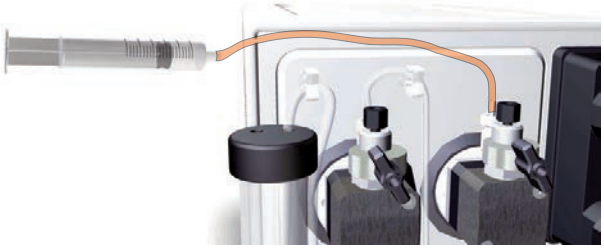
Prime the sample pump piston rinsing system

Follow the steps below to fill the pump piston rinsing system with rinsing solution. For the tubing configuration of the rinsing system, see the illustration above.

Step	Action
1	Unscrew and remove the rinsing liquid tube from the holder.



2	Fill the rinsing liquid tube with 50 mL of 20% ethanol.
3	Put the rinsing liquid tube back in the holder and fasten it by screwing it into place.

Step	Action
4	Insert the inlet tubing to the piston rinsing system into the fluid in the rinsing liquid tube. ***Note:*** *Make sure that the inlet tubing reaches close to the bottom of the rinsing liquid tube.*
5	Connect a 25 to 30 mL syringe to the outlet tubing of the Sample pump piston rinsing system. Draw liquid slowly into the syringe. 
6	Disconnect the syringe and discard its contents.
7	Insert the outlet tubing into the fluid in the rinsing liquid tube.
8	Fill the rinsing liquid tube so that the tube contains 50 mL of 20% ethanol.

7.6.2 Check the flow restrictor

Maintenance interval

Check the back pressure for the flow restrictor every month.

Location of the Flow restrictor

The recommended positions for Flow restrictor **FR-902** are:

- Connected between the Conductivity monitor **C9** and the outlet valve.
- Connected to the **ToR** and **FrR** ports of the pH valve.

Check Flow restrictor connected between conductivity monitor and outlet valve

Follow the steps below to check the back pressure of Flow restrictor **FR-902**, when it is connected between the conductivity monitor and the outlet valve.

Step	Action
1	Immerse the piece of inlet tubing marked **A1** in distilled water, and insert the piece of tubing from Outlet valve port **W** into a waste container.
2	In the ***System Control*** module, select the ***System pump*** (e.g. System pump A) in the ***Process Picture***.
3	Select ***System flow*** and set the ***Flow rate*** to 2.5 mL/min. ***Note:*** *When using the blue tubing i.d. 0.25 mm, set the flow rate to 0.25 mL/min.*
4	Note the ***PreC pressure*** displayed in the ***Run Data*** pane. ***Tip:*** *If **PreC pressure** is not displayed, click the **Customize** icon. In the **Customize** dialog box, under the **Run Data Groups** tab, select **PreC pressure**.*
5	Click on the ***Pause*** icon to pause the flow.
6	Replace the Flow restrictor with a female/female union connector. ***Tip:*** *The female/female union connector is included in the accessory kit.*
7	Click the ***Continue*** icon to restart the flow.
8	Note the ***PreC pressure*** displayed in the ***Run Data*** pane.

Step	Action
9	Calculate the difference between the two pressure values.
10	Check that the pressure difference is within the range 0.2 ± 0.05 MPa. If this is not the case, the flow restrictor must be replaced, see *Section 7.5.8 Replace flow restrictor, on page 416*.

Check Flow restrictor connected to pH valve

Follow the steps below to check the back pressure of Flow restrictor **FR-902**.

Step	Action
1	Immerse the piece of inlet tubing marked **A1** in distilled water, and insert the piece of tubing from Outlet valve port **W** into a waste container.
2	In the ***System Control*** module, select the ***System pump*** (e.g. System pump A) in the ***Process Picture***.
3	Select ***System flow*** and set the ***Flow rate*** to 2.5 mL/min. ***Note:*** *When using the blue tubing i.d. 0.25 mm, set the flow rate to 0.25 mL/min.*
4	Note the ***PreC pressure*** displayed in the ***Run Data*** pane. ***Tip:*** *If* ***PreC pressure*** *is not displayed, click the* ***Customize*** *icon. In the* ***Customize*** *dialog box, under the* ***Run Data Groups*** *tab, select* ***PreC pressure****.*
5	In the ***Manual instructions*** dialog box: • Select ***Flowpath →pH valve***, and set the pH electrode to ***Off-line*** and the flow restrictor to ***Off-line***. • Click ***Execute***.
6	Note the ***PreC pressure*** displayed in the ***Run Data*** pane.
7	Calculate the difference between the two pressure values noted in step 4 and step 6.
8	Check that the pressure difference is within the range 0.2 ± 0.05 MPa. If this is not the case, the flow restrictor must be replaced, see *Section 7.5.8 Replace flow restrictor, on page 416*.

7.6.3 Storage of the pH electrode

Maintenance interval

When pH monitoring is not used, the pH electrode can be stored in storage solution inside the pH flow cell. If pH monitoring is not used for a week or longer, inject new storage solution into the pH flow cell or replace the pH electrode with the dummy electrode that was installed in the pH valve on delivery.

Required material

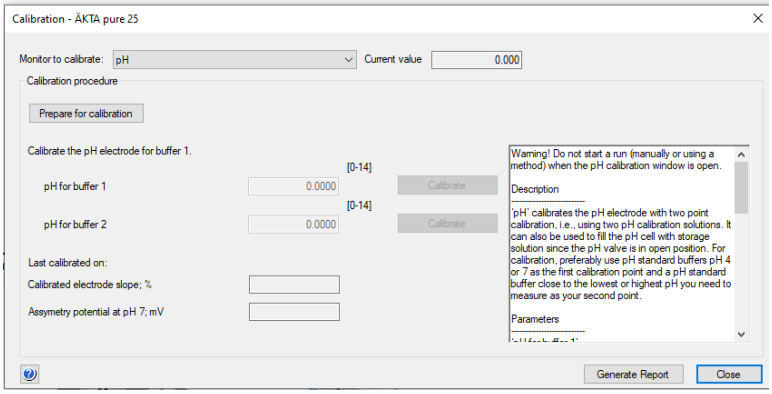
The following materials are required:

- Syringe, 25 to 30 mL
- Storage solution (1:1 mixture of standard buffer, pH 4, and 1 M KNO_3)

Preparing for storage

Follow the steps below to fill the pH flow cell with storage solution. The calibration function is used to switch the position of the pH valve. However, no calibration is performed.

Step	Action
1	In the ***System Control*** module, on the ***System*** menu click ***Calibrate***.



2	In the ***Calibration*** dialog box, click ***pH*** on the ***Monitor to calibrate*** drop-down list.
3	Click ***Prepare for Calculation***. *Result:* The pH valve switches to the calibration position.
4	Prepare at least 10 mL storage solution by mixing equal volumes of a standard buffer, pH 4, and a 1 M Potassium Nitrate (KNO_3) solution.

Step	Action
5	Fill a syringe with approximately 10 mL of the storage solution. Connect the syringe to the pH valve port **Cal**, and inject the storage solution.
	
6	Click ***Close***. *Result:* The pH valve switches back to the default position and the ***Calibration*** dialog box closes. No calibration is performed.
7	Disconnect the syringe from the pH valve.

8 Troubleshooting

About this chapter

This chapter describes troubleshooting and corrective actions for ÄKTA pure.

In this chapter

Section		See page
8.1	Introduction to troubleshooting	454
8.2	Troubleshooting: General Checklist	456
8.3	Troubleshooting: Monitors	458
8.4	Troubleshooting: Valves	474
8.5	Troubleshooting: Fraction collectors	475
8.6	Troubleshooting: Pumps	490
8.7	Troubleshooting: Other components	498
8.8	Troubleshooting: Instrument communication	511
8.9	Troubleshooting: Method development	513
8.10	Error codes	514

8.1 Introduction to troubleshooting

Introduction

This section describes troubleshooting procedures for ÄKTA pure and how to generate a System error report for service purposes. Subsequent sections in this chapter present general troubleshooting checklists, module-specific problems and corrective actions.

Troubleshoot the software

For software related troubleshooting, see the following table and the list of literature below:

Problem	Possible cause	Action
Text in the *Process Picture* pane in the *System control* module looks strange	The operating system of the computer does not have the text font Calibri True.	Install the font Calibri True Type or switch to an operating system that includes the font.
	The scaling in Windows is set to more than 100%.	Change to ***100%*** in ***Settings*** →***System*** →***Display*** →***Scale and layout***

- *UNICORN Method Manual*
- *UNICORN Evaluation Manual*
- *UNICORN Administration and Technical Manual*

Troubleshooting procedure

Follow the steps below to troubleshoot ÄKTA pure:

Step	Action
1	Always start by checking the General checklist. See *Section 8.2 Troubleshooting: General Checklist, on page 456*.
2	In this document, search for solutions in the section corresponding to the problem.
3	Make the recommended corrective actions.
4	If problems remain after corrective actions, generate a System error report and contact your local Cytiva representative.

Generate a System error report

A System error report can be generated during a troubleshooting case with information about the problem and can also include methods, logs, and results. The report can then be sent to Service for action.

Follow the steps below to generate a System error report for information to Service:

Step	Action
1	In the ***System Control*** module, on the ***System*** menu, click ***Create System Error Report***.
2	**a.** Click ***Next***. **b.** Type in information about the problem, click ***Next***. **c.** Select methods, logs, and/or result files and attach them to the system error report. **d.** Select the location for the report and click **Finish** to generate the report. The file name of the zip file will be `Report_YYYYMMDD.zip` and the default folder location is: `C:\Program Files (x86)\Cytiva \UNICORN\UNICORN 7.7\`.
3	E-mail the report to Cytiva Service department.

8.2 Troubleshooting: General Checklist

Introduction

Check the items in the following topics before starting more in-depth troubleshooting work.

System checks

- Is the correct system selected in UNICORN ***System Control***? For more details, see *Section 5.3 Start UNICORN and connect to system, on page 217*.

Monitor checks

- Is the UV monitor set to the correct wavelength? Check the wavelengths that are used in the method. For the predefined methods the wavelengths are set in the ***Method Settings*** phase. For more details, see *UNICORN Method Manual*.
- Is the air sensor sensitivity set to normal in UNICORN ***System Control*** →***System Settings*** to avoid unnecessary stops due to minor air bubbles?

Instrument communication

- Have Node IDs been set correctly for all instrument modules? To check and change Node IDs, see *Node ID, on page 96*.

Flow path checks

- Is all tubing connected correctly? See *Section 9.3 Tubing and connectors, on page 536* and *Section 5.2 Prepare the flow path, on page 208*.
- Is there leakage at any of the connections?
- Is any tubing folded or twisted?
- Is the inlet tubing correctly immersed in the buffer solution, i.e. beneath the liquid surface but not too close to the flask bottom?
- Have Fraction collector F9-R, Fraction collector F9-C and/or Fraction collector F9-T been correctly prepared? For more details, see relevant sections in *Chapter 3 ÄKTA pure external modules, on page 118* and *Chapter 4 System configuration, on page 170*.
- Are the inlet filters and the online filters clean or are they generating a back pressure higher than normal? If this is the case, replace the inlet filters and the online filters. For more details, see *Section 7.5.9 Replace the online filter, on page 417* and *Section 7.5.10 Replace the inlet filters, on page 418*.
- Does the positioning of the columns correspond to the selections made in the method? For more details, see *Section 5.5 Connect a column, on page 233*.

Purification checks

- Have all columns been cleaned and prepared according to the column recommendations?
- Have the samples been adjusted to binding buffer conditions?
- Have the samples been clarified by centrifugation and/or filtration prior to sample loading?
- Are the correct buffers used for the chosen columns and proteins?
- Check buffers for precipitations. Adjust to room temperature.
- Are the chosen columns suitable for the chosen target proteins?
- Do the buffers have correct pH? The pH of some buffers changes with the temperature.
- Are the UV-wavelengths used by the method appropriate with respect to used buffers and proteins? For more details see the method handbooks available from Cytiva.

8.3 Troubleshooting: Monitors

In this section

Section		See page
8.3.1	UV monitor U9-M and UV detector U9-D	459
8.3.2	UV monitor U9-T and U9-L	463
8.3.3	Conductivity monitor	467
8.3.4	pH monitor and pH valve	470
8.3.5	Pressure monitors	472

8.3.1 UV monitor U9-M and UV detector U9-D

Problem	Possible cause	Action
The UV monitor is not found by the instrument	Communication problem	Contact Service.
	The cable between the UV monitor and the instrument control unit (ICU) is not connected	Remove the UV monitor and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
No UV signal	The lamp is turned off	Turn on the lamp with the ***Manual instruction →Monitors →UV lamp***.
Too low UV lamp intensity	The detector is not correctly fitted	Contact Service.
	Unclean optical fiber connectors	Clean the connectors. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
	Dirt on optical sensors in detector	Remove visible dirt on detector photo diodes. See *Clean the optical fiber connectors, on page 413*.
	Worn-out or broken lamp	Contact Service.
No light transmission through the UV cell	Wrong wavelength for current buffer	Change wavelength or buffer.
	Dirt in the UV flow cell	Clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392*.
	Unclean optical fiber connectors	Clean the connectors. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
	Broken UV flow cell	Replace UV flow cell. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
Autozero out of accepted range	Wrong wavelength for current buffer	Change wavelength or buffer.
	Unclean optical fiber connectors	Clean the connectors. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
The internal temperature of the UV monitor is too high	The air intake on the rear or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.

Problem	Possible cause	Action
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
UV cell path length unreadable	No UV flow cell is attached	Attach UV cell. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
	UV flow cell is not correctly installed	Verify that the UV cell is correctly installed. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
	The UV flow cell is broken	Replace the cell. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
Ghost peaks	Air in the UV flow cell	Use the Flow restrictor. If using a pH valve, use the instruction to manually set the Flow restrictor inline (***Flow path*** →***pH valve*** →***Restrictor in-line***), or select the Flow restrictor in the ***Method Settings*** phase of a method. Remove the air by flushing the cell with water or buffer. If persistent, clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392*.
	Air in buffers	De-gas if necessary.
	Dirt in the UV flow cell	Clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392*.
	Dirt in the flow path	Clean the system in accordance to *Section 7.4.2 Perform System CIP, on page 366*. Clean the column in accordance to *Section 7.4.3 Perform Column CIP, on page 377*.
Baseline drift or noisy signal	No restrictror inline	Use the Flow restrictor. If using a pH valve, use the instruction to manually set the Flow restrictor inline (***Flow path*** →***pH valve*** →***Restrictor in-line***), or select the Flow restrictor in the ***Method Setting s*** phase of a method.
	Air in the UV flow cell	Use the Flow restrictor. Remove the air by flushing the cell with water or buffer. If persistent, clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392* .

Problem	Possible cause	Action
	Air in buffers	De-gas if necessary.
	Buffer temperature	Make sure that both the instrument and the buffers have reached the ambient temperature.
	Impure buffers	Check if the signal is noisy with water.
	Unclean optical fiber connectors	Clean the connectors. See *Section 7.5.6 Replace the UV monitor U9-M flow cell, on page 411*.
	Dirt in the UV flow cell	Perform a System CIP. See *Section 7.4.2 Perform System CIP, on page 366* . If necessary, manually clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392* .
	Wrong type of UV cell is used	Select another flow cell.
Unstable signal	Bad pump function	Check that the pump is operating properly. See *Section 8.6.2 Examples of pump pressure curves, on page 494* for example of pump pressure curves.
	Poor mixing function	Check the mixer chamber size and change the chamber if necessary. See *Section 5.2.2 Select mixer chamber, on page 211*. Check the function of the mixer. Place a stirrer bar in the palm of your hand. Hold the hand above the mixer. The stirrer should move when the mixer is activated. Check that the mixer chamber is free from solids. To open the mixer, see *Section 7.5.9 Replace the online filter, on page 417* .
The UV curve shows a gradient that is inverted compared to the expected gradient	Large difference in refractive index between buffer A and buffer B	Due to light spreading effects in the UV cell, the buffer with the highest UV absorption shows the lowest UV absorption in the chromatogram, and the buffer with the lowest UV absorption shows the highest UV absorption. This can occur if there is a large difference in refractive index between buffer A and buffer B and the UV is run at high sensitivity.

Problem	Possible cause	Action
The ratio curve shows spikes	The ratio curve shows a spike when the UV signal changes from negative values to positive values, or *vice versa*	Autozero the UV monitor with the buffer used during the run. Autozero with ***Manual instructions***, ***Process Picture***, or in the used method.
Faulty ratio levels at low UV absorbance	Absorbance zero level faulty	Autozero the UV monitor with the buffer used during the run. Autozero with ***Manual instructions***, ***Process Picture***, or in the used method.

8.3.2 UV monitor U9-T and U9-L

Problem	Possible cause	Action
No UV signal	The lamp is turned off	Turn the lamp on with the ***Manual instruction →Monitors →UV lamp***.
The UV monitor is not found by the instrument	Communication problem	Contact Service.
	The cable between the UV monitor and the instrument control unit (ICU) is not connected	Remove the UV monitor and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
	Wrong Node ID	Check the Node ID of the module. If necessary, change the Node ID. See *Node ID, on page 96*.
Autozero out of accepted range	Wrong UV flow cell for current buffer	Change to a shorter UV flow cell or change buffer.
	The UV flow cell is not correctly installed	Check that the UV flow cell is fitted correctly, see *Section 7.5.7 Replace the UV monitor U9-T and U9-L flow cell, on page 414*.
	Broken UV flow cell	Replace the cell, see *Section 7.5.7 Replace the UV monitor U9-T and U9-L flow cell, on page 414*.
The internal temperature of the UV monitor is too high	The air intake on the rear or on the right side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
Maximum absorbance that can be measured by the detector is reached	Wrong UV flow cell for current buffer	Change to a shorter UV flow cell or change buffer.
	The UV flow cell is not correctly installed	Check that the UV flow cell is fitted correctly, see *Section 7.5.7 Replace the UV monitor U9-T and U9-L flow cell, on page 414*.
	Dirt in the UV flow cell	Clean the UV flow cell, see *Section 7.4.10 Clean the UV flow cell, on page 392*.
	Broken UV flow cell	Replace the cell, see *Section 7.5.7 Replace the UV monitor U9-T and U9-L flow cell, on page 414*.

Problem	Possible cause	Action
Ghost peaks	Air in the UV flow cell	Use the Flow restrictor. If using a pH valve, use the instruction to manually set the Flow restrictor inline (***Flow path*** →***pH valve*** →***Restrictor in-line***), or select the Flow restrictor in the ***Method Settings*** phase of a method. Remove the air by flushing the cell with water or buffer. If persistent, clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392* .
	Air in buffers	De-gas the buffers.
	Dirt in the UV flow cell	Clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392* .
	Dirt in the flow path	Clean the system in accordance to *Section 7.4.2 Perform System CIP, on page 366*. Clean the column in accordance to *Section 7.4.3 Perform Column CIP, on page 377*.
Baseline drift or noisy signal	No flow restrictror inline	Use the Flow restrictor. If using a pH valve, use the instruction to manually set the Flow restrictor inline (***Flow path*** →***pH valve*** →***Restrictor in-line***), or select the Flow restrictor in the ***Method Settings*** phase of a method.
	Air in the UV flow cell	Use the Flow restrictor. Remove the air by flushing the cell with water or buffer. If persistent, clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392*.
	Air in buffers	De-gas the buffers.
	Buffer temperature	Make sure that both the instrument and the buffers have reached the ambient temperature.
	Impure buffers	Check if the signal is noisy with water.
	Dirt in the UV flow cell	Perform a System CIP. See *Section 7.4.2 Perform System CIP, on page 366* . If necessary, manually clean the UV cell. See *Section 7.4.10 Clean the UV flow cell, on page 392* .
	Wrong type of UV cell is used	Select another flow cell.

Problem	Possible cause	Action
Unstable signal	Bad pump function	Check that the pump is operating properly. See *Section 8.6.2 Examples of pump pressure curves, on page 494* for example of pump pressure curves.
	Poor mixing function	Check the mixer chamber size and change the chamber if necessary. See *Section 5.2.2 Select mixer chamber, on page 211* . Check the function of the mixer. Place a stirrer bar in the palm of your hand. Hold the hand above the mixer. The stirrer should move when the mixer is activated. Check that the mixer chamber is free from solids. To open the mixer, see *Section 7.5.9 Replace the online filter, on page 417* .
The UV curve shows a gradient that is inverted compared to the expected gradient	Large difference in refractive index between buffer A and buffer B	Due to light spreading effects in the UV cell, the buffer with the highest UV absorption shows the lowest UV absorption in the chromatogram, and the buffer with the lowest UV absorption shows the highest UV absorption. This can occur if there is a large difference in refractive index between buffer A and buffer B and the UV is run at high sensitivity.
Distorted protein peaks in IEX gradients (for example step gradients).	Rapid changes of the refractive index	The refractive index of the buffer changes rapidly in quick IEX gradients. The rapid change may cause light spreading effects and disturb the shape of the protein peaks in the **U9-T** or **U9-L** 2 mm flow cell. Run with reversed flow direction through the 2 mm flow cell: 1. Use the correct tubing length, see *Connect tubing, on page 90*. 2. Connect the inlet tubing at the bottom and the outlet tubing at the top of the flow cell. 3. Update the delay volume.
The ***Ratio UV 260/280*** curve of the UV monitor **U9-T** shows spikes	The ***Ratio UV 260/280*** curve shows a spike when the UV signal changes from negative values to positive values	Autozero the UV monitor **U9-T** with the buffer used during the run. Autozero with ***Manual instructions***, ***Process Picture***, or in the used method.

Problem	Possible cause	Action
Faulty ratio levels at low UV absorbance	Absorbance zero level faulty	Autozero the UV monitor with the buffer used during the run. Autozero with ***Manual instructions***, ***Process Picture***, or in the used method.

8.3.3 Conductivity monitor

Problem	Possible cause	Action
The conductivity part of the system performance test failed	Test was performed at cold room temperature and the conductivity temperature compensation factor was not optimized	In the ***System Settings*** dialog box, select ***Conductivity*** →***Cond temp compensation*** and set the ***Compensation factor*** to 2.1%.
The conductivity monitor is not found by the instrument	The cable between the conductivity monitor and the instrument control unit (ICU) is not connected	Remove the conductivity monitor and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
	Wrong Node ID	Check the Node ID of the module. If necessary, change the Node ID. See *Node ID, on page 96*.
The cell constant measurement has been aborted	Internal errors	See error log. Restart instrument and retry. If this problem recurs, generate a System error report and contact Service.
Unstable conductivity	Air in the conductivity flow cell	Flush the conductivity flow cell with water.
	Solids in the conductivity flow cell	Clean the conductivity cell. See *Section 7.4.11 Clean the conductivity flow cell, on page 395*.
Temperature out of range for calibration	This error can only occur when the temperature compensation is turned on. The error will occur when the temperature is outside the range 2°C to 40°C	Make sure the temperature of the calibration solution is within 2°C to 40°C.
Baseline drift of noisy signal	Air in the Conductivity flow cell	Use the Flow restrictor. Remove the air by flushing the flow cell with water or buffer.
	Leaking tubing connections	Tighten the connectors. If necessary, replace the connectors.
	Unclean conductivity flow cell	Clean the conductivity flow cell. See *Section 7.4.11 Clean the conductivity flow cell, on page 395*.

Problem	Possible cause	Action
	Poor mixing function	Check the mixer chamber size and change chamber if necessary, see *Section 5.2.2 Select mixer chamber, on page 211*. Check the motor operation of the mixer. Place a magnet close to the mixer chamber during run. The magnet should vibrate. Check that the mixer chamber is free from solids. To replace the online filter, see *Section 7.5.9 Replace the online filter, on page 417*.
Conductivity measurement with the same buffer appears to decrease/increase over time	Unclean conductivity flow cell	Clean the conductivity cell, see *Section 7.4.11 Clean the conductivity flow cell, on page 395*.
	The ambient temperature may have decreased/increased	Use a temperature compensation factor. The temperature compensation factor is found in ***System Control*** →***System Settings*** →***Conductivity.*** Instruction regarding the factor is also found in *Section 7.3.3 Calibrate the conductivity monitor, on page 355*.
	The conductivity monitor needs to be calibrated	Check calibration with a solution with known conductivity. Calibrate the conductivity monitor, see *Section 7.3.3 Calibrate the conductivity monitor, on page 355*.
Waves on the gradient	Bad pump function	Check that the pump is operating properly. See *Section 8.6.2 Examples of pump pressure curves, on page 494* for example of pump pressure curves.
	Air in the flow path	Purge the pumps. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
	Poor mixing function	Check that the correct mixer chamber size is used. See *Section 5.2.2 Select mixer chamber, on page 211* for recommendations. To change the Mixer, see *Section 7.5.4 Replace the mixer, on page 408*. Check the motor operation of the mixer. Place a magnet close to the mixer chamber during run. The magnet should vibrate. Check that the mixer chamber is free from solids. To open the mixer, see *Section 7.5.9 Replace the online filter, on page 417*.

Problem	Possible cause	Action
Ghost peaks appear in the gradient profile	A charged particle has been detected	Prepare the sample so that charged particles are eliminated.
	Air bubbles are passing through the flow cell	Check for loose tubing connections. Use the flow restrictor.
Non-linear gradients or slow response to %B changes	Dirt in the tubing	Make sure that the tubing has been washed properly.
	Bad pump function	Make sure that the pump operates properly. See *Section 8.6.2 Examples of pump pressure curves, on page 494* for example of pump pressure curves.
	The mixer chamber is too large	Change to a mixer chamber with a smaller volume. See *Section 5.2.2 Select mixer chamber, on page 211* for recommendations. To change the mixer, see *Section 7.5.4 Replace the mixer, on page 408*.
Incorrect or unstable reading	The temperature compensation factor is not properly set	Use a temperature compensation factor. The temperature compensation factor is found in ***System Control*** →***System Settings*** →***Conductivity.*** Instruction regarding the factor is also found in *Section 7.3.3 Calibrate the conductivity monitor, on page 355*.
	The column is not equilibrated	Equilibrate the column. Use the method phase ***Equilibration***. If necessary, clean the column. Use the predefined method ***Column CIP***. See *Section 7.4.3 Perform Column CIP, on page 377* .
	Poor mixing function	Check that the correct mixer chamber size is used. See *Section 5.2.2 Select mixer chamber, on page 211* for recommendations. To change the mixer, see *Section 7.5.4 Replace the mixer, on page 408*. Check the motor operation of the mixer. Place a magnet close to the mixer chamber during run. The magnet should vibrate. Check that the mixer chamber is free from solids. To open the mixer, see *Section 7.5.9 Replace the online filter, on page 417*.

8.3.4 pH monitor and pH valve

Problem	Possible cause	Action
The pH monitor is not found by the instrument	The cable between the pH valve and the instrument control unit (ICU) is not connected	Remove the pH valve and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
The internal valve temperature is too high	The air intake on the rear or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
Unstable pH signal	Calibration time out	Check the connections between pH electrode and pH monitor. Regenerate the pH electrode. Place the electrode in deionized water for 30 minutes followed by 30 minutes in a buffer with pH 4. If persistent, replace the pH electrode. See *Section 7.5.3 Replace the pH electrode, on page 407*.
	Bad pH electrode	Regenerate the pH electrode. Place the electrode in deionized water for 30 minutes followed by 30 minutes in a buffer with pH 4. Clean the pH electrode. See *Section 7.4.9 Clean the pH electrode, on page 389*. If persistent, replace the pH electrode. See *Section 7.5.3 Replace the pH electrode, on page 407*.
	Wrong mixer size for the used flow rate	Use the recommended mixer size for the used flow rate. See *Section 5.2.2 Select mixer chamber, on page 211*.
Drift of pH signal when the pH electrode has been removed from storage solution	Decreasing salt concentration in the electrode membrane due to osmosis to buffer	Regenerate the pH electrode. Place the electrode in deionized water for 30 minutes followed by 30 minutes in a buffer with pH 4.

Problem	Possible cause	Action
Temperature reading error	The temperature compensation of the pH monitor is turned off	Contact Service.
It is not possible to inject calibration solution	Waste tubing is twisted or blocked	Untwist the tubing. Perform System CIP to clean waste tubing. See *Section 7.4.2 Perform System CIP, on page 366* . Change the tubing.
Alarm in UNICORN: ***(Alarm) The pH cell can only be run at pressures below 0.8 MPa. Please check the tubing or lower the flow through the pH cell.*** ***Note:*** *The pressure limit 0.8 MPa is for the post-column pressure.*	High pressure in the pH cell	Decrease the flow rate. Bypass the pH electrode (see *Ports and flow paths of the pH valve, on page 76*) and measure pH in fractions manually.
Leaking pH valve	The dummy electrode was dry when it was installed in the valve	1. Remove the dummy electrode. 2. Wet the dummy electrode properly with distilled water. 3. Insert the dummy electrode into the pH valve. 4. Rotate the dummy electrode before securing it with the nut.

8.3.5 Pressure monitors

Problem	Possible cause	Action
Pressure offset	The monitors have lost their calibration	Calibrate the pressure monitors. See *Section 7.3.2 Calibrate the pressure monitors, on page 351*.
	The temperature has changed	Wait until the temperature has stabilized and calibrate the pressure monitors.
Excessively high pressure values	Dirt in the online filter in the mixer	Replace the online filter in the mixer. See *Section 7.5.9 Replace the online filter, on page 417*.
	Solids in the flow path	Use the predefined method System CIP to clean the flow path, see *Section 7.4.2 Perform System CIP, on page 366*. Clean the column, see *Section 7.4.3 Perform Column CIP, on page 377*. If persistent, replace the column.
	0.25 mm i.d. tubing kit is mounted	A smaller tubing inner diameter gives higher back pressure. The back pressure for the 0.25 mm i.d. tubing kit is normally 16 times higher than for the 0.50 mm i.d. tubing kit used at the same running conditions. The maximum system flow rate for 0.25 mm i.d. tubing kit is 10 mL/min.
	Too high flow rate	The flow rate for the used column and/or tubing kit is too high. Lower the flow rate.
The pressure monitors are not found by the instrument	The cable between the pressure monitor and the instrument control unit (ICU) is not connected.	Remove the monitor and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
The internal temperature of the pressure monitor is too high	The air intake on the rear or on the left side of the chromatography instrument or the sample pump is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.

Problem	Possible cause	Action
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
Irregular pressure curves	Air bubbles are passing through or are trapped in the pump	Check that there is a sufficient volume of buffer present in the flasks. Check all connections for leaks. Check pump pressure curves. See *Section 8.6.2 Examples of pump pressure curves, on page 494* for examples of pump pressure curves. Purge the pumps, see *Section 5.4 Prime inlets and purge pump heads, on page 220* or *Section 5.4.2 Sample pump, on page 228*.
	The check valve does not function correctly	Remove any solids in the valves by cleaning the check valves according to the instructions in *Section 7.4.12 Clean the pump head check valves, on page 397*.
	Piston seal is leaking	Replace the piston seal of a system pump according to the instructions in *Section 7.5.12 Replace pump piston seals of Pump P9 or P9H, on page 422*. Replace the piston seal of the sample pump according to the instructions in *Section 7.5.13 Replace pump piston seals of Pump P9-S, on page 431*.
	Blockage of flow path	Use the predefined method ***Prepare System*** to flush through to clear blockage. If necessary, replace tubing. See *Section 7.5.1 Replace tubing and connectors, on page 402*. Check the online filter in the mixer. It can be clogged if unfiltered buffers or samples are applied. To replace the online filters, see *Section 7.5.9 Replace the online filter, on page 417*. Check the inlet filters. They can be clogged if unfiltered buffers or samples are applied. To replace the filters, see *Section 7.5.10 Replace the inlet filters, on page 418*. Check the column. It can be clogged if unfiltered buffers or samples are applied. To clean a column, see *Section 7.4.3 Perform Column CIP, on page 377*.

8.4 Troubleshooting: Valves

General

The following table lists the general problems that may occur for the different valves.

Problem	Possible cause	Action
The valve is not found by the instrument	The cable between the valve and the instrument control unit (ICU) is not connected	Remove the valve and make sure that the cable is connected. See *Hardware installation of a module, on page 96*.
	Wrong Node ID	Check the valve Node ID. If necessary, change the Node ID. See *Node ID, on page 96*.
The internal valve temperature is too high	The air intake on the rear or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
The valve is not switching or is switching to wrong position	Hardware error	Generate a System error report and contact Service.
External leakage	Hardware error	Generate a System error report and contact Service.
Internal leakage	Broken valve	Replace the valve. See *Section 2.5 Installation of internal modules, on page 96*.

Inlet valves

The Inlet valves include Inlet valve V9-IA, V9H-IA, V9-IB, V9H-IB, as well as Sample inlet valve V9-IS and V9H-IS.

Problem	Possible cause	Action
Faulty air sensor in the valve	Hardware error	Restart the instrument with the power switch. If this error is recurrent, generate a System error report and contact Service.

8.5 Troubleshooting: Fraction collectors

In this section

Section		See page
8.5.1	Fraction collector F9-C	476
8.5.2	Fraction collector F9-R	484
8.5.3	Fraction collector F9-T	486

8.5.1 Fraction collector F9-C

Problem	Possible cause	Action
Fraction collector not detected by UNICORN	The UniNet-9 cable between the fraction collector and the ÄKTA system is not properly connected	Get a System error report and contact Service.
	A fuse in the instrument control unit (ICU) is broken	The ICU needs to be changed. Generate a System error report and contact Service.
Fraction collector arm is blocked or internal fault in the fraction collector	Obstruction inside the fraction collector	Switch off the instrument and check for obstruction inside the fraction collector. Try to move the fractionation arm by hand. Switch on the instrument. If this error is recurrent, generate a System error report and contact Service.
The accumulator is jammed or there is an internal error in the instrument	Salt crystals or protein residuals block the accumulator	Restart the instrument and perform an accumulator wash.
	Mechanical error	If this error is recurrent, generate a System error report and contact Service.
Fraction collection tube or well is overfilled and fractionation movements are lost	Too many commands are pending in the fraction collector	The reason could be that too many fraction collector instructions have been sent. Wait for a while and try again.
The fraction collector failed to detect the code on the Cassette	Cassette tray or tube rack is inserted in the wrong direction	Take it out and insert it in the correct direction.
	Unclean Cassette type code reader	Clean the dispenser head and its four diode windows using a cloth and a mild cleaning agent or 20% ethanol. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381* for more information.
	Unclean Cassette type codes	Clean the Cassette type codes. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381* for more information. If this error is recurrent, set the Fraction collector configuration manually in UNICORN. In ***System Control***, select ***System*** →***Settings***. Navigate to***Fraction collector*** →***Cassette configuration*** and select ***Manual***.

Problem	Possible cause	Action
The Cassette tray is loose in the fraction collector	The Tray guides in the fractionation chamber are missing	Replace the Tray guides. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
	The Tray catch is broken	Contact service.
Calibration of the Drop sync sensor failed	Unclean Drop sync sensor diode windows	Clean the Drop sync sensor diode windows. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381* for location of the Drop sync sensor diode windows and cleaning instructions. If this error is recurrent, generate a System error report and contact Service.
Drop sync does not work	Liquid spatter in the fraction collector	Change to another type of tubes or deep well plates or collect fraction volumes of maximum two thirds of the volume of the tubes. Clean the diode windows of the drop sync sensor more frequently than once a week, using a cloth and a mild cleaning agent or 20% ethanol.
The Dispenser head failed to detect a drop	Air in the flow path	If this error is recurrent, generate a System error report and contact Service. Check the flow path for air. Fill system and purge pumps according to *Section 5.4 Prime inlets and purge pump heads, on page 220*.
	Unclean Drop sync sensor diode windows	Clean the diode windows of the Drop sync sensor. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*. If this error is recurrent, generate a System error report and contact Service.
	Too high flow rate	Decrease the flow rate.
The Dispenser head failed to detect a drop and has switched to tube change without Drop sync	Too high flow rate	Decrease the flow rate.
	Air in the flow path	Check the flow path for air. Fill system and purge pumps according to *Section 5.4 Prime inlets and purge pump heads, on page 220*. If this error is recurrent, generate a System error report and contact Service.
	Unclean diode windows in the Drop sync sensor	Clean the diode windows of the Drop sync sensor. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381* . If this error is recurrent, generate a System error report and contact Service.

Problem	Possible cause	Action
The Dispenser head failed to detect the flow properly and has switched to tube change with reduced accumulator functionality	Air in the flow path	Check the flow path for air. Fill system and purge pumps according to *Section 5.4 Prime inlets and purge pump heads, on page 220*. If this error is recurrent, generate a System error report and contact Service.
	Unclean diode windows in the Drop sync sensor	Clean the diode windows of the Drop sync sensor. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*. If this error is recurrent, generate a System error report and contact Service.
Tubes do not fit in the Cassette	Wrong tube dimensions are used	Check that the used tubes have the right dimensions. See *Section 3.2.4 Fraction collector tubes and bottles, on page 132* for information about tubes and Cassettes.
	QuickRelease function is worn out	Order a new Cassette. See *Chapter 10 Ordering information, on page 649* for ordering information.
Deep well plate does not fit in the Cassette	Unsupported deep well plate model	Check that the deep well plates are supported. See *Section 3.2.5 Deep well plates, on page 133*.
The Cassette does not fit the Cassette tray	The Cassette is turned in the wrong direction	See *Prepare and insert the Cassette tray, on page 266* for information of how to place the Cassettes.
	Objects or dirt under the Cassette	Remove the object or dirt.
Cassette tray in wrong position	The Tray guides in the fractionation chamber are missing	Replace the Tray guides. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
	The Cassette tray is facing the wrong direction	Make sure that the front of the tray (marked with the Cytiva-logotype) is facing outwards. See *Prepare and insert the Cassette tray, on page 266*.
	Dirt under the Cassette tray	Remove the dirt.
The loaded Cassette tray cannot be inserted into the fractionation collector	The Cassette tray is facing the wrong direction	Check that the Cytiva-logotype is facing outwards when the tray is inserted into the fraction collector.

Problem	Possible cause	Action
	Some of the tubes or plates are incorrectly placed in the Cassettes	Check that all tubes and plates are correctly inserted in the Cassettes. See *Prepare and insert the Cassette tray, on page 266*.
	Some of the tubes or plates have the wrong dimensions	Check that the deep well plates and the tubes used are of the right type. See *Section 3.2.4 Fraction collector tubes and bottles, on page 132* and *Section 3.2.5 Deep well plates, on page 133* for information about supported tubes and plates.
Quick scan or Full scan does not work	The Tray guides in the fractionation chamber are missing	Replace the Tray guides. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
	The Cassette tray is facing the wrong direction	Check that the Cytiva-logotype is facing outwards.
	The Cassette type codes are unclean	Clean the the Cassette type codes.
	The QuickRelease of a Cassette is in open position	Close the Cassette. See *Prepare and insert the Cassette tray, on page 266*.
	The Cassette code reader diode window is unclean	Clean the Cassette code reader diode window. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
	The automatic scanning is turned off in UNICORN	Make sure that the automatic scanning is turned on in UNICORN software. In ***System Control***, select System:Settings. Navigate to***Fraction collector*** →***Cassette configuration*** and select ***Automatic***.
	Wells in deep well plates are pre-filled to a volume above 25% of the total well volume	Full scan will not work with pre-filled wells during these conditions.
	Hardware error	Generate a System error report and contact Service.
The interior of the fraction collector is dark	The light has been turned off in UNICORN	Turn on the light in UNICORN. In ***System Control***, select System:Settings. Navigate to ***Fraction collector*** →***Fraction collector lamp*** and select ***Lamps On***.
	The lamp is broken	Contact Service.

Problem	Possible cause	Action
The waste or the interior of the fraction collector is flooded	The waste tubing is positioned so that the flow is obstructed	Untwist the waste tubing.
	The waste container is placed in a position higher than the waste outlet	Place the waste container in a position lower than the waste outlet.
	The waste tubing is blocked	Clean or replace the waste tubing.
The liquid leaving the nozzle does not strike the waste funnel	Check the position of the waste funnel	Refit the waste funnel.
The tubing in the fraction collector is blocked	Salt residuals in the tubing	Perform a ***Fraction collector wash***, see *Section 7.4.4 Clean Fraction collector F9-C, on page 381*. If persistent, contact Service.
The fraction volume found in the tubes or wells are smaller than expected	Leakage on the wet side of the instrument	Localize the leakage and take care of the leakage, for example by tightening connectors.
	Air in pumps	Purge pumps. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.
	Bad pump function	See troubleshooting of pumps in *Section 8.6 Troubleshooting: Pumps, on page 490*.
	Leakage inside the Frac chamber	Contact Service.
Liquid on the floor of the fraction collector	The fingertight connector on the Dispenser head is not tight enough	Tighten the connector. Replace the connector if the leakage is persistent.
	The waste tubing or the waste funnel is blocked	Make sure that there is no blockage or clogging and that the waste tubing not is bent.
	Leakage in the Frac arm	Contact service.
Spillage by the fraction collector during fractionation	Unclean diode windows in the Drop sync sensor or unclean nozzle	Clean the Drop sync diode windows and the nozzle. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*. Use the Fraction collector cleaning position.

Problem	Possible cause	Action
	Too high flow rate during usage of Drop sync	Use a flow rate below 2 mL/min.
	One or more Cassettes have empty positions	Make sure that all Cassette positions contain tubes or plates.
	The tubes are flooded	Make sure that the fraction volume is adapted to the tube volume. Fraction tubes have not been replaced by empty tubes when the Fraction collector was opened.
It is not possible to fractionate	The door of the fraction collector is not properly closed	Close the door.
The fraction collector fractionates in the wrong well or tube	Quick scan has not detected the correct Cassette	Clean the Cassette type code of the Cassette. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
	Unsupported deep well plate is used	Make sure that approved deep well plates are used. See *Section 3.2.5 Deep well plates, on page 133*.
	Wells in deep well plates are prefilled to a volume above 25% of the total well volume	Full scan will not work with prefilled wells during these conditions.
	The deep well plate is not correctly positioned in the Cassette	See *Prepare and insert the Cassette tray, on page 266* for information of how to place the deep well plate.
	Dirt on the nozzle or Drop sync sensor diode windows. Dirt may effect where the drops fall	Clean nozzle and Drop sync sensor diode windows. See *Section 7.4.4 Clean Fraction collector F9-C, on page 381*.
Error message when the door of the fraction collector is opened during a run	Some parts in a method require that the door of the fraction collector is closed	Do not open the door when the Fractionation indicator is lit.

Problem	Possible cause	Action
Fraction collector wash is reported as ***Completed*** when it has been aborted	• The ***Fraction collector wash*** instruction is aborted when the fraction collector door is opened during state pause. • When selecting to continue the run the instruction is reported as ***Completed***, even though it should state ***Cancelled***	Do not open the door during the ***Fraction collector wash*** instruction. If the door has been opened, restart the ***Fraction collector wash*** instruction to make sure that the Fraction collector wash has been performed as recommended.
The instrument does not fractionate via the Outlet valve ports	The Detector - Outlet valve delay volume has been set to zero	1. In ***System Control***, select ***System*** →***Settings*** →***Fraction collections*** →***Delay volumes***. 2. Correct the Detector - Outlet valve delay volume.
When starting a method run during a manual run, the fraction collector generates an error message that wrong Cassettes are in place, even though the correct Cassettes are present in the Fraction collector	A manual run is ongoing	End a manual run before starting a method run.

Problem	Possible cause	Action
High pressure alarm when collecting fractions with the fraction collector	• Run uses too high flow rates for the tubing kit • Blocked or twisted tubing	• Decrease the flow rate or use Outlet valve fractionation, or • replace the following tubing with tubing of larger inner diameter (i.d.): - the tubing between the outlet valve and the fraction collector and - the internal tubing of the fraction collector. ***Note:*** *Update the delay volume. In the* ***System Control*** *software module, select* ***System →Settings →Tubing and Delay volumes*** *and update the value for* ***Delay volume →Monitor to frac****.*
Spillage between fractions	• The Frac arm is moving from Cassette placed in position 1 and 2 to waste • The combination of high flow rates and liquids with low surface tension might lead to spillage in the Fraction collector	If possible, place the Cassettes close to waste, i.e., position 5 or 6 during fractionation. Lower the flow rate when using liquids with low surface tension.
The fractionation starts in the first row again	The door of the fraction collector has been opened between two runs. When the instrument is in state ***Ready*** and the door is opened and closed, the Fraction collector content is reset	If fractionation will continue after the last fraction of the first run, do not open the door of the fraction collector between two runs.

8.5.2 Fraction collector F9-R

Problem	Possible cause	Action
Fraction collector not detected by UNICORN	The UniNet-9 cable between the fraction collector and the ÄKTA system is not properly connected	Make sure that the cable is connected.
	Broken or bad cable	Replace with a new cable.
	Wrong node ID	Check the Node ID of the fraction collector. If necessary, change the Node ID. See *Section 9.16 Node IDs, on page 645*.
The internal temperature of the fraction collector is too high	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact service.
The fraction collector failed to detect a drop and has changed tube without drop sync.	To high flow rate for drop sync	Decrease the flow rate or disable drop sync.
	Air in the flow path	Check the flow path for air. Fill system and purge pumps. If this error is recurrent, generate a System error report and contact service.
	Unclean drop sync sensor	Clean the drop sensor photocell located above the tube sensor with a damp cloth.
	The tubing is not correctly mounted in the tubing holder nut	Check that the tubing is correctly mounted, see *Section 3.3.3 Connect the Fraction collector F9-R, on page 139*.
The tube sensor has not detected a new tube.	The fraction collector movement is blocked	Make sure that the fraction collector can move and is free from obstructions.
The delay queue is full or there is a tube change overload.	The flow rate is too high	Reduce the flow rate.
	The fraction volume is too small	Collect larger fractions.
	Too many fraction collector instructions have been sent	Wait for a while and try again.

Problem	Possible cause	Action
Fraction numbering does not start at 1 when the fractionation is restarted after a ***No tube*** error	Fraction numbering continues from where it was at the time of the ***No tube*** error	Manually reset the fraction number in the ***System settings*** menu.

8.5.3 Fraction collector F9-T

Problem	Possible cause	Action
Fraction collector not detected by UNICORN	The UniNet-9 cable between the fraction collector and the ÄKTA system is not properly connected	Make sure that the cable is connected.
	Broken or bad cable	Replace with a new cable.
	Wrong Node ID	Check the Node ID of the fraction collector. If necessary, change the Node ID. See *Section 9.16 Node IDs, on page 645*.
Drop sync sensor fails to detect drops	The drop sync sensor needs to be cleaned	Clean the drop sync sensor. See *Section 7.4.8 Clean the Fraction collector F9-T drop sync sensor, on page 388* for cleaning instructions.
	Air in the flow path	Check the flow path for air. Fill system and purge the pump. Refer to *ÄKTA pure Operating Instructions (29022997)*.
	Too high flow rate	Decrease the flow rate. Refer to *Fraction Collector F9-T Operation Instructions (29478336)* for appropriate flow rates for different nozzles and plate types.
	Blocked drop sync sensor	Check the drop sync sensor, or if the tubing nozzle is used, that the tubing has correct protrusion for drop sync sensor functionality.
The drop sync sensor signal LED did not manage to adjust the LED power to get a correct drop sync signal	The drop sync sensor needs to be cleaned	Clean the drop sync sensor. See *Section 7.4.8 Clean the Fraction collector F9-T drop sync sensor, on page 388*.
	Blocked drop sync sensor	Remove what is blocking the drop sync sensor, such as salt contamination. ***Note:*** *If the tubing nozzle is used, it must extend 4 mm or it may block the sensor.*
Plates or tubes do not fit in the plate or tube holder	Wrong plates or tubes are used	Check recommended plates or tubes in *Section 3.4 Fraction collector F9-T, on page 143*.
Drops does not hit the tubes inserted in 24 deep-well plates	Tubes are positioned incorrectly in the 24 deep-well plate	Correct positioning of tubes in 24 deep-well plates is described in *Section 3.4 Fraction collector F9-T, on page 143*.

Problem	Possible cause	Action
	Drop position adjustment is not correct	Check that drop position is correct. In the ***System Control*** software module select ***System Settings*** →***System*** →***Adjust Drop Position (F9-T)***.
The green LED light on the dispenser head is off	The LED is switched off in the UNICORN software	Switch on the LED in ***System settings***.
The tubing/nozzle in the Fraction collector is blocked	Salt residuals in the tubing/nozzle	Cleaning of the Fraction collector tubing is described in *Fraction Collector F9-T Operation Instructions (29478336)*.
	The tubing is bent	Replace the tubing.
	The nozzle needs to be replaced	Replacing the nozzle is described in *Fraction Collector F9-T Operation Instructions (29478336)*.
The fraction volume found in the tubes or wells are smaller than expected	Leakage in the flow path	Localize and take care of the leakage, for example by tightening connectors.
	Air in the flow path	Check the flow path for air. Fill system and purge the pump, refer to *ÄKTA pure Operating Instructions (29022997)* for more information.
	Bad pump function	See troubleshooting of pumps in *Section 8.6 Troubleshooting: Pumps, on page 490*.
Liquid on the plate holder or on plates beside wells	The finger tight connector on the nozzle is not tight enough	Tighten the connector. Replace the connector if the leakage is persistent.
	Wrong plate/tube type set for the selected plate position	Check plate/tube type set in the method or manual run.
	Wrong drop position set in ***System control*** →***Adjust Drop Position (F9-T)***	Check that drop position is correct. In the ***System Control*** software module select ***System Settings*** →***System*** →***Adjust Drop Position (F9-T)***.
	The tubes are flooded	Make sure that the fraction volume is adapted to the well/tube volume or that they have been replaced by empty ones. If using 96 microplates with a well volume of less than 300 µL, make sure to change to more narrow tubing, with an inner diameter of 0.25 mm or less and adjust delay volume accordingly.

Problem	Possible cause	Action
Spillage by the Fraction collector during fractionation	The drop sync sensor needs to be cleaned.	Cleaning of Fraction collector drop sync sensor is described in *Section 7.4.8 Clean the Fraction collector F9-T drop sync sensor, on page 388*.
	The nozzle needs to be cleaned	Remove the nozzle from the fractionation arm. Unscrew the two nozzle parts to detach the nozzle from the tubing. Clean it in distilled water, mild cleaning agent or 20% ethanol. Replace if necessary.
	Too high flow rate during usage of drop sync function	See *Section 5.8.1 Fractionation overview, on page 261* for more information on the drop sync function.
	Wrong plate/tube type set for the selected plate position	Check plate/tube type set in the method or manual run.
	Wrong drop position set in ***System Control***	Check that drop position is correct. In the ***System Control*** software module select ***System Settings*** →***System*** →***Adjust Drop Position (F9-T)***. Reset it to default value or adjust it to fit the chosen plate/tube.
	The combination of high flow rates and liquids with low surface tension might lead to spillage in the Fraction collector	Lower the flow rate when using liquids with low surface tension.
Spillage in Fraction collector when the fractionation arm is moving from the plate in position 1 to that in position 2	Too high flow rate during fractionation arm move from plate 1 to plate 2	Check the setting in ***System Control*** →***System Settings*** →***Fraction collector*** →***Flow when moving from plate 1 to plate 2***.
Mismatch between chromatogram and actual fraction number	The delay volume has not been properly set	Adjust the delay volume. In the ***System Control*** software module, select ***System*** →***Tubing and Delay volumes*** and adjust the value for ***Delay volume: Monitor to frac***. See *Section 9.13 Delay volumes, on page 635* for more information.

Problem	Possible cause	Action
The Fraction collector fractionates in the wrong well or tube	Unsupported deep well plate/tubes are used	Check that the used plates or tubes have the right dimensions. See *Plates, on page 149* and *Tubes, on page 149* for information on recommended plates and tubes.
	Wrong plate/tube type set for the selected plate position	Check that the set plates/tubes in the method match those placed in the Fraction collector.
	Plates/tubes are not reset when replaced with new	When starting a ***Method run***, check that plates/tubes are reset in the ***Start Protocol***.
Error upon start of a method run, indicating that the wrong plate/tubes are in place, even though the correct plate/tubes are present in the Fraction collector	The plate/tube type in the text method is not the same as that set in ***Method settings***	End the Method and make sure that the same plate/tube types are set in the text method as in ***Method settings***.
High pressure alarm when collecting fractions with the fraction collector	Too high flow rate during fractionation	Decrease the flow rate or replace the tubing between the outlet valve and the Fraction collector with tubing of larger inner diameter. ***Note:*** *Adjust the delay volume. In the* ***System Control*** *software module, select* ***System →Tubing and Delay volumes*** *and adjust the value for* ***Delay volume: Monitor to frac****.*
The fractionation arm does not return to **Home** after completed run	***Frac behavior when ending a run*** is set to ***Remain without reset*** in ***System Settings →Fraction collection***	To change Fraction collector behavior when ending a run, adjust the settings in ***System Settings →Fraction collection →Frac behavior when ending a run***.
Plates/tubes are not reset after ending a run	***Frac behavior when ending a run*** is set to ***Remain without reset*** or ***Go home without reset*** in ***System Settings →Fraction collection***	Choose ***Go home and reset*** in ***System Settings →Fraction collection →Frac behavior when ending a run***.

8.6 Troubleshooting: Pumps

In this section

Section		See page
8.6.1	Pumps	491
8.6.2	Examples of pump pressure curves	494
8.6.3	Remove persistent air bubbles	497

8.6.1 Pumps

Problem	Possible cause	Action
Liquid is leaking between the pump head and the side panel	Piston seal or rinsing membrane incorrectly fitted or worn	Replace or reinstall the seal or the membrane. For System pumps and Sample pump **S9H**, see *Section 7.5.12 Replace pump piston seals of Pump P9 or P9H, on page 422*. For Sample pump **S9**, see *Section 7.5.13 Replace pump piston seals of Pump P9-S, on page 431*.
Low eluent flow and noise	Air in pumps	Purge the pumps. See *Section 5.4 Prime inlets and purge pump heads, on page 220* or *Section 5.4.2 Sample pump, on page 228*.
	Bad piston spring	Disassemble the pump head and examine the piston spring. If the spring is corroded, check piston seal and rinse membrane. Make sure that the pump piston rinsing system is always used when working with aqueous buffers containing salt.
	Bad pump piston	If the piston is damaged, replace it according to *Section 7.5.14 Replace pump pistons, on page 439*.
	Bad pump piston seal	Replace the piston seal and rinse membrane according to *Section 7.5.12 Replace pump piston seals of Pump P9 or P9H, on page 422* or *Section 7.5.13 Replace pump piston seals of Pump P9-S, on page 431*.
Leakage around a connector	Leaking connection and/or crystallized material around a connector	Unscrew the connector and check if it is worn or incorrectly fitted. If so, replace the connector. Gently finger tighten the connector.
Erratic pump pressure	• Air trapped in the pump heads • Partially blocked solvent filters • Leaking connections • Piston seal leakage • Check valve malfunction • Piston damaged	See *Section 8.6.2 Examples of pump pressure curves, on page 494* for examples of pump pressure curves.

Problem	Possible cause	Action
The pump is not found by the instrument	The cable between the system pump and the Instrument control unit (ICU) is not connected.	Contact Service.
	The cable between the sample pump and the ÄKTA pure instrument is not connected.	Connect the cable.
	A fuse in the instrument ICU is broken.	Contact Service.
The internal temperature of the pump is too high	The air intake on the rear or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
High pressure alarm	The pressure has increased due to increased viscosity	The viscosity increases in cold room. Lower the flow when performing runs in a cold room.
High pressure alarm when pressure control is activated	The parameter selected for pressure control is not the most appropriate one	The pressure control is based on either the pre-column pressure or the delta-column pressure. To change the parameter for pressure control, select ***Pre column pressure*** or ***Delta column pressure*** from the ***Pressure control*** drop-down list in the ***Instruction box*** of the instruction of interest.
	The flow is too high	Lower the flow.
Abnormal difference in system pressure compared to pre-column pressure	Clogged online filter	Replace the online filter, see *Section 7.5.9 Replace the online filter, on page 417*.
Internal pump error combined with high pressure	The flow path is blocked	Remove obstructions in the flow path. For example, remove stop plugs and replace constricted tubing.

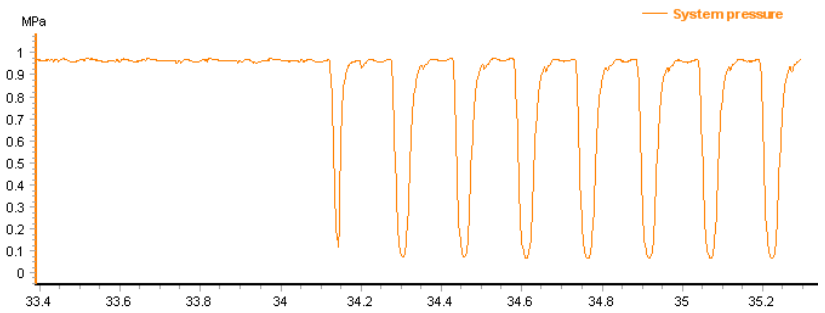
Problem	Possible cause	Action
Internal pump error at normal pressure	Blocked pump restrictor	Contact Service.
Too slow pressure build up when pressure control is active	Too low ***I factor*** in the ***Pressure control parameters*** instruction	In the ***Manual Instructions*** dialog box, increase the ***I factor*** of the ***Pressure control parameters*** instruction.
Too slow pressure build up when using constant pressure flow	Too low ***I factor*** in the ***Constant pressure flow parameters*** instruction	In the ***Manual Instructions*** dialog box, increase the ***I factor*** of the ***Constant pressure flow parameters*** instruction.
Pressure overshoot or oscillating pressure when pressure control is active	Too high ***I factor*** in the ***Pressure control parameters*** instruction	In the ***Manual Instructions*** dialog box, decrease the ***I factor*** of the ***Pressure control parameters*** instruction.
Pressure overshoot or oscillating pressure when using constant pressure flow	Too high ***I factor*** in the ***Constant pressure flow parameters*** instruction	In the ***Manual Instructions*** dialog box, decrease the ***I factor*** of the ***Constant pressure flow parameters*** instruction.

8.6.2 Examples of pump pressure curves

The table below shows some examples of pump system pressure curves obtained when errors have occurred. The examples can be useful in troubleshooting of the system pumps and the sample pump. The system pressure monitor R9 has higher resolution than the other pressure monitors, and is therefore recommended for troubleshooting purposes.

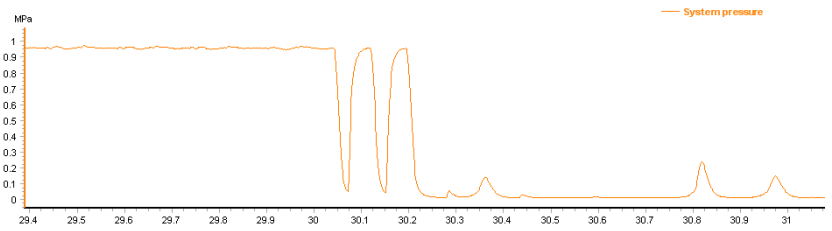
Incident: 30 µl of air enters pump.

Action: Purge pump. See *Section 5.4 Prime inlets and purge pump heads, on page 220*. If the problem is persistent, see *Section 8.6.3 Remove persistent air bubbles, on page 497*.



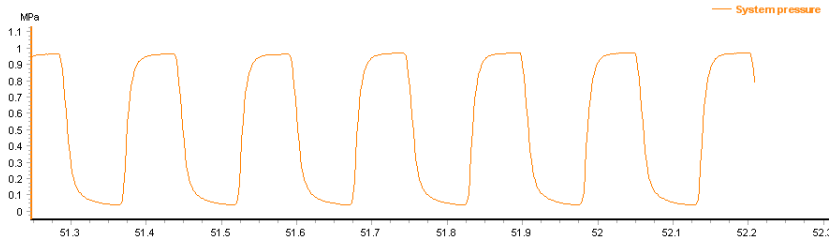
Incident: A large volume of air enters pump.

Action: Purge pump. See *Section 5.4 Prime inlets and purge pump heads, on page 220*.



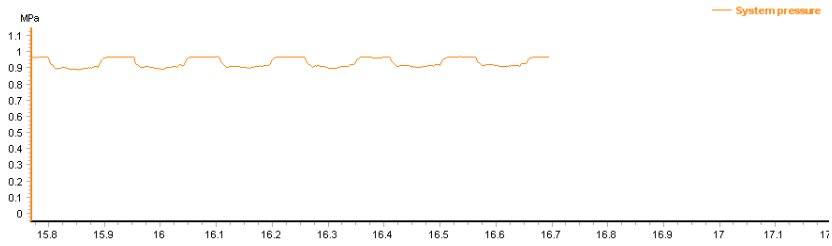
Incident: Blocked outlet check valve.

Action: Clean the check valve, See *Section 7.4.12 Clean the pump head check valves, on page 397*.



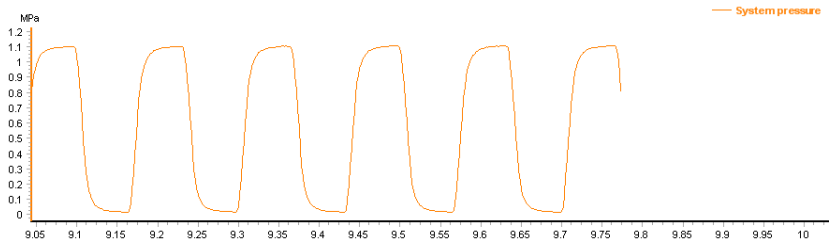
Incident: Inlet check valve is loose.

Action: Tighten the check valve. See *Section 7.5.11 Replace the pump head check valves, on page 419*.



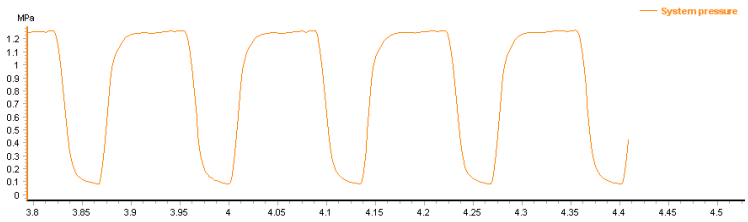
Incident: Leaking inlet check valve.

Action: Replace the check valve. See *Section 7.5.11 Replace the pump head check valves, on page 419*.



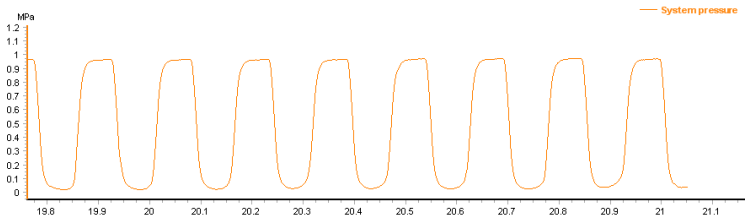
Incident: Leaking outlet check valve.

Actions: Replace the check valve. See *Section 7.5.11 Replace the pump head check valves, on page 419*.



Incident: One inlet is blocked.

Action: Clean inlet tubing. For example, perform a System CIP.



8.6.3 Remove persistent air bubbles

After purging the pump (see *Section 5.4 Prime inlets and purge pump heads, on page 220*), check that all air bubbles have been removed by analyzing the system pressure curve (see examples above). If the pressure curve indicates that there are still air bubbles present, repeat the purging process. If the problem persists, follow the instructions below to purge the pump with methanol (see *Section 5.4 Prime inlets and purge pump heads, on page 220* for detailed instructions for the purge procedure). Alternatively, follow the procedure in *Break in the new pump piston seal, on page 429*.

Step	Action
1	Connect the pump inlet to a vessel containing 100% methanol.
2	Use a syringe to prime both pump heads with methanol (see *Section 5.4 Prime inlets and purge pump heads, on page 220* for details).
3	Set the pump flow to 2.5 mL/min for ÄKTA pure 25, or 10 mL/min for ÄKTA pure 150, or 0.25 mL/min for ÄKTA pure micro.
4	Let the flow run until the disturbances in the pressure curve disappear.
5	Remove the methanol: **a.** Stop the pump and move the inlet tubing to water. Make sure that no air is introduced into the system. **b.** Set the pump flow to 1 mL/min for ÄKTA pure 25, or 5 mL/min for ÄKTA pure 150, or 0.25 mL/min for ÄKTA pure micro. **c.** Run for 5 minutes.
6	Purge the pump again using an appropriate buffer.

8.7 Troubleshooting: Other components

In this section

Section		See page
8.7.1	General hardware: All modules	499
8.7.2	Mixer	500
8.7.3	Module Panel	502
8.7.4	Superloop	503
8.7.5	Cabinet	504
8.7.6	Power and ICU	505
8.7.7	External air sensors	507
8.7.8	I/O-box E9	508
8.7.9	Instrument control panel	510

8.7.1 General hardware: All modules

Problem	Possible cause	Action
Modules cannot be found by the instrument	The cable between a module and the ICU is not connected	Remove the module and make sure that the cable is connected.
	Two similar modules have been added to the instrument, for example two Inlet valve A	Remove one of the modules with the same Node ID.
	The Node ID for one or more of the modules is incorrect, for example an Inlet valve A2 has the same Node ID as Inlet valve A. The instrument then considers them to have the same identity.	Remove Inlet valve A2 and change the Node ID according to *Section 9.16 Node IDs, on page 645*.
An unknown instrument module is connected to the system	The Node ID for one or more of the modules is incorrect	Check Node ID and change the Node ID according to *Section 9.16 Node IDs, on page 645*.

8.7.2 Mixer

Problem	Possible cause	Action
Leakage	Leaking tubing connections	Check the tubing connections. Tighten or replace if necessary. Check the O-ring. Replace it if it is damaged. See *Section 7.5.5 Replace the O-ring inside the mixer, on page 409*. Check the Mixer chamber. Replace it if the liquid has penetrated the Mixer chamber walls and sealings. See *Section 7.5.4 Replace the mixer, on page 408*. See *Chapter 10 Ordering information, on page 649*.
Unstable gradients	Bad mixing of eluents	Check the function of the Mixer. Place a stirrer bar in the palm of your hand. Hold the hand above the Mixer. The stirrer should move when the Mixer is activated. Check the Mixer chamber size and change chamber if necessary. See *Section 7.5.4 Replace the mixer, on page 408*.
Noisy UV signal	Bad mixing of eluents	Check the function of the Mixer. Place a stirrer bar in the palm of your hand. Hold the hand above the Mixer. The stirrer should move when the Mixer is activated. Check the Mixer chamber size and change chamber if necessary, see *Section 5.2.2 Select mixer chamber, on page 211*.
The Mixer chamber was not recognized	The Mixer chamber is not correctly installed	Verify that the Mixer chamber is correctly installed. See *Section 7.5.4 Replace the mixer, on page 408*. If the error is recurrent, replace the Mixer chamber. See *Chapter 10 Ordering information, on page 649*.
The internal Mixer temperature is too high	The air intake on the rear or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.

Problem	Possible cause	Action
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.

8.7.3 Module Panel

Problem	Possible cause	Action
The instrument is unable to find some of the modules	A Module Panel is missing and the position is left empty	Install the missing Module panel.
	The cable between the Module Panel and the instrument control unit (ICU) is not connected	Remove the Module Panel and make sure that the cable is connected.

8.7.4 Superloop

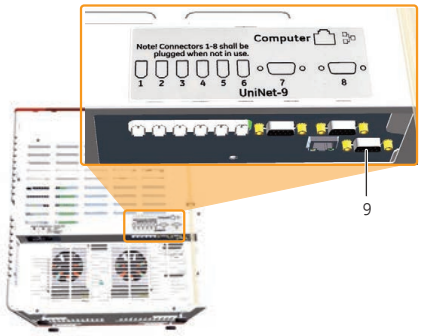
Problem	Possible cause	Action
Overpressure during filling	The Superloop is filled to the maximum capacity. The pressure is not released anywhere.	Manually turn Injection valve to ***Manual load*** position.

8.7.5 Cabinet

Problem	Possible cause	Action
The temperature of the instrument or an instrument component is too high	The air intake on the back or on the left side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Broken fans	Contact Service.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
Liquid from the Buffer tray is flowing onto the table	The waste tubing from the Buffer tray is loose	Contact Service.

8.7.6 Power and ICU

Problem	Possible cause	Action
The instrument cannot be turned on	The power cord is not connected	Connect the power cord to the wall outlet and to the electrical inlet on the instrument. Make sure that the cord is attached using the clip, thereby preventing the cord from coming loose.
	No electric current in the wall outlet	Make sure that there is electric current in the wall outlet.
	A fuse in the instrument control unit (ICU) is broken	Contact Service.
	The instrument is overheated	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
One or more modules are automatically turned off	One or some of the minor modules use too much current. Minor modules include all modules except the Fraction collector, the UV monitor and the pumps.	The current is cut off by a temperature sensitive component. The instrument can be restarted when the temperature has decreased. If persistent, generate a System error report and contact Service.
The instrument cannot be seen in UNICORN	The network cable is not connected	Connect the cable, see *UNICORN Administration and Technical Manual*.
One or more module(s) is not found by the instrument	The cable between a module and the ICU is not connected.	Remove valves and check that cables are connected.

Problem	Possible cause	Action
	One or more jumpers are loose or missing.	Check the connections on the back of the instrument. Empty positions for **UniNet-9** connectors 1 to 8 must have connected jumpers. See the illustration below. The connector 9 must be protected with a plastic lid and must never have a jumper connected to it. 
The internal instrument temperature is too high	The air intake at the rear or on the right side of the instrument is covered	Make sure that none of the air intakes on the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.

8.7.7 External air sensors

Problem	Possible cause	Action
The external air sensor is not found by the instrument	The cable between the external air sensor and the instrument control unit (ICU) is not connected	Check the back of the instrument and make sure that the cable is connected.
	The external air sensor has not been selected in UNICORN	Select the external air sensor in UNICORN. Refer to the installation instructions delivered together with the external air sensor.
Air is introduced into the flow path	One of the connections is not tight enough	Tighten the connectors.
Liquid is leaking from the external air sensor	One of the connections is not tight enough	Tighten the connectors.
The internal temperature of the air sensor is too high	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.

8.7.8 I/O-box E9

Problem	Possible cause	Action
The internal temperature of the I/O-box is too high	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.
The I/O-box is not detected by the system	Wrong Node ID	Make sure that the Node ID is (00) for the primary box, and (01) for a potential secondary box. See *Section 9.16 Node IDs, on page 645*.
The system does not detect Digital signals correctly	Cables incorrectly connected	Make sure that the cables are connected correctly, earth to earth etc.
Digital In always "0", regardless of input signals	Digital Out connections switched	Check that the digital Out cables are correctly connected, earth to earth etc.
External digital equipment does not respond to set changes in Digital Out	Cables incorrectly connected	Make sure that the cables are connected correctly, earth to earth etc.
	To isolate the problem: 1. Connect Digital Out (e.g. pin 6) to Digital In (pin 1,2,3, or 4). 2. Change the Digital Out 1 signal between "1" and "0". Verify that Digital out follows.	If not: Contact Service. If it does: The problem probably lies within the connected equipment.
Analog In does not measure expected voltage	Autozero on the wrong level	Reset Autozero.
	Digital Out connection error	Make sure that Digital Out is connected correctly, earth to earth etc.
Noisy analog signal	Too long cable between the external equipment and E9	Use as short cable as possible. Use shielded cable. Connect the cable shield to the D-sub connector's shield.

Problem	Possible cause	Action
Analog In does not measure expected voltage	Analog In is not calibrated	Contact Service.
Analog Out does not generate expected voltage	Analog Out is not calibrated	Contact Service.

8.7.9 Instrument control panel

Problem	Possible cause	Action
The internal temperature of the instrument control panel is too high	The air intake on the rear or on the right side of the instrument is covered.	Make sure that none of the air intakes of the instrument are covered.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Hardware error	Switch off the instrument and wait until the temperature has decreased. Restart the instrument. If this error is recurrent, generate a System error report and contact Service.

8.8 Troubleshooting: Instrument communication

Problem	Possible cause	Action
Multiple error messages in UNICORN: ***Lost modules***	A cable to a module (including Module Panels) is not connected	Connect the cable: 1. Switch off the instrument. 2. Check all modules and connections. 3. Switch on the instrument. 4. In the displayed dialog box in UNICORN, select the option ***Restart the system only*** and click ***OK***.
	A **UniNet-9** connector is not plugged	Check that all **UniNet-9** connectors that are not in use are plugged.
UNICORN has lost communication with the instrument server	The UNICORN client has lost connection to the instrument server during a temporary overload of the processor	Restart the UNICORN client to regain control. The run continues and no data will be lost.
Warning message in UNICORN: ***Instrument module is missing***	The module is not functioning properly	In the displayed dialog box in UNICORN, select the option ***Restart the system only*** and click ***OK***. If the problem still remains, replace the module or contact Service.
Warning messages in UNICORN: ***(Warning) Two instrument modules have the same Node ID***	Two or several modules have the same Node ID	Change to correct Node IDs: 1. Switch off the instrument. 2. Check the Node ID for all modules, see *Section 9.16 Node IDs, on page 645*. 3. Switch on the instrument. 4. In the displayed dialog box in UNICORN, select the option ***Restart the system only*** and click ***OK***.

Problem	Possible cause	Action
Warning messages in UNICORN: ***(Warning) Gate (12)→Internal instrument error***	One module has an incorrect Node ID	Change the Node ID: 1. Switch off the instrument. 2. Check the Node ID for all modules, see *Section 9.16 Node IDs, on page 645*. 3. Switch on the instrument. 4. In the displayed dialog box in UNICORN, select the option ***Restart the system only*** and click ***OK***.

8.9 Troubleshooting: Method development

Problem	Possible cause	Action
Phase properties or text instructions are not available in the method editor as expected	The module required for the instruction has not been enabled in the component selection in the administration module	Review the component list.
	The wrong system has been selected when creating the new method	Make sure that the right system has been selected in the "new method" dialog box.
UNICORN notification: ***This method was created for a different configuration. Do you want to adapt method or keep text method?***	A method has been created for a system that now has a different configuration	1. Open the method in the method editor. 2. Select to either adapt the method to the new configuration or Keep the text method unchanged and manually replace the text instructions that are not available for the new configuration. Refer to *Open and save methods for different systems, on page 288* for more information.
An old method is to be transferred to a new system configuration.	N/A	1. Open the method in the method editor. 2. Save as and select the new system. or If the configuration is different on the new system, select to either adapt the method to the new configuration or to keep the text method unchanged and manually replace the text instructions that are not available. Refer to *Open and save methods for different systems, on page 288* for more information.

8.10 Error codes

Introduction

This section describes the error codes that can appear for the different modules, together with corrective actions.

All modules

Error code	Description	Action
0 - 19	Internal instrument error	Restart the instrument. If recurrent please contact Service.

Instrument control unit

Error code	Description	Action
21 - 69	Internal instrument error	Restart the instrument. If recurrent please contact Service.

Valve

Error code	Description	Action
20, 24	Internal instrument error	Restart the instrument. If recurrent please contact Service.
22	Valve not finding position	Restart the instrument. If recurrent please contact Service.
23	Faulty air sensor	Restart the instrument. If recurrent please contact Service.
25	High temperature	See *Section 8.4 Troubleshooting: Valves, on page 474*.

Pressure monitor

Error code	Description	Action
20, 21, 24-27	Internal instrument error	Restart the instrument. If recurrent please contact Service.

Error code	Description	Action
23	High temperature	See *Section 8.3.5 Pressure monitors, on page 472*.

Air sensor

Error code	Description	Action
20	High temperature	See *Section 8.7.7 External air sensors, on page 507*.

Mixer

Error code	Description	Action
25	Mixer motor error	Restart the instrument. If recurrent please contact Service.
26	Internal instrument error	Restart the instrument. If recurrent please contact Service.
27	High temperature	See *Section 8.7.2 Mixer, on page 500*.

pH monitor

Error code	Description	Action
20, 21	Internal instrument error	Restart the instrument. If recurrent please contact Service.
25	No factory calibration	Contact Service.
26	High temperature	See *Section 8.3.4 pH monitor and pH valve, on page 470*.

Conductivity monitor

Error code	Description	Action
20-27	Internal instrument error	Restart the instrument. If recurrent please contact Service.
28	High temperature	See *Section 8.3.3 Conductivity monitor, on page 467*.

Error code	Description	Action
29	Temperature data error	Restart the instrument. If recurrent please contact Service.
32-34	No factory calibration	Contact Service.

Pump

Error code	Description	Action
51-53	Internal pump error	Check that there is no blockage of the pump outlet. Restart the instrument. If recurrent please contact Service.
54	High temperature	See *Section 8.6 Troubleshooting: Pumps, on page 490*.

UV monitor U9-M

Error code	Description	Action
21, 25, 26, 31	Grating error	Restart the instrument. If recurrent please contact Service.
22, 23, 32	Block filter error	Restart the instrument. If recurrent please contact Service.
24	Internal instrument error	Restart the instrument. If recurrent please contact Service.
27	Spectrum calibration error	Restart the instrument. If recurrent please contact Service.
28, 29	Lamp error	Restart the instrument. If recurrent please contact Service.
30	High temperature	Switch off the instrument, then check the troubleshooting section, see *Section 8.3.1 UV monitor U9-M and UV detector U9-D, on page 459*.

UV detector U9-D

Error code	Description	Action
24, 26, 28, 29, 31, 33	Internal instrument error	Restart the instrument. If recurrent please contact Service.
25	Too low light intensity	Check that the detector and flow cell are fitted correctly. If warning reappears, contact Service.
27	Too high light intensity	Check that the detector and flow cell are fitted correctly. If warning reappears, contact Service.
30	Too high light intensity, R channel	Check that the detector is fitted correctly. If warning reappears, contact Service.
32	Too high S light intensity, S channel	Check that the flow cell is fitted correctly. If warning reappears, contact Service.
34	No light detected	Check optical pathway and restart the instrument. If recurrent please contact Service.
35	Too low light intensity, R channel	Check that the detector is fitted correctly. If warning reappears, contact Service.
36	Too low light intensity, S channel	No light through flow cell. Check solution absorption and that the cell is fitted correctly.

UV monitor U9-T

Error code	Description	Action
51	High temperature	Switch off the instrument, then check the troubleshooting section, see *Section 8.3.2 UV monitor U9-T and U9-L, on page 463*.
52	The 280 nm lamp is close to its lifetime limit	Contact Service.
54	Autozero, the 280 nm lamp is out of range	Autozero requested for 280 nm when AU value is larger than 2.

Error code	Description	Action
55	The 280 nm lamp has reached its lifetime limit	Contact Service.
57	Autozero, the 260 nm lamp is out of range	Autozero requested for 260 nm when AU value is larger than 2.
58	Too low light intensity, S channel	No light through flow cell. Check solution absorption and that the cell is fitted correctly.
59	Internal instrument error	Restart the instrument. If recurrent please contact Service.
60	Internal instrument error	Restart the instrument. If recurrent please contact Service.
61	The 260 nm lamp is close to its lifetime limit	Contact Service.
62	The 260 nm lamp has reached its lifetime limit	Contact Service.
64	Internal instrument error	Check that UV cell is installed correctly, that the ports is connected or has a stop plug. Restart instrument. If recurrent, please contact Service.
65	Internal instrument error	Check that UV cell is installed correctly, that the ports is connected or has a stop plug. Restart instrument. If recurrent, please contact Service.
66	Internal instrument error	Check that UV cell is properly installed. Restart instrument. If error remains, please contact Service.

UV monitor U9-L

Error code	Description	Action
51	High temperature	Switch off the instrument, then check the troubleshooting section, see *Section 8.3.2 UV monitor U9-T and U9-L, on page 463*.

Error code	Description	Action
52	Low lamp intensity	Contact Service.
54	Autozero out of range	Autozero requested when AU value is larger than 2.
55	Low lamp intensity	Contact Service.
58	Too low light intensity, S channel	No light through flow cell. Check solution absorption and that the cell is fitted correctly.
59	Internal instrument error	Restart the instrument. If recurrent please contact Service.
60	Internal instrument error	Restart the instrument. If recurrent please contact Service.
64	Internal instrument error	Check that UV cell is installed correctly. Restart instrument. If recurrent, please contact Service.

Fraction collector F9-C

Error code	Description	Action
20 - 22	Internal instrument error	Restart the fraction collector with the power switch on the ÄKTA pure instrument. If recurrent, generate a system error report and contact service.
32	The fraction collector failed to operate the accumulator	Restart the fraction collector with the power switch on the ÄKTA pure instrument. If recurrent, generate a system error report and contact service.
33 - 35, 37 - 40, 55	Fraction collector configuration error	The instrument restarts. If the error is recurrent, generate a system error report and contact service.
47 - 48	The fraction collector failed to detect the code on the cassette or the type of plate	Clean the cassette code reader, the cassette type code on the cassette and the plate.

Error code	Description	Action
53	Calibration of the drop-sync sensor failed	Clean the Drop sync sensor, see user documentation for the location of the sensor. If this error is recurrent, please contact service.
54	Fraction collector movement is blocked. Either the door is open or there are communi-cation problems	Close the door of the fraction collector.
57 - 59	Internal fraction collector error	The instrument restarts. If the error is recurrent, generate a system error report and contact service.
63	Tube change is too fast	Lower the flow rate or increase the fractionation volume.
68, 72	Fraction collector arm movement is blocked	The instrument restarts. If the error is recurrent, generate a system error report and contact service.

Fraction collector F9-R

Error code	Description	Action
20	High temperature	See *Section 8.5 Troubleshooting: Fraction collectors, on page 475*.
21	Drop sync warning	Clean the sensor and remove air bubbles in the flow path.
22	Tube sensor error	Check that the tube sensor is adjusted properly.
23	Delay queue full	Increase the fraction size. Fraction size must be greater than 1/10 of the delay volume.
24, 26	Internal instrument error	Restart the instrument. If recurrent please contact Service.
25, 28	Too fast tube change	Increase the fraction size or lower the flow rate.
27	Drop sync error	Clean the drop sensor, see *Cleaning the DropSync sensor, on page 385*.

Fraction collector F9-T

Error code	Description	Action
20	The internal temperature of the Fraction collector is too high	See *Section 8.5.3 Fraction collector F9-T, on page 486*.
21	The Fraction collector failed to detect a drop	See *Section 8.5.3 Fraction collector F9-T, on page 486*.
23	The fractionation volume is too small compared to the delay volume. New position changes will be lost.	Increase fractionation volume.
26	Delayed position change	Avoid mixing fast and slow longer movements of the fraction collector. Check also that the drop sync works properly.
27	An invalid mode change was requested	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.
28	Too fast position changes	Reduce flow or increase the fractionation volume.
29	Axis not verified. Start fractionation was attempted without a successful Find Zero Position	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.
30	Position filled	Reduce fractionation time or flow.
31	Error in one or both optical sensors on the y axis	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.
32	Restart position error. An error occurred when trying to move to the next start position.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.

Error code	Description	Action
33	Encoder has identified a loss of steps	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.
35	Find zero position failed	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report and contact service.
36	Drops were detected during two movements in a row	Decrease the flow rate or turn drop sync off, see *Fraction collector F9-T, on page 582*.

Instrument control panel

Error code	Description	Action
51	High temperature	See *Section 8.7.9 Instrument control panel, on page 510*.

I/O-box

Error code	Description	Action
20	High temperature	See *Section 8.7.8 I/O-box E9, on page 508*.
21	Analog in signal below -2V	Check the external equipment connected to the I/O-box.
22	Analog in signal above 2V	Check the external equipment connected to the I/O-box.
23-28	Internal instrument error	Restart the instrument. If recurrent please contact Service.

9 Reference information

About this chapter

This chapter lists the allowed environmental and operational ranges for ÄKTA pure. Refer to *ÄKTA pure Product Documentation* for detailed technical specifications.

In this chapter

Section		See page
9.1	System specifications	524
9.2	Module specifications	526
9.3	Tubing and connectors	536
9.4	Recommended tubing kits for prepacked columns	546
9.5	Chemical resistance guide	549
9.6	Wetted materials	555
9.7	Predefined methods and phases	557
9.8	System settings	567
9.9	Manual instructions	593
9.10	Available Run data	625
9.11	Available Curves	630
9.12	Injection volumes and peak broadening	634
9.13	Delay volumes	635
9.14	Component volumes	640
9.15	Pressure control	643
9.16	Node IDs	645

9.1 System specifications

System specifications

Parameter	Data
System configuration	Benchtop system, external computer
Control system	Refer to *cytiva.com/UNICORNcompatibility*
Connection between PC and instrument	Ethernet
Dimensions (W x D x H)	535 × 470 × 630 mm
Weight (excluding computer)	Up to 53 kg
Power supply	100 to 240 VAC, 50 to 60 Hz
Power consumption	300 VA (typical) 25 VA (power-save)
Enclosure protective class	IP 21
Tubing and connectors	• Inlet: FEP tubing, i.d. 1.6 mm, 5/16 to 24 UNF connections • Pump to injection valve: PEEK tubing, i.d. 0.75 mm (ÄKTA pure micro: i.d. 0.25 mm), 10-32 UNF connections • After Injection valve: PEEK tubing, i.d. 0.50 mm (ÄKTA pure micro: i.d. 0.13 mm), 10 to 32 UNF connections • Outlet and waste: ETFE tubing, i.d. 1.0 mm, Fingertight connector, 1/16" • Optional tubing kits: i.d. 0.25 mm, i.d. 0.75 mm, i.d. 1.0 mm

Environmental ranges

Parameter	Data
Storage and transport temperature range	-25°C to +60°C for 48 hours

Parameter	Data
Chemical environment	See *Section 9.5 Chemical resistance guide, on page 549*.

Operating ranges

Parameter	Data
Operating temperature range	4°C to 35°C
Relative humidity	20% to 95%, non-condensing

Equipment noise level

Equipment	Acoustic noise level
ÄKTA pure instrument	< 60 dB(A)

9.2 Module specifications

Introduction

This section specifies the operating data of the components in ÄKTA pure. For general data for the system, see *System specifications, on page 524*.

System pumps

Parameter	Data
Pump type	Piston pump, metering type
Flow rate range	ÄKTA pure 25: 0.001 to 25 mL/min (up to 50 mL/min during column packing) ÄKTA pure 150: 0.01 to 150 mL/min (up to 300 mL/min during column packing)
Pressure range	ÄKTA pure 25: 0 to 20 MPa (2900 psi) ÄKTA pure 150: 0 to 5 MPa (725 psi)
Viscosity range	ÄKTA pure 25: 0.35 to 10 cP (5 cP above 12.5 mL/min) ÄKTA pure 150: 0.35 to 5 cP
Flow rate specifications	ÄKTA pure 25: • Accuracy: ± 1.2% • Precision: RSD < 0.5% (Conditions: 0.25 to 25 mL/min, < 3 MPa, 0.8 to 2 cP) ÄKTA pure 150: • Accuracy: ± 1.5% • Precision: RSD < 0.5% (Conditions: 1.0 to 150 mL/min, < 3 MPa, 0.8 to 2 cP)

Sample pump

Parameter	Data
Pump type	Piston pump, metering type
Dimensions (W x D x H)	215 x 370 x 210 mm
Weight	11 kg
Flow rate range	ÄKTA pure 25: 0.001 – 50 mL/min ÄKTA pure 150: 0.01 to 150 mL/min
Pressure range	ÄKTA pure 25: 0 to 10 MPa (1450 psi) ÄKTA pure 150: 0 to 5 MPa (725 psi)
Viscosity range	0.7 to 10 cP
Flow rate specifications	ÄKTA pure 25: • Accuracy: ± 2% • Precision: RSD < 0.5% (Conditions: 0.25 – 50 mL/min, < 3 MPa, 0.8 – 3 cP) ÄKTA pure 150: • Accuracy: ± 2% • Precision: RSD < 0.5% (Conditions: 1.0 – 150 mL/min, < 3 MPa, 0.8 – 3 cP)

Valves

Parameter	Data
Type	Rotary valves
Number of valves	Up to 12
Functions	Standard: Injection Options: Inlet A, Inlet B, Sample inlet, Extra inlet, Mixer by-pass, Loop selection, Column selection, pH, Outlet, Versatile

Inlet options

Parameter	Data
Inlet A	1, 2 or 7 inlets
Inlet B	1, 2 or 7 inlets
Sample inlet	Up to 7 sample inlets and 1 buffer inlet

Outlet options

Parameter	Data
Number of outlets	1 or 10

Mixer

Parameter	Data
Mixing principle	Chamber with magnetic stirrer
Mixer volume	ÄKTA pure 25: 0.6, 1.4 or 5 mL ÄKTA pure 150: 1.4, 5 or 15 mL

Gradient formation

Parameter	Data
Gradient flow rate range	ÄKTA pure 25: 0.1 to 25 mL/min ÄKTA pure 150: 0.5 to 150 mL/min ÄKTA pure micro/Micro kit: 0.05 to 2 mL/min

Parameter	Data
Gradient composition accuracy	ÄKTA pure 25: ± 0.6% (Conditions 5 to 95% B. 0.5 to 25 mL/min, 0.2 to 2 MPa, 0.8 to 2 cP) ÄKTA pure 150: ± 0.8% (Conditions 5 to 95% B. 2 to 150 mL/min, 0.2 to 2 MPa, 0.8 to 2 cP)

Pressure monitors

Parameter	Data
Number of sensors	Up to 4
Placement of sensors	Standard: The System pressure monitor is located after the System pump Options: • The Pre-column pressure monitor and the Post-column pressure monitor are integrated in Column valve **V9-C** or **V9H-C**. • The Sample pressure monitor is located after the Sample pump.

External air sensor options

Parameter	Data
Number of sensors	Up to 7
Placement	• Integrated in inlet valve A, inlet valve B and sample inlet valve • After the injection valve • Before the system pumps • Before the sample pump
Sensing principle	Ultrasonic

UV monitor options

Parameter	Data
Number of monitors	Up to 2
Wavelength range	**U9-M**: 190 to 700 nm in steps of 1 nm, up to 3 wavelengths **U9-T**: 280 nm, or 280 nm and 260 nm **U9-L**: 280 nm
Absorbance range	-6 to 6 AU
Resolution	0.001 mAU
Linearity	**U9-M**: within ± 2% at 0 to 2 AU **U9-T**: within ± 3% at 0 to 2 AU **U9-L**: within ± 5% at 0 to 2 AU
Drift	**U9-M** (2 mm cell at 280 nm): ≤ | 0.2 mAU | AU/h **U9-T** (2 mm cell at 260 nm and 280 nm): ≤ | 0.2 mAU | AU/h **U9-L** (2 mm cell): ≤ | 0.2 mAU | AU/h
Noise	**U9-M**: < 0.08 mAU **U9-T**: < 0.06 mAU **U9-L**: < 0.1 mAU
Operating pressure	0 to 2 MPa
Lamp operating time	**U9-M**: > 5000 h **U9-T**: > 4000 h **U9-L**: > 10 000 h

Parameter	Data
Flow cells: **U9-M**	Standard: Optical path length 2 mm Cell volume 2 µL Total volume 11 µL Micro: Optical path length 2 mm Illuminated volume 0.8 µL Total volume 2.6 µL Optional: Optical path length 10 mm Cell volume 8 µL Total volume 12 µL Optical path length 5 mm Cell volume 7 µL Total volume 12 µL Optical path length 0.5 mm Cell volume 1 µL Total volume 10 µL
Flow cells: **U9-T**	Standard: Optical path length 2 mm Cell volume 2 µL Total volume 30 µL Optional: Optical path length 5 mm Cell volume 6 µL Total volume 20 µL Optical path length 0.4 mm Cell volume 1 µL Total volume 17 µL

Parameter	Data
Flow cells: **U9-L**	Standard: Optical path length 2 mm Cell volume 2 µL Total volume 30 µL Optional: Optical path length 5 mm Cell volume 6 µL Total volume 20 µL Optical path length 0.4 mm Cell volume 1 µL Total volume 17 µL

Conductivity monitor options

Parameter	Data
Conductivity reading range	0.01 to 999.99 mS/cm
Accuracy	± 0.01 mS/cm or ± 2%, whichever is greater, (within 0.3 to 300 mS/cm)
Operating pressure	0 to 5 MPa
Flow cell volume	Conductivty cell **C9n** 22 µL Conductivty cell **C9M** 5.4 µL
Temperature monitor range	0°C to 99°C
Temperature monitor accuracy	± 1.5°C within 4°C to 45°C

pH monitor option

Parameter	Data
pH reading range	0 to 14

Parameter	Data
Accuracy	± 0.1 pH unit within pH 2 to 12, temperature within ± 3°C from calibration temperature
Operating pressure	0 to 0.5 MPa
Flow cell volume	ÄKTA pure 25: 76 µL ÄKTA pure 150: 129 µL

Outlet valve fractionation option

Parameter	Data
Number of outlets	10
Fraction volumes	0.01 to 20 000 mL
Delay volume (UV – outlet valve)	ÄKTA pure 25: • 125 µL • 66 µL with optional tubing kit (i.d. 0.25 mm) ÄKTA pure 150: • 296 µL • 245 µL with optional tubing kit (i.d. 0.5 mm) ÄKTA pure micro/ Micro kit: • 12 µL

Fraction collector options

Parameter	Data
Number of fraction collectors	Up to two. The second fraction collector must be an **F9-R**.
Number of fractions	**F9-C**: Up to 576 **F9-R**: Up to 175 **F9-T**: Up to 192

Parameter	Data
Vessel types	**F9-C**: • Deep well plates, 96, 48 or 24 wells • Tubes 3, 5, 8, 15, 50 mL • Bottle, 250 mL **F9-R**: 3, 5, 8, 15 or 50 mL tubes **F9-T**: • Deep well plates, 96, 48 or 24 wells • Tubes 0.5, 1.5, 2, 15, 50 mL
Fraction volumes	**F9-C**: 0.1 to 250 mL **F9-R**: 0.1 to 50 mL **F9-T**: 0.2 to 50 mL
Spillage-free mode	**F9-C**: Automatic, Drop sync or Accumulator **F9-R**: Drop sync **F9-T**: Drop sync
Flammable liquids	**F9-C**: no **F9-R**: yes **F9-T**: yes
Delay volume (UV – dispenser head)	ÄKTA pure 25: **F9-C**: 435 µL, 214 µL with optional tubing kit (i.d. 0.25 mm) **F9-R**: 205 µL, 86 µL with optional tubing kit (i.d. 0.25 mm) **F9-T**: 215 µl, 96 µl with optional tubing kit (i.d. 0.25 mm) ÄKTA pure 150: **F9-C**: 876 µL, 508 µL with optional tubing kit (i.d. 0.5 mm) **F9-R**: 473 µL, 278 µL with optional tubing kit (i.d. 0.5 mm) ÄKTA pure micro/Micro kit: **F9-R**: 18 µl **F9-T**: 18 µl
Dimensions (W x D x H)	**F9-C**: 390 x 585 x 320 mm **F9-R**: 320 x 400 x 250 mm **F9-T**: 320 x 270 x 190 mm

Parameter	Data
Weight	**F9-C**: 21 kg **F9-R**: 5 kg **F9-T**: 4 kg

I/O box

Parameter	Data
Number of ports	2 analog in, 2 analog out 4 digital in, 4 digital out
Analog range	In ± 2 V Out ± 1 V

9.3 Tubing and connectors

Tubing types

The table below shows the tubing types used in ÄKTA pure.

Description	Color	Scope of use	Volume/cm
PEEK, o.d. 1/16", i.d. 0.13 mm	Red	High pressure tubing for Micro kit	0.13 µL
PEEK, o.d. 1/16", i.d. 0.25 mm	Blue	High pressure tubing Reference capillary 1 Tubing Kit 0.25	0.5 µL
PEEK, o.d. 1/16", i.d. 0.50 mm	Orange	High pressure tubing Tubing kit 0.5 (standard)	2.0 µL
PEEK, o.d. 1/16", i.d. 0.75 mm	Green	High pressure tubing Tubing kit 0.75	4.4 µL
PEEK, o.d. 1/16", i.d. 1.0 mm	Beige	High pressure tubing Tubing kit 1.0	7.8 µL
FEP, o.d. 1/8", i.d. 1.6 mm	Trans-parent	Inlet tubing	20.0 µL
FEP, o.d. 3/16", i.d. 2.9 mm	Trans-parent	Inlet tubing for high flow rate and high viscosity	66.0 µL
ETFE, o.d. 1/16", i.d. 0.75 mm	Trans-parent	Narrow inlet tubing (optional)	4.4 µL
ETFE, o.d. 1/16", i.d. 1.0 mm	Trans-parent	Outlet and waste tubing	7.8 µL
Silicone, o.d. 12 mm, i.d. 8 mm	Trans-parent	Waste tubing from Buffer tray	0.5 mL
Silicone, o.d. 4.1 mm, i.d. 2.1 mm	Trans-parent	Pump rinse solution tubing	35 µL

Note: *Many different sizes/types of tubing can be connected to a chromatography system. Tubing with a smaller inner diameter (i.d.) holds less delay volume and will therefore generate less dilution of the protein peak. Narrow tubing, however, increases the system pressure, especially when running at high flow rates. The tubing used must match the application needs. See* *Section 9.4 Recommended tubing kits for prepacked columns, on page 546* *for more information.*

Note: *When using the high pressure tubing kit (i.d. 1.0 mm) to allow high flow rates in combination with high viscosity solutions, use an inlet tubing with a larger i.d. than the standard tubing to prevent air bubble formation in the tubing.*

Tubing connectors

The table below shows the tubing connectors used in ÄKTA pure.

Description	Use with tubing...
Fingertight connector, 1/16"	• PEEK, o.d. 1/16", i.d. 0.25 mm • PEEK, o.d. 1/16", i.d. 0.50 mm • PEEK, o.d. 1/16", i.d. 0.75 mm • PEEK, o.d. 1/16", i.d. 1.0 mm • ETFE, o.d. 1/16", i.d. 1.0 mm
Tubing connector 5/16" + Ferrule (blue), 1/16"	ETFE, o.d. 1/16", i.d. 0.75 mm
Tubing connector 5/16" + ferrule (blue) 3/16"	FEP o.d. 3/16" i.d. 2.9 mm
Tubing connector 5/16" + Ferrule (yellow), 1/8"	FEP, o.d. 1/8", i.d. 1.6 mm

Other connectors

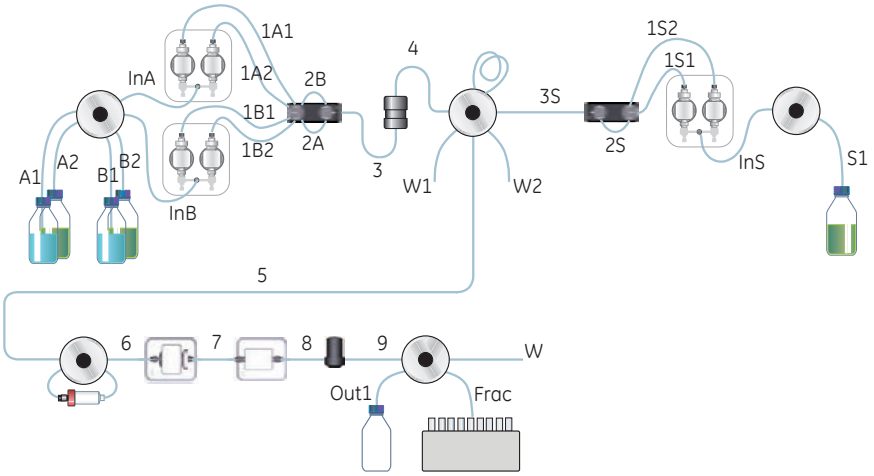
The table below shows other connectors used in ÄKTA pure.

Description	Scope of use
Stop plug 1/16"	Stop plug for valve ports
Luer female	Syringe connector for pH valve and Injection valve

Tubing labels

The illustration below shows the tubing labels for a typical system configuration.

For information about labels for the ÄKTA pure Micro kit, see *Install the tubing kit, on page 112*.



Inlet tubing

The table below shows the labels, standard diameters, and standard lengths of the inlet tubing.

Label	Description	Tubing		Length (mm)	Volume (mL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
A1-**A2** and **B1**-**B2**	Inlets to Inlet valve AB	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500	3.0	9.9
A1-**A7**	Inlets to Inlet valve A	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500	3.0	9.9
B1-**B7**	Inlets to Inlet valve B	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1500	3.0	9.9
InA	From Inlet valve A or Inlet valve AB to System pump A	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	300	0.6	2.0

Label	Description	Tubing		Length (mm)	Volume (mL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
InB	From Inlet valve B or Inlet valve AB to System pump B	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	300	0.6	2.0
S1-**S7**	Inlets to Sample inlet valve V9-IS or V9H-IS	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1000	2.0	6.6
Buff	Inlet to Sample inlet valve V9-IS or V9H-IS	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	1000	2.0	6.6
InS	From Sample inlet valve V9-IS or V9H-IS to Sample pump S9 or S9H, respectively	FEP, o.d. 1/8", i.d. 1.6 mm	FEP, o.d. 3/16", i.d. 2.9 mm	580	1.2	3.8

Note: *Narrow inlet tubing, ETFE, o.d. 1/16", i.d. 0.75 mm, is available for* **S1**-**S7**.

High pressure tubing

The tables below shows the labels, standard diameters, and standard lengths of the standard high pressure tubing and the optional high pressure tubing.

Standard high pressure tubing

Label	Description	Tubing		Length (mm)	Volume (µL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
1A1	System pump A left to Restrictor A	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270
1A2	System pump A right to Restrictor A	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270
2A	Restrictor A to Pressure monitor	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	115	50	90
1B1	System pump B left to Restrictor B	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270
1B2	System pump B right to Restrictor B	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270
2B	Restrictor B to Pressure monitor	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	115	50	90
1S1	Sample pump left to Restrictor S	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270
1S2	Sample pump right to Restrictor S	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	340	150	270

Label	Description	Tubing		Length (mm)	Volume (µL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
2S	Restrictor S to Sample pressure monitor	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	115	50	90
3S	Sample pressure monitor to injection valve.	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	530	230	420
3	Pressure monitor to Mixer	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	400	180	310
4	Mixer to Injection valve	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	200	90	160
5	Injection valve to Column valve	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	160[1]	30	70
6	Column valve to UV monitor	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	160[2]	30	70
7	UV monitor to Conductivity monitor	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	170	30	80
8	Conductivity monitor to Flow restrictor	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	95	20	40
9	Flow restrictor to Outlet valve	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	135	30	60

Label	Description	Tubing		Length (mm)	Volume (µL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
Frac	Outlet valve to Fraction collector F9-C	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	350	70	160
	Outlet valve to Fraction collector F9-R	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	400	80	310
	Outlet valve to Fraction collector F9-T	PEEK, o.d. 1/16", i.d. 0.50 mm	N/A	400	80	N/A

[1] To perform a run with the Column valve **V9-Cm**, a longer tubing **5** is needed. Replace the 160 mm tubing from the UV monitor with tubing that is 180 mm long. Update the tubing length in ***System settings***.
[2] To perform a run with the Column valve **V9-Cm**, a longer tubing **6** is needed. Replace the 160 mm tubing from the UV monitor with tubing that is 180 mm long. Update the tubing length in ***System settings***.

Note: *Tubing of different inner diameter can be used for tubing* **Frac** *and tubing* **5** *to* **9**. *An inner diameter (i.d.) of 0.5 mm is standard, but tubing kits of 0.25 mm i.d., 0.75 mm i.d. and 1.0 mm i.d. are also available. Tubing* **3S** *is not included in the kits, but must be changed to 1.0 mm i.d. if the Tubing kit 1.0 is used.*

Note: *For information about tubing for the micro kit, see* *Install the tubing kit, on page 112*.

Optional high pressure tubing

Label	Description	Tubing		Length (mm)	Volume (µL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
3-1	Pressure monitor to Mixer valve	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	160	70	130
3-2	Mixer valve to Mixer	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	330	150	260

Label	Description	Tubing		Length (mm)	Volume (µL)	
		ÄKTA pure 25	ÄKTA pure 150		ÄKTA pure 25	ÄKTA pure 150
3-3	Mixer valve to Injection valve	PEEK, o.d. 1/16", i.d. 0.75 mm	PEEK, o.d. 1/16", i.d. 1.0 mm	260	120	200
8pH	Conductivity monitor to pH valve	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	180	40	80
9pH	pH valve to Outlet valve	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	160	30	70
L1	Injection valve port **LoopF** to Loop valve port **F**	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	160	30	70
L2	Injection valve port **LoopE** to Loop valve port **E**	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	160	30	70
1R	From pH flow cell to flow restrictor	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	80	20	40
2R	From flow restrictor tp pH flow cell	PEEK, o.d. 1/16", i.d. 0.50 mm	PEEK, o.d. 1/16", i.d. 0.75 mm	80	20	40

Note: *Tubing of different inner diameter can be used for tubing* **8pH**, **9pH**, **1R** *and* **2R**. *An inner diameter (i.d.) of 0.5 mm is standard, but tubing kits of 0.25 mm i.d., 0.75 mm i.d. and 1.0 mm i.d. are also available.*

Reference capillary

The table below shows the label, diameter, and standard length of the reference capillary. The capillary is used during the System performance tests.

Label	Description	Tubing	Length (mm)	Volume (µL)
Ref 1	Reference capillary	PEEK, o.d. 1/16", i.d. 0.25 mm	400	20

Outlet tubing

The table below shows the labels, diameters, and standard lengths of the outlet tubing. The tubing is not mounted on delivery.

Label	Description	Tubing	Length (mm)	Volume (mL)
Out	Outlets from Outlet valve **V9-Os** or **V9H-Os**	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2
Out1 - **Out10**	Outlets from Outlet valve **V9-O** or **V9H-O**	V9-O: ETFE, o.d. 1/16", i.d. 1.0 mm V9H-O: FEP o.d.1/8", i.d. 1.6 mm	1500	1.2 3.0

Waste tubing

The table below shows the labels, diameters, and standard lengths of the waste tubing. The waste tubing is mounted on delivery.

Label	Description	Tubing	Length (mm)	Volume (mL)
W1	System pump waste	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2
W2	Sample pump waste	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2
W3	pH valve waste	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2
W	System waste	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2
N/A	Top tray waste	Silicone, o.d. 12 mm, i.d. 8 mm	1500	80

Label	Description	Tubing	Length (mm)	Volume (mL)
N/A	Fraction collector F9-C waste	Silicone, o.d. 12 mm, i.d. 8 mm	1350	70

9.4 Recommended tubing kits for prepacked columns

This section specifies recommended tubing kits for prepacked columns used with ÄKTA pure.

The recommendations are also valid for the "Increase" type of SEC columns.

Note: *Where alternative tubing kits are listed for a given column, the* ***Pressure control*** *option may be used instead of changing the tubing kit to adapt to different run conditions. Refer to* *Section 9.15 Pressure control, on page 643* *for details.*

Column name	Recommended Tubing Kits		Comments
	Standard system[1]	Advanced system[2]	
All HiTrap 1 mL (including HisTrap™, GSTrap™, StrepTrap™)	0.5	0.5 with ***Pressure control*** or 0.75	N/A
All HiTrap 5 mL (including HisTrap, GSTrap except 4B, StrepTrap)	0.5 or 0.75	0.75	0.75 with ***Pressure control*** for maximum flow rate combined with high viscosity.
GSTrap 4B, 5 mL	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
All HiScreen	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
RESOURCE™ Q, S, ETH, ISO, PHE and RPC 1 mL	0.5	0.5	N/A
RESOURCE RPC 3 mL	0.5	0.5	N/A
RESOURCE Q, S 6 mL	0.5 or 0.75	0.75	0.75 with ***Pressure control*** for maximum flow rate combined with high viscosity.
SOURCE™ 15RPC ST 4.6/100	0.5	0.5	Avoid combination of column valve **V9-C** and pH position in valve **V9-pH**. Resolution may be improved with 0.25.

Column name	Recommended Tubing Kits		Comments
	Standard system[1]	Advanced system[2]	
SOURCE 15Q. S, PHE 4.6/100 PE	0.5	0.5	Avoid combination of column valve **V9-C** and pH position in valve **V9-pH**. Resolution may be improved with 0.25.
Mini Q™, Mini S™ 4.6/50 PE	0.25 or 0.5	0.25 or 0.5	Use 0.25 for maximum resolution. Avoid column valve **V9-C** and pH position in **V9-pH**.
Mono Q™, Mono S™, Mono P HR 5/5	0.5	0.5	Avoid combination of column valve **V9-C** or **V9H-C** and pH position in valve **V9-pH** or **V9H-pH**. Resolution may be improved with 0.25.
Mono P HR 5/20	0.5	0.5	N/A
Mono Q, S HR 10/10	0.5	0.5	N/A
Mono Q, Mono S HR 16/10	0.5 or 0.75	0.5 or 0.75	N/A
Superdex™ Peptide, 75, 200 Superose™ 6, 12 HR 10/30	0.5	0.5	N/A
Mono Q, Mono S 4.6/100 PE	0.5	0.5	Avoid combination of column valve **V9-C** or **V9H-C** and pH position in valve **V9-pH** or **V9H-pH**. Resolution may be improved with 0.25.
Mono Q, Mono S, Mono P 5/50 GL	0.5	0.5	Avoid combination of column valve **V9-C** or **V9H-C** and pH position in valve **V9-pH** or **V9H-pH**. Resolution may be improved with 0.25.
Superdex 200 5/150 GL	0.25	0.25	Avoid column valve **V9-C** and pH position in **V9-pH**.

Column name	Recommended Tubing Kits		Comments
	Standard system[1]	Advanced system[2]	
Superdex 3.2/300	0.25, or 0.13 with Micro kit	Not recommended	For best result, use ÄKTA pure micro or Micro kit without any additional modules in the flow path.
Mono P 5/200 GL	0.5	0.5	N/A
Mono Q, Mono S 10/100 GL	0.5 or 0.75	0.5 or 0.75	N/A
Capto HiRes 5/50 and 10/100	0.25	0.25	Avoid column valve **V9-C** and pH position in **V9-pH**.
Superdex Peptide, 75, 200, Superose 6, 12 10/300 GL	0.5	0.5	N/A
All HiPrep™ 16/10 (including GSTPrep™ FF, HisPrep™ FF)	0.5 or 0.75	0.75	N/A
HiPrep 16/60 Sephacryl™ S 100-500 HR	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
HiPrep 26/10 Desalting HiPrep 26/10 Sepharose™ 6 FF	0.5 with Pressure Control or 0.75	0.75 with ***Pressure control*** or 1.0	N/A
HiPrep 26/60 Sephacryl S 100-500 HR	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
HiLoad™ 16/10 Phenyl Sepharose HP, Q Sepharose HP, SP Sepharose HP	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
HiLoad 16/60 Superdex 30 pg, 75 pg, 200 pg	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A
HiLoad 26/10 Phenyl Sepharose HP, Q Sepharose HP, SP Sepharose HP	0.5 or 0.75	0.75	N/A
HiLoad 26/60 Superdex 30 pg, 75 pg, 200 pg	0.5 or 0.75	0.5 with ***Pressure control*** or 0.75	N/A

[1] Standard system configuration, normal flow rates, moderate sample and buffer viscosities.
[2] Complex system configuration (for additional tubing, use the same tubing dimension as the Tubing Kit), maximum flow rates, high viscosities (≥3 cP).

9.5 Chemical resistance guide

Introduction

This section provides general information about biocompatibilty and detailed information about chemical resistance of the ÄKTA pure instrument.

In this section

Section		See page
9.5.1	General information about biocompatibility and chemical resistance	550
9.5.2	Chemical resistance specifications	551

9.5.1 General information about biocompatibility and chemical resistance

Biocompatibility

The ÄKTA pure instrument is designed for maximum biocompatibility, with biochemically inert flow paths constructed mainly from titanium, PEEK and highly resistant fluoropolymers and fluoroelastomers. Titanium is used as far as possible to minimize contribution of potentially deactivating metal ions such as iron, nickel and chromium. There is no standard stainless steel in the flow path. Plastics and rubber materials are selected to avoid leakage of monomers, plasticizers or other additives.

Cleaning chemicals

Strong cleaning works well with 2 M sodium hydroxide, 70% acetic acid or the alcohols methanol, ethanol and isopropyl alcohol. If sodium hypochlorite is used as sanitizing agent instead of 2 M sodium hydroxide, use a concentration up to 10%.

Organic solvents

Reversed phase chromatography of proteins works well with 100% acetonitrile and additives trifluoroacetic acid (TFA) up to 0.2% or formic acid up to 5%.

Strong organic solvents like ethyl acetate, 100% acetone or chlorinated organic solvents must not be used. These might cause swelling of plastic material and reduce the pressure tolerance of PEEK tubing. For this reason, flash chromatography and straight ("normal") phase chromatography is generally not recommended on the system.

Assumptions made

The ratings are based on the following assumptions:

- Synergy effects of chemical mixtures have not been taken into account.
- Room temperature and limited overpressure is assumed.

Note: *Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.*

9.5.2 Chemical resistance specifications

Introduction

This section provides detailed information about chemical resistance of the ÄKTA pure instrument to some of the most commonly used chemicals in liquid chromatography. Regarding exposure to solutions not covered by this information, contact your Cytiva representative for recommendations.

Note: *A user can be exposed to large volumes of chemical substances over a long time period. Material Safety Data Sheets (MSDS) provide the user with information regarding characteristics, human and environmental risks and preventive measures. Make sure to have the MSDS available from your chemical distributor and/or databases on the internet.*

Aqueous buffers

The specified aqueous buffers are suitable for continuous use.

Chemical	Concentration	CAS no/EC no
Aqueous buffers pH 2-12	N/A	N/A

Strong chemicals and salts for CIP

The following chemicals are suitable for up to 2 hours contact time at room temperature.

Chemical	Concentration	CAS no/EC no
Acetic acid	70%	75-05-8/ 200-835-2
Decon 90	10%	N/A
Ethanol	100%	75-08-1/ 200-837-3
Methanol	100%	67-56-1/ 200-659-6
Hydrochloric acid	1 M	7647-01-0/ 231-595-7
Isopropanol	100%	67-63-0/ 200-661-7
Sodium hydroxide	2 M	1310-73-2/ 215-185-5
Sodium hydroxide/ethanol	1 M/40%	N/A
Sodium chloride	4 M	7647-14-5/ 231-598-3
Sodium hypochlorite	10%	7681-52-9/231-668-3

Solubilization and denaturing agents

The following chemicals are suitable for continuous use, as additives in separation and purification methods.

Chemical	Concentration	CAS no/EC no
Guanidinium hydrochloride	6 M	50-01-1/ 200-002-3
Sodium dodecyl sulfate (SDS)	1%	151-21-3/ 205-788-1
Triton X-100	1%	9002-93-1/ 618-344-0
Tween 20	1%	9005-64-5/ 500-018-3
Urea	8 M	57-13-6/ 200-315-5

Chemicals used in reversed phase chromatography (RPC)

The following chemicals are suitable for continuous use.

Chemical	Concentration	CAS no/EC no
Acetonitrile[1]	100%	75-05-8/ 200-835-2
Acetonitrile/Tetrahydrofuran[1]	85%/15%	109-99-9/ 203-726-8
Acetonitrile/water/Trifluoroacetic acid (TFA)[2]	Max 0.2% TFA	N/A
Ethanol	100%	75-08-1/ 200-837-3
Isopropanol	100%	67-63-0/ 200-661-7
Methanol	100%	74-93-1/ 200-659-6
Water/organic mobile phase/formic acid	Max 5% formic acid	N/A

[1] Organic solvents can penetrate weaknesses in PEEK tubing walls more easily than water based buffers. Special care must therefore be taken with prolonged use of organic solvents close to pressure limits. Depending on pressure, tubing between pump head and pressure monitor needs to be changed. See *Section 7.5.1 Replace tubing and connectors, on page 402* for more information.

[2] Mobile phase system.

Note: *It is recommended to replace the mixer sealing ring with the highly resistant O-ring (product code 29011326) if the system is to be exposed to organic solvents or high concentrations of organic acids, such as acetic acid and formic acid, for a longer period of time.*

Salts and additives for hydrophobic interaction chromatography (HIC)

The following chemicals are suitable for continuous use.

Chemical	Concentration	CAS no/EC no
Ammonium chloride	2 M	12125-02-9/ 235-186-4
Ammonium sulfate	3 M	7783-20-2/ 231-984-1
Ethylene glycol	50%	107-21-1/ 203-473-3
Glycerol	50%	56-81-5/ 200-289-5

Reducing agents and other additives

The following chemicals are suitable for continuous use.

Chemical	Concentration	CAS no/EC no
Arginine	2 M	74-79-3/ 200-811-1
Benzyl alcohol	2%	100-51-6/ 202-859-9
Dithioerythritol (DTE)	100 mM	6892-68-8/ 229-998-8
Dithiothreitol (DTT)	100 mM	3483-12-3/ 222-468-7
Ethylenediaminetetraacetic acid (EDTA)	100 mM	60-00-4/ 200-449-4
Mercaptoethanol	20 mM	37482-11-4/ 253-523-3
Potassium chloride	4 M	7447-40-7/ 231-211-8

Long term storage

The following chemicals are suitable for long term storage.

Chemical	Concentration	CAS no/EC no
Ethanol	20%	75-08-1/ 200-837-3
Benzyl alcohol[1]	2%	100-51-6/ 202-859-9

[1] It is recommended to store the system in 20% ethanol, but 2% benzyl alcohol can be used as an alternative to ethanol.

Other substances

The following chemicals are suitable for continuous use.

Chemical	Concentration	CAS no/EC no
Acetone	10%	67-64-1/ 200-662-2
Ammonia	30%	7664-41-7/ 231-635-3
Dimethyl sulphoxide (DMSO)	5%	67-68-5/ 200-664-3
Ethanol	20%	75-08-1/ 200-837-3
Phosphoric acid	0.1 M	7664-38-2/ 231-633-2

9.6 Wetted materials

Material definitions

The tables below list the materials that come into contact with process fluids in the ÄKTA pure system.

Primary flow path: Material

Material	Abbreviation
Ethylene ChloroTriFluoroEthylene	ECTFE
Ethylene TetraFluoroEthylene	ETFE
Fluorinated Ethylene Propylene	FEP
Fluorinated Propylene Monomer	FPM/FKM
Fully Fluorinated Propylene Monomer	FFPM/FFKM
PolyChloroTriFluoroEthylene	PCTFE
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyTetraFluoroEthylene	PTFE
PolyVinylidene DiFluoride	PVDF
UltraHighMolecularWeightPolyEthylene	UHMWPE
Aluminum oxide	N/A
Elgiloy	N/A
Hastelloy® C-276	N/A
Hastelloy C-22	N/A
Quartz glass	N/A
Ruby	N/A
Sapphire	N/A
Titanium grade 2	N/A

Pump rinse system: Material

Material	Abbreviation
EthylenePropyleneDiene M-class rubber	EPDM
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyPhenylene Sulfide	PPS
PolyVinylidene DiFluoride	PVDF
Silicone	N/A

9.7 Predefined methods and phases

Introduction

A predefined method contains a set of phases, each phase reflecting a specific stage of a chromatography or maintenance run. Additional phases from the phase libraries can be added to an existing method, or phases that are not required can be removed from a method.

The predefined purification methods have default values with suitable running conditions for the chosen column type such as flow and pressure limits. Other settings (e.g., sample application technique, sample volume, elution profile and fractionation) are set on the ***Phase Properties*** pane in the appropriate phases.

This section describes the predefined methods and phases.

A method is built up by a number of phases. Each phase represents a major process step in the method, for example, equilibration or elution. Predefined methods, that include all the phases necessary to run the system, are available for different chromatography techniques and also for system cleaning.

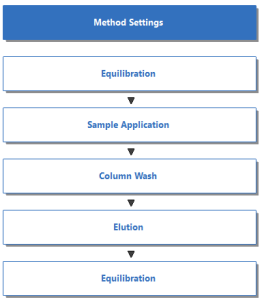
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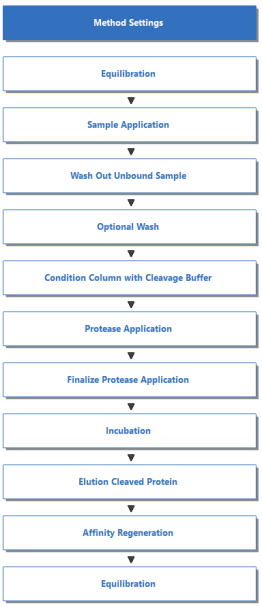
Section		See page
9.7.1	Predefined purification methods	558
9.7.2	Predefined maintenance methods	564
9.7.3	Predefined phases	565

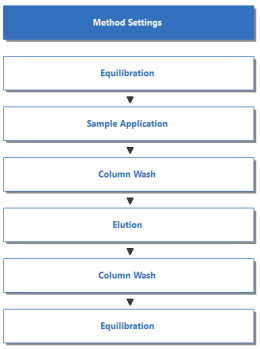
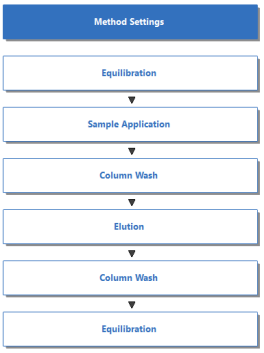
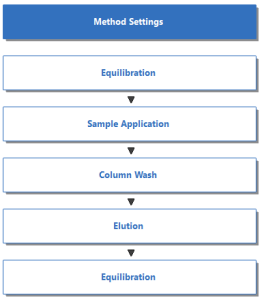
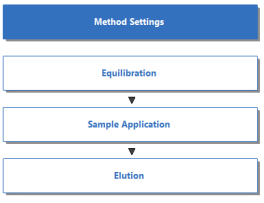
9.7.1 Predefined purification methods

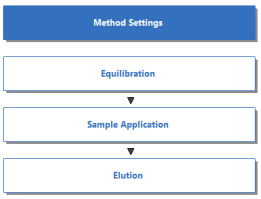
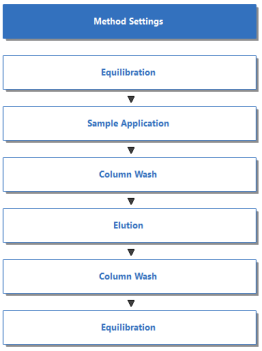
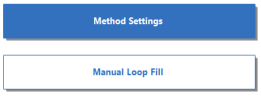
The ***Method Editor*** has predefined methods for different separation techniques. The methods include a number of relevant phases.

The table below describes the available predefined purification methods and which phases that are included.

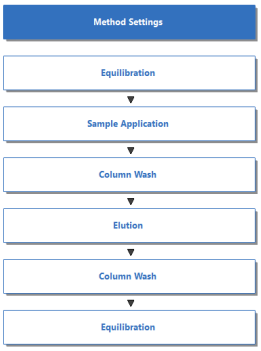
Predefined purification method	Principle	Included phases
Affinity Chromatography (AC)	After equilibration and sample application, the protein of interest is adsorbed to the column ligand. After a wash to remove unbound sample, elution is performed either by using a buffer containing a competitor to displace the protein of interest, or by changing the pH or ionic strength. Finally, the column is re-equilibrated with start buffer.	
Affinity Chromatography (AC) - HiTrap Fibro Prisma	This method is specifically adapted for running the HiTrap Fibro PrismA unit without a mixer. To cycle the run, add runs using scouting. After equilibration and sample application, the protein of interest is adsorbed to the unit ligand. After a wash to remove the unbound sample, elution is performed, e.g. by changing the pH. Finally, a CIP of the unit is performed and the unit is re-equilibrated.	

Predefined purification method	Principle	Included phases
Affinity Chromatography (AC) with Tag Removal	After equilibration, the sample of tagged protein is applied. After washing to remove unbound sample the column is equilibrated with cleavage buffer, a protease is applied and the flow is stopped. The cleaved protein and the protease are eluted by starting the flow. The next step regenerates the column, eluting the tag, uncleaved protein and protease (if tagged). Finally, the column is re-equilibrated. **Required components** The required components for this method are Inlet valve (2-ports or 7-ports) and Mixer valve, or the sample pump. **Required solutions** The method phases are pre-configured to use the following solutions in the following inlets and loop: Inlet A1: Equilibration buffer Inlet A2 or sample inlet: Sample Inlet B1: Cleavage buffer Inlet B2: Affinity Regeneration buffer Loop: Protease ***Note:*** *Select **Inject sample directly onto the column** in the sample application phase.* ***Note:*** *The protease application phase is preconfigured to empty the loop with the protease with a volume of 0.7 mL. The recommended protease volume is 0.7 CV (column volume). If a column volume different than 1 mL is used, this value needs to be changed to correspond to 0.7 CV.*	

Predefined purification method	Principle	Included phases
Anion Exchange Chromatography (AIEX)	After equilibration and sample application, negatively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g. NaCl). Finally, the column is washed and re-equilibrated with start buffer.	
Cation Exchange Chromatography (CIEX)*	After equilibration and sample application, positively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g. NaCl). Finally, the column is washed and re-equilibrated with start buffer.	
Chromatofocusing (CF)	After equilibration and sample application, elution is performed using a pH gradient. The proteins separate and elute according to their isoelectric points. Finally, the column is re-equilibrated.	
Desalting (DS)	After equilibration and sample application, the proteins are eluted isocratically. This technique is commonly used for buffer exchange.	

Predefined purification method	Principle	Included phases
Size Exclusion Chromatography (SEC)	After equilibration and sample application, proteins separate and elute according to their size (largest first).	
Hydrophobic Interaction Chromatography (HIC)	After equilibration and sample application (use a buffer containing a high salt concentration, for example 2 M ammonium sulfate) hydrophobic proteins are adsorbed to the column ligand. After a wash to remove unbound sample, elution is performed using a gradient of decreasing salt concentration. Finally, the column is washed and re-equilibrated with start buffer.	
Manual Loop Fill	The sample application loops of the loop valve are manually filled with samples. The method will guide the user through the process by pausing and displaying on-screen instructions. Up to five loops can be filled with different samples. The loops are filled in descending order. Partial or complete loop fill can be chosen. Automatic washing of loops and flow path are integrated in the method. The required components for this phase are a column valve and a loop valve.	

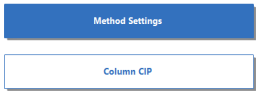
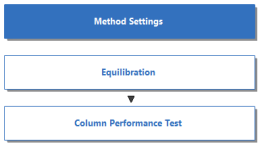
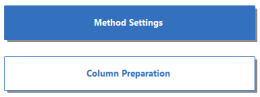
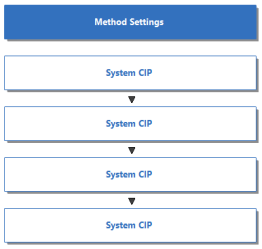
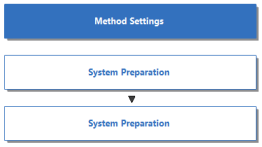
Predefined purification method	Principle	Included phases
NHS-coupling	A column packed with NHS-activated Sepharose is washed with 1 mM HCl, followed by immediate application of the protein for covalent coupling onto the column. After incubation the non-bound protein is washed out and the remaining active groups are deactivated with ethanolamine buffer, followed by further washes. **Required components** The required component for this method is an Inlet valve (2-ports or 7-ports). **Required solutions** The method phases are preconfigured to use the following solutions in the following inlets and sample loop: Inlet A1: Coupling buffer, for example 0.2 M $NaHCO_3$ + 0.5 M NaCl pH 8.3. Inlet A2: Activation solution, for example 1 mM HCl. Inlet B1: High pH buffer, for example 0.5 M Ethanolamine, 0.5 M NaCl pH 8.3. Inlet B2: Low pH buffer, for example 0.1 M Sodium acetate, 0.5 M NaCl pH 4.0. Sample loop: Ligand in coupling buffer. ***Note:*** *The NHS coupling phase is preconfigured to empty the loop with the ligand with a volume of 1 mL. The recommended ligand volume is 1 CV. If a column volume different than 1 mL is used, this value needs to be changed to correspond to 1 CV.*	

Predefined purification method	Principle	Included phases
Reversed Phase Chromatography (RPC)	After equilibration and sample application, hydrophobic proteins adsorb to the column ligand. After a wash to remove unbound sample, elution is performed by generating a gradient of a non-polar, organic solvent such as acetonitrile. Finally, the column is washed and re-equilibrated.	

9.7.2 Predefined maintenance methods

A number of predefined methods for preparation and cleaning are available. These maintenance methods are used to prepare the system, clean the system, and to fill the system with storage solution.

The table below describes the available predefined maintenance methods.

Predefined maintenance method	Principle	Included phases
Column CIP	The column is filled with a cleaning solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several cleaning solutions can be used. Suggestions for cleaning steps are available for a number of column types.	
Column Performance Test	After equilibration of the column, sample is injected via a loop and eluted isocratically. A non-adsorbing sample like acetone or salt must be used. After the run, calculate column performance in the ***Evaluation*** module. The efficiency of the column is determined in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s). The result is logged in the column logbook.	
Column Preparation	The column is filled with buffer solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several preparation solutions can be used.	
System CIP	The system is filled with cleaning solution. Select for example inlets, outlets and column positions to be cleaned. Three ***System CIP*** phases are included in the method to facilitate the use of three different cleaning solution. Additional ***System CIP*** phases can be added from the ***Phase Library*** if desired.	
System Preparation	The system is filled with preparation solution. Select for example inlets, outlets and column positions to be prepared. Two ***System Preparation*** phases are included in the method. Additional ***System Preparation*** phases can be added from the ***Phase Library*** if desired.	

9.7.3 Predefined phases

The following table describes the predefined phases.

Phase Name	Description
Method Settings	The first, and mandatory, phase in any method. Defines common parameters used in the subsequent phases.
Column CIP	Cleans the column after purification runs by rinsing the column with a cleaning solution to remove nonspecifically bound proteins. By adding steps, several cleaning solutions can be used sequentially.
Column performance test	Tests the efficiency of a packed column in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s).
Column Preparation	Prepares the column before use by removing the storage solution and equilibrating the column. By adding steps, several preparation solutions can be used sequentially.
Column Wash	Washes out unbound sample after sample application or removes strongly bound proteins after elution.
Conditional Frac Stop	This phase is used to end an ongoing fractionation that has been started in a previously called phase in the method. Use together with ***Conditional Fractionation***.
Conditional Fractionation	This phase is used to start and/or stop a fractionation either directly or conditionally.
Elution	Elutes the sample from the column. Defines parameters for the elution and fractionation settings.
Equilibration	Equilibrates the column before purification, or re-equilibrates the column after purification. Allows for washing the ***Inlet A***, ***Inlet B***, and the ***Accumulator*** of the Fraction collector F9-C.
Manual Loop Fill	Is used to manually fill the additional sample application loops mounted on the loop valve. The filling options are: • Partial loop fill • Complete loop fill
Miscellaneous	Can be added to any method at suitable places. The instructions can help the user to better organize the graphical output of the results or introduce a controlled delay in the method run.

Phase Name	Description
Sample Application	Applies sample to the column. Defines the sample application technique, the sample volume, and the handling of flow-through.
System CIP	Cleans the system after purification runs by rinsing the system with a cleaning solution. One cleaning solution is used per phase.
System Preparation	Prepares the system before a run by removing storage solution and filling the system and inlets with buffer solution. One preparation solution is used per phase.
User Defined	Use this phase to add text instructions to a phase method.

9.8 System settings

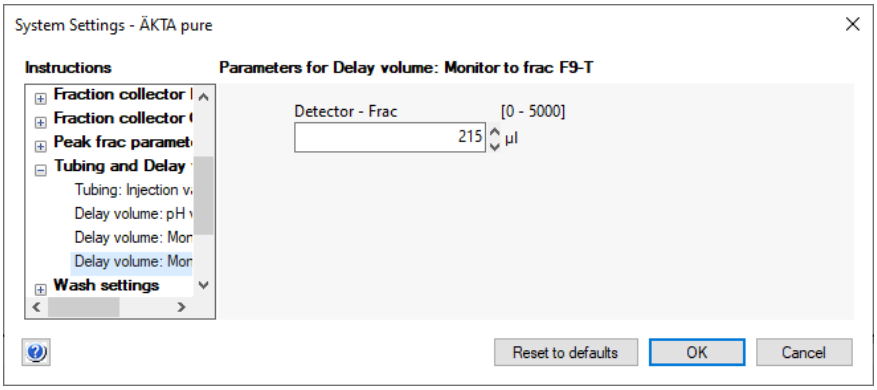
In this section

Section		See page
9.8.1	Introduction	568
9.8.2	System settings - UV	569
9.8.3	System settings - Conductivity	572
9.8.4	System settings - Versatile valve	574
9.8.5	System settings - pH	575
9.8.6	System settings - Pressure alarms	576
9.8.7	System settings - Air sensor	578
9.8.8	System settings - I/O-box	580
9.8.9	System settings - Fraction collection	581
9.8.10	System settings - Tubing and Delay volumes	585
9.8.11	System settings - Wash settings	587
9.8.12	System settings - Watch parameters	589
9.8.13	System settings - Advanced	591
9.8.14	System settings - Data collection	592

9.8.1 Introduction

The ***System Settings*** function is used to set the parameters for the available instructions.

An example of the ***System Settings*** dialog for editing the system settings is shown below.



9.8.2 System settings - UV

The tables below describes the UV related system settings available for ÄKTA pure.

UV monitor U9-M

Instruction name	Description
Alarm UV1	***Alarm UV 1*** enables or disables the alarm for the UV 1 signal from the UV monitor **U9-M**. When enabled, it sets the alarm limits for the UV 1 signal from the UV monitor **U9-M**. When the signal falls outside the set limits, an alarm is issued and the method will be paused. ***Note:*** *It is not possible to set an alarm signal for the UV 2 or UV 3 signals from UV monitor* **U9-M**.
Wavelength	***Wavelength*** sets the wavelengths for UV monitor **U9-M**. The corresponding curves in the chromatogram are marked with the set wavelengths. ***Note:*** *For best performance, do not use more wavelengths than necessary for the application.* ***Note:*** *At low wavelengths, the eluent can have significant absorbance of its own.*
Noise reduction UV	***Noise reduction UV*** filters the noise in the UV signal from the UV monitor **U9-M**. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.

UV monitor U9-T

Instruction name	Description
Alarm UV 1_280	***Alarm UV 1_280*** enables or disables the alarm for the 280 nm UV signal from the UV monitor **U9-T**. When enabled, it sets the alarm limits for the UV signal for the UV monitor **U9-T**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.

Instruction name	Description
Alarm UV 2_260	***Alarm UV 2_260*** enables or disables the alarm for the 260 nm UV signal from the UV monitor **U9-T**. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-T**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 1_280 2nd	***Alarm UV 1_280 2nd*** enables or disables the alarm for the 280 nm UV signal from the UV monitor **U9-T**, 2nd. When enabled, it sets the alarm limits for the UV signal from the UV monitor **U9-T**, 2nd. When the signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 2_260 2nd	***Alarm UV 2_260 2nd*** enables or disables the alarm for the 260 nm UV signal from the UV monitor **U9-T**, 2nd. When enabled, it sets the alarm limits for the UV signal for the UV monitor **U9-T**, 2nd. When the signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Wave-length	***Wavelength*** sets the wavelengths for the UV monitor **U9-T**. The corresponding curves in the chromatogram are marked with the set wavelength. ***Note:*** ***Wavelength*** *can be set to 280 nm, or 280 nm and 260 nm. The default system setting is 280 nm.*
Wave-length 2nd	***Wavelength 2nd*** sets the wavelengths for the UV monitor **U9-T**, 2nd. The corresponding curves in the chromatogram are marked with the set wavelength. ***Note:*** ***Wavelength 2nd*** *can be set to 280 nm, or 280 nm and 260 nm. The default system setting is 280 nm.*
Noise reduction UV	***Noise reduction UV*** filters the noise in the UV signal from the UV monitor **U9-T**. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.
Noise reduction UV 2nd	***Noise reduction UV 2nd*** filters the noise in the UV signal from the UV monitor **U9-T**, 2nd. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.

UV monitor U9-L

Instruction name	Description
Alarm UV	***Alarm UV*** enables or disables the alarm for the UV signal from the UV monitor **U9-L**. When enabled, it sets the alarm limits for the UV signal for the UV monitor **U9-L**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 2nd	***Alarm UV 2nd*** enables or disables the alarm for the UV signal from the UV monitor **U9-L**, 2nd. When enabled, it sets the alarm limits for the UV signal for the UV monitor **U9-L**, 2nd. When the signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Noise reduction UV	***Noise reduction UV*** filters the noise in the UV signal from the UV monitor **U9-L**. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.
Noise reduction UV 2nd	***Noise reduction UV 2nd*** filters the noise in the UV signal from the UV monitor **U9-L**, 2nd. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.

9.8.3 System settings - Conductivity

The table below describes the conductivity related system settings available for ÄKTA pure.

Instruction name	Description
Alarm conduc-tivity	***Alarm conductivity*** enables or disables the conductivity alarm. When enabled, it sets the alarm limits for the conductivity signal. When the conductivity falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm conductivity 2nd	***Alarm conductivity 2nd*** enables or disables the conductivity alarm for the secondary conductivity monitor. When enabled, it sets the alarm limits for the conductivity signal. When the conductivity falls outside the set limits, an alarm will be triggered and the method will be paused.
Relative scale cond	***Relative scale cond*** facilitates monitoring of a gradient, for which the user sets the conductivity values for 0% and 100%. The ***Relative scale cond*** can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity). ***Note:*** *The **Relative scale cond** in descending manner is especially useful for conductivity visualization in RPC and HIC, where the conductivity curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).*
Relative scale cond 2nd	***Relative scale cond 2nd*** facilitates monitoring of a gradient with the secondary conductivity monitor, for which the user sets the conductivity values for 0% and 100%. The ***Relative scale cond 2nd*** can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity). ***Note:*** *The **Relative scale cond 2nd** in descending manner is especially useful for conductivity visualization in RPC and HIC, where the conductivity curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).*

Instruction name	Description
Cond temp compensation	***Cond temp compensation*** is used to adjust the conductivity values to a reference temperature in order to compare conductivity values between runs that have been performed at different temperatures. Setting the compensation factor to 0% turns this function off.
Cond temp compensation 2nd	***Cond temp compensation 2nd*** is used to adjust the conductivity values of the secondary conductivity monitor to a reference temperature in order to compare conductivity values between runs that have been performed at different temperatures. Setting the compensation factor to 0% turns this function off.

9.8.4 System settings - Versatile valve

The table below describes the Versatile valve related system settings available for ÄKTA pure.

Instruction name	Description
Versatile valve 1 default position	Sets the default position of Versatile valve 1. ***Note:*** *Four sets of positions are available. In positions **1-3** and **2-4** only a single flow channel can be used. In positions **1-4 and 2-3** and **1-2 and 3-4** the flow can be directed through two channels simultaneously.*
Versatile valve 2 default position	Sets the default position of Versatile valve 2. ***Note:*** *Four sets of positions are available. In positions **1-3** and **2-4** only a single flow channel can be used. In positions **1-4 and 2-3** and **1-2 and 3-4** the flow can be directed through two channels simultaneously.*
Versatile valve 3 default position	Sets the default position of Versatile valve 3. ***Note:*** *Four sets of positions are available. In positions **1-3** and **2-4** only a single flow channel can be used. In positions **1-4 and 2-3** and **1-2 and 3-4** the flow can be directed through two channels simultaneously.*
Versatile valve 4 default position	Sets the default position of Versatile valve 4. ***Note:*** *Four sets of positions are available. In positions **1-3** and **2-4** only a single flow channel can be used. In positions **1-4 and 2-3** and **1-2 and 3-4** the flow can be directed through two channels simultaneously.*

9.8.5 System settings - pH

The table below describes the pH related system settings available for ÄKTA pure.

Instruction name	Description
Alarm pH	***Alarm pH*** enables or disables the pH alarm. When enabled, it sets the alarm limits for the pH signal. When the pH falls outside the set limits, an alarm will be triggered and the method will be paused.

9.8.6 System settings - Pressure alarms

The table below describes the pressure alarm related system settings available for ÄKTA pure.

Instruction name	Description
Alarm system pressure	***Alarm system pressure*** sets the alarm limits for the system pressure. When enabled and the system pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. Default values for the alarm limits are set by the values in the column list when a column is selected in the method and ***Alarm system pressure*** is set as a variable. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously and then falls below the ***Low alarm*** limit. ***Note:*** *Setting the* ***Low alarm*** *or the system flow rate to 0 deactivates the low pressure alarm.*
Alarm sample pressure	***Alarm sample pressure*** sets the alarm limits for the sample pressure. When enabled and the sample pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. Default values for the alarm limits are set by the values in the column list when a column is selected in the method and ***Alarm sample pressure*** is set as a variable. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously and then falls below the ***Low alarm*** limit. ***Note:*** *Setting the* ***Low alarm*** *or the system flow rate to 0 deactivates the low pressure alarm.*
Alarm delta column pressure	***Alarm delta column pressure*** sets the alarm limits for the delta column pressure (pre-column pressure minus post-column pressure). When enabled and the delta column pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously then falls below the ***Low alarm*** limit. ***Note:*** *Setting the* ***Low alarm*** *to 0 deactivates the low pressure alarm.* ***Note:*** *Instruction* ***Alarm delta column pressure*** *is available only when Column valve* **V9-C** *or* **V9H-C** *(5-columns) is selected in the component list.*

Instruction name	Description
Alarm pre-column pressure	***Alarm pre column pressure*** sets the alarm limits for the pre-column pressure. When enabled and the pre-column pressure falls outside the set pressure limits, an alarm is issued and the method will be paused. Default values for the alarm limits are set by the values in the column list when a column is selected in the method and ***Alarm pre column pressure*** is set as a variable. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously then falls below the ***Low alarm*** limit. ***Note:*** *Setting the **Low alarm** to 0 deactivates the low pressure alarm.*

9.8.7 System settings - Air sensor

The table below describes the air sensor related system settings available for ÄKTA pure.

Instruction name	Description
Alarm inlet A air sensor	***Alarm inlet A air sensor*** enables or disables the air sensor alarm for the built-in air sensor at inlet A. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm inlet B air sensor	***Alarm inlet B air sensor*** enables or disables the air sensor alarm for the built-in air sensor at inlet B. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm inlet S air sensor	***Alarm inlet S air sensor*** enables or disables the air sensor alarm for the built-in air sensor at the sample inlet. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm external air sensor X	***Alarm external air sensor X*** enables or disables the alarm for the optional air sensor number X. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Sensitivity inlet A air sensor	***Sensitivity inlet A air sensor*** is used together with the ***Alarm inlet A air sensor*** instruction and sets the sensitivity of the built-in air sensors at inlet A. ***Normal*** (30 µl) is used to detect when a buffer or sample vessel is empty. ***High*** (10 µl) is used to detect small air bubbles.
Sensitivity inlet B air sensor	***Sensitivity inlet B air sensor*** is used together with the ***Alarm inlet B air sensor*** instruction and sets the sensitivity of the built-in air sensors at inlet B. ***Normal*** (30 µl) is used to detect when a buffer or sample vessel is empty. ***High*** (10 µl) is used to detect small air bubbles.
Sensitivity inlet S air sensor	***Sensitivity inlet S air sensor*** is used together with the ***Alarm inlet S air sensor*** instruction and sets the sensitivity of the built-in air sensors at the sample inlet. ***Normal*** (30 µl) is used to detect when a buffer or sample vessel is empty. ***High*** (10 µl) is used to detect small air bubbles.

Instruction name	Description
Sensitivity external air sensor X	***Sensitivity external air sensor X*** is used together with the ***Alarm external air sensor X*** instruction and sets the sensitivity of the optional air sensor number X. The optional air sensor can be located either before any of the inlets A or B or after the injection valve. ***Normal*** (30 µl) is used to detect when a buffer or sample vessel is empty. ***High*** (10 µl) is used to detect small air bubbles. - When located before an inlet, the default sensitivity is ***Normal***. - When located after the injection valve, the default sensitivity is ***High*** and the pump currently pumping onto the column is used for calculating the air volume for the external air sensor. ***Note:*** *Using an air sensor after the injection valve is only useful when running at lower pressures. High pressure dissolves any small air bubbles present.*

9.8.8 System settings - I/O-box

The table below describes the I/O-box related system settings available for ÄKTA pure.

Instruction name	Description
Digital out X	***Digital out X*** sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Noise reduction analog in X	***Noise reduction analog in X*** filters the noise in the analog signal in port number X.
Alarm analog in X	***Alarm analog in X*** enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm digital in X	***Alarm digital in X*** enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in 'Value' occurs, an alarm will be triggered and the method will be paused.
Configure analog out X	***Configure analog out X*** enables the user to send one of the pre-defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

9.8.9 System settings - Fraction collection

The tables below describe the fraction collection related system settings available for ÄKTA pure.

Fraction collector F9-C

Instruction name	Description
Fractionation settings	***Fractionation settings*** comprises fractionation mode and fractionation order. ***Fractionation mode*** (***Automatic***, ***Accumulator*** or ***Drop-Sync***). ***Fractionation order*** (***Row-by-row***, ***Column-by-column***, ***Serpentine-row***, ***Serpentine-column***). For fractionation mode ***DropSync***, only the ***Serpentine*** option is available.
Last tube filled	***Last tube filled***: Action when last tube is filled (pause, direct the flow to one of outlets or direct the flow to waste.
Cassette configuration	***Cassette configuration***: ***Automatic*** or ***Manual*** configuration. If ***Automatic*** is selected, a Quick scan or a Full scan will be performed when the door of the fraction collector is closed to determine which type of cassettes and plates are used. If ***Manual*** is selected, used plates and tubes in each tray position are entered.
Fraction collector lamp	***Fraction collector lamp***: Lamps in the fraction collector chamber on or off.

Fraction collector F9-R

Instruction name	Description
Fractionation settings	***Drop sync*** synchronises tube change to drop release. The available settings are on or off. It is recommended to use ***Drop sync*** for flow rates below 2 mL/min. Higher flow rates can however be used, depending on the properties (for example viscosity) of the liquid.
Fractionation numbering mode	Determines whether the fraction number is reset at the end of a method or not. ***Note:*** *The default setting is* ***Reset***.

Instruction name	Description
Fractionation settings frac 2	***Drop sync*** on or off. It is recommended to use this setting for flow rates below 2 mL/min. Higher flow rates can be however be used, depending on the properties (e.g. viscosity) of the liquid.
Fractionation numbering mode frac 2	Determines whether fraction number for the second fraction collector is reset at the end of a method or not. ***Note:*** *The default setting is* ***Reset****.*

Fraction collector F9-T

Instruction name	Description
Drop sync	Drop sync synchronize movement after drop release. The available settings are ***On***, ***Off***, and ***Auto***. The Auto setting turns the drop synchronization on for flow rates below 3 mL/min, and turns it off for flows above 3 mL/min. This is done for all plate types except 96 deep well, where the limit is 5 mL/min. Do not use the Auto setting together with a micro nozzle because the micro nozzle only form drops up to 1.5 mL/min.
Collection of pre-fractionation volume	Options for ***Collection of pre-fractionation volume***: • ***Auto*** sets the pre-fractionation volume to be collected in a separate well/tube position if the fraction volume is below 5 mL. If the fraction volume is 5 mL or more, the pre-fractionation volume will be collected in the same tube/well as the first fraction. • ***Combined with first*** fraction is used when the pre-fractionation volume shall be collected in the same position as the first fraction. ***Note:*** *If the sum of pre-fractionation plus fraction volume exceed the maximum volume for the well/tube, the pre-fractionation volume will automatically be collected in a separate well/tube.* • ***Separate position*** is used when the pre-fractionation volume shall be collected in a position that is different from the first fraction.

Instruction name	Description
Last position filled action	***Last position filled*** action determines what action must be taken after the last position has been filled during fractionation. The options for ***Last position filled action*** are: • ***Out 1*** directs the flow to outlet 1 after the last position has been filled. • ***Pause*** sets the method to pause after the last position has been filled. • ***Waste*** directs the flow to waste after the last position has been filled. ***Note:*** *It is possible to enable collection in more than two plates when* ***Last position filled action*** *is set to* ***Pause****.*
Flow when moving from plate 1 to plate 2	***Flow when moving from plate 1 to plate 2*** sets system behavior when moving between plates. The options for ***Flow when moving from plate 1 to plate 2*** are: • ***Keep flow***, keeps the flow rate when the fractionation arm is moving from plate 1 to plate 2. For high flow rates, drops may be lost during movement. • ***Direct flow to waste,*** directs the flow to waste when the fractionation arm is moving from plate 1 to plate 2. When the fractionation arm reaches its position the outlet valve will turn to frac again. • ***Auto*** chooses ***Keep flow*** or ***Direct flow to waste*** depending on flow rate. ***Keep flow*** is selected for flow rates below 1.5 mL/min, ***Direct flow to waste*** for flow rates above or equal to 1.5 mL/min.
Frac behavior when ending a run	***Frac behavior when ending a run*** sets what shall happen after the run has ended. The options for ***Frac behavior when ending a run*** are: • ***Go home without reset*** moves the fractionation arm to its home position without resetting the plate(s). • ***Go home and reset*** moves the fractionation arm to the home position and resets the plate(s). • ***Remain without reset*** keeps the fractionation arm at its last position without resetting the plate.
LED mode	***LED mode*** turns the green light in the fractionation arm On or Off during fractionation.

System settings - Peak fractionation parameters

The table below describes the system settings available for the peak fractionation parameters during fraction collection.

Instruction name	Description
Peak fractionation parameters	***Peak fractionation parameters*** sets the detection parameters for peak collection, i.e. the monitor which is used as ***Signal source*** and when a peak starts and ends. This information is used by the instructions ***Peak fractionation***, ***Peak fractionation frac 2***, and ***Peak frac in outlet valve*** in order to start or end the peak collection.

9.8.10 System settings - Tubing and Delay volumes

The tables below describes the system settings related to tubing and delay volumes, available for ÄKTA pure.

Instruction name	Description
Tubing: Injection valve to column	The instruction is used in calculations of pre-column pressure when Column valve **V9-C** or **V9H-C** (5-columns) is not mounted onto the system. In such cases, there is no pre-column pressure sensor present. The estimated pre-column pressure is either calculated by using the measured system pressure and the length of the tubing between the system pressure sensor and the column valve or by using the measured sample pressure and the length of the tubing between the sample pressure sensor and the column valve depending on the position of the injection valve. The instruction is available only when Column valve **V9-C** or **V9H-C** (5-columns) is not selected in the component list.
Tubing: Sample pump to injection valve	The instruction is used in calculations of pre-column pressure when Column valve **V9-C** or **V9H-C** (5-columns) is not mounted onto the system. In such cases, there is no pre-column pressure sensor present. The estimated pre-column pressure is either calculated by using the measured system pressure and the length of the tubing between the system pressure sensor and the column valve or by using the measured sample pressure and the length of the tubing between the sample pressure sensor and the column valve depending on the position of the injection valve. The instruction is available only when Column valve **V9-C** or **V9H-C** (5-columns) is not selected and a sample pump (**S9** or **S9H**) is selected in the component list.
Delay volume: pH valve	The instruction is used to calculate the delay volume between the monitor and the Outlet valve. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. It is recommended not to alter the default values for restrictor and pH cell delay volumes when standard modules and standard tubing for flow restrictor are used.

Instruction name	Description
Delay volume: Monitor to outlet valve	The instruction is used to define the delay volume between the monitor and the Outlet valve. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The delay volume must be changed when changing tubing to another I.D. or length or when removing or adding components.
Delay volume: Monitor to frac (Only applicable to fraction collectors F9-C and F9-R)	The instruction is used to define the delay volume between the monitor and the Fraction collector. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The instruction is available only when the Fraction collector is selected in the component list.
Delay volume: Monitor to frac 2 (Only applicable to fraction collector F9-R)	The instruction is used to define the delay volume between the monitor and the second Fraction collector F9-R. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The instruction is available only when the second Fraction collector is selected in the component list.
Delay volume: Monitor to frac F9-T (Only applicable to fraction collector F9-T)	The instruction defines the delay volume between the UV monitor and the Fraction collector F9-T, excluding the pH valve but including the tubing to the pH valve.

9.8.11 System settings - Wash settings

The table below describes the wash related system settings available for ÄKTA pure.

Instruction name	Description
System wash settings	***System wash settings*** sets the flow rate used for ***System wash***. ***Note:*** *The volume for system wash is set in the* ***System wash*** *instruction.* ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.* ***Note:*** *Adjust the flow rate during the system wash so that the system pressure does not exceed 2 MPa.*
System pump wash settings	***System pump wash settings*** sets the flow rate and the wash volume used during system pump washes. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*
Sample pump wash settings	***Sample pump wash settings*** sets the flow rate and the wash volume used during sample pump washes. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*
Fraction collector wash settings (Only for Fraction collector **F9-C**)	***Fraction collector wash settings*** sets the flow rate during ***Fraction collector wash***.
Mixer by-pass wash settings	***Mixer by-pass wash settings*** sets the flow rate used during ***Mixer by-pass wash*** and defines wash volumes for mixer by-pass wash options. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*

Instruction name	Description
Loop wash settings (requires a column valve **V9-C**, **V9-Cm** or **V9-Cs**)	***Loop wash settings*** sets the flow rate used during ***Loop wash***. ***Note:*** *The volume for system wash is set in the* ***Loop wash*** *instruction.* ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*

9.8.12 System settings - Watch parameters

The table below describes the watch parameter settings available for ÄKTA pure.

Instruction name	Description
Watch UV parameters	***Watch UV parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the UV signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch UV 2nd parameters	***Watch UV 2nd parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the UV 2nd signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV1/UV2 parameters (Only for UV monitor **U9-M**)	Sets the ***Delta peak*** and ***Accepted fluctuation*** limit of the ratio UV2/UV1 signal for some of the ***Test*** parameters in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV 260/280 parameters (Only for UV monitor **U9-T**)	Sets the ***Delta peak*** and ***Accepted fluctuation*** limit of the ratio UV 260/280 signal for some of the ***Test*** parameters in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV 260/280 2nd parameters (Only for UV monitor **U9-T**, 2nd)	Sets the ***Delta peak*** and ***Accepted fluctuation*** limit of the ratio UV 260/280 2nd signal for some of the ***Test*** parameters in the ***Watch*** and ***Hold until*** instructions.
Watch conductivity parameters	***Watch conductivity parameters*** sets the accepted fluctuation and ***Delta peak*** limit of the conductivity signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch conductivity 2nd parameters	***Watch conductivity 2nd parameters*** sets the accepted fluctuation and ***Delta peak*** limit of the secondary conductivity signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch pH parameters	***Watch pH parameters*** sets the value for the accepted fluctuation of the pH signal used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until***.
Watch flow parameters	***Watch flow parameters*** sets the value for the accepted fluctuation of the flow rate signal used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until*** with signal ***System flow***.

Instruction name	Description
Watch pressure parameters	***Watch pressure parameters*** sets the value for the accepted fluctuation of the pressure signals used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until***.
Watch analog in parameters	***Watch analog in parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the analog signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.

9.8.13 System settings - Advanced

The table below describes the advanced system settings available for ÄKTA pure.

Instruction name	Description
Power-save	***Power-save*** sets the instrument into power saving mode. When the function is enabled, the instrument enters power-saving mode after having been in state ***Ready*** for a certain time period. The instrument turns into state ***Ready*** when a method run, a method queue or a manual run ends. The time interval before the instrument enters power-saving mode is defined by the user.
Instrument control panel	***Instrument control panel*** locks/unlocks the control panel located on the front side of the instrument. When unlocked, the buttons on the Instrument control panel are active and can be used to control a few basic functions of the instrument. When the Instrument control panel is locked, no functions are available.
Pressure control parameters	By using ***Pressure control*** the method can be run with the set flow rate without the risk of method stop due to pressure alarm. Pressure control is enabled in the instruction ***System flow*** or ***Sample flow***. ***Pressure control parameters*** provides the P and I factors used in the regulator and can be adjusted for different columns.
Constant pressure flow parameters	***Constant pressure flow parameters*** sets the values for the P and I factors needed to keep a constant pressure by varying the flow rate. The signal used for pressure control is set in the instruction ***Constant pressure flow***.
Max flow during valve turn	***Max flow during valve turn*** sets the maximum flow rate used during the turning of the injection and outlet valve in order to avoid high pressure alarms.
Method progressing flow	***Method progressing flow*** sets which flow (automatic, system flow, sample flow) the progress of the method is calculated after. In automatic mode, the position of the injection valve determines if the system flow or the sample flow is used.
Sample pump setting	***Sample pump setting*** enables sample pump flow while the injection valve is in manual load position.

9.8.14 System settings - Data collection

The table below describes the Data collection related system settings available for ÄKTA pure.

Instruction name	Description
Data collection	The ***Data collection*** settings determine the maximum number of data points collected for a given curve. Data reduction occurs if the maximum number of data points is exceeded. To avoid data reduction, set the maximum number of data points to be collected to 180000 or insert a ***New Chromatogram*** instruction in the method. ***Note:*** *The default setting is 54000 data points, which corresponds to 1.5 h for a signal of 10 Hz.*

9.9 Manual instructions

About this chapter

This section contains information about the manual instructions available in ÄKTA pure.

In this section

Section		See page
9.9.1	General instruction	594
9.9.2	Manual instructions - Pumps	595
9.9.3	Manual instructions - Flow path	599
9.9.4	Manual instructions - Monitors	602
9.9.5	Manual instructions - Alarms	605
9.9.6	Manual instructions - Fraction collector F9-R	609
9.9.7	Manual instructions - Fraction collector F9-C	610
9.9.8	Manual instructions - Fraction collector F9-T	612
9.9.9	Manual instructions - Fraction collector Outlet	614
9.9.10	Manual instructions - Peak fractionation parameters	615
9.9.11	Manual instructions - I/O-box	616
9.9.12	Manual instructions - Wash settings	617
9.9.13	Manual instructions - Watch parameters	619
9.9.14	Manual instructions - Advanced	621
9.9.15	Manual instructions - Other	624

9.9.1 General instruction

Follow the instructions below to perform a manual run using ***Manual Instructions***.

Step	Action
1	In the ***System Control*** module, select ***Manual*** →***Execute Manual Instructions***.
2	In the ***Manual instructions*** dialog box: **a.** Click the + symbol to show the instructions for the instruction group that you want to modify. **b.** Select the instruction that you want to modify. **c.** Enter the new values for the instruction.
3	To execute several instructions at the same breakpoint, select and edit an instruction and click ***Insert***. Repeat for several instructions.
4	To update parameter fields during method run, check the ***Auto update...*** box.
5	To perform the instructions, click ***Execute***.

All available manual instructions are described in the following subsections.

9.9.2 Manual instructions - Pumps

The table below describes the pump related manual instructions available for ÄKTA pure.

Instruction name	Description
System flow	***System flow*** defines the system flow rate. Flow rate can be set either as volumetric or as linear flow. A column type must be selected before using linear flow.
Sample flow	***Sample flow*** defines the system flow rate. Flow rate can be set either as volumetric or as linear flow. A column type must be selected before using linear flow.
Gradient	***Gradient*** sets a gradient (linear or stepwise) using the system pumps A and B. ***Note:*** *Set gradient length value to 0 to perform a step gradient.*
Pump A wash	***Pump A wash*** is used to change buffers in the inlet tubing, pump and mixer. ***Note:*** *Pressing **End** during **Pump wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **Pump wash** will terminate the wash and the run will continue from the point at which the pump wash instruction was executed.* ***Note:*** *An instruction issued when a **Pump wash** is in progress will not be executed until the wash is completely finished and all valves have turned back to the previous positions.* ***Pump wash*** cannot be executed when the system is in state ***HOLD***.

Instruction name	Description
Pump B wash	***Pump B wash*** is used to change buffers in the inlet tubing, pump and mixer. ***Note:*** *Pressing **End** during **Pump wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **Pump wash** will terminate the wash and the run will continue from the point at which the **Pump wash** instruction was executed.* ***Note:*** *An instruction issued when a **Pump wash** is in progress will not be executed until the wash is completely finished and all valves have turned back to the previous positions.* ***Note:*** ***Pump wash** cannot be executed when the system is in state **HOLD**.*
Sample pump wash	***Sample pump wash*** is used to change buffers in the sample inlet tubing and sample pump. ***Note:*** *Pressing **End** during **Sample pump wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **Sample pump wash** will terminate the wash and the run will continue from the point at which the **Sample pump wash** instruction was executed.* ***Note:*** *An instruction issued when a **Sample pump wash** is in progress will not be executed until the wash is completely finished and all valves have turned back to the previous positions.* ***Note:*** ***Sample pump wash** cannot be executed when the system is in state **HOLD**.* ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.*

Instruction name	Description
Mixer by-pass wash	***Mixer by-pass wash*** is used to wash or fill the flow path (mixer valve and tubing) that is used for sample application with system pump. It is possible to choose a more extensive or a less extensive wash option. ***Note:*** *Pressing **End** during **Mixer by-pass wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **Mixer by-pass wash** will terminate the wash and the run will continue from the point at which the **Mixer by-pass wash** instruction was executed.* ***Note:*** *An instruction issued during a **Mixer by-pass wash** will not be executed until the wash is completely finished and the valves have turned back to the previous positions.* ***Note:*** ***Mixer by-pass wash** cannot be executed when the system is in state **HOLD**.*
Loop wash	***Loop wash*** is used to wash the Loop valve. It is possible to wash a single sample application loop, all loops or only the by-pass position. ***Loop wash*** instruction is not available, if a Column valve is not mounted on the system. ***Note:*** *Pressing **End** during **Loop wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **Loop wash** will terminate the wash and the run will continue from the point at which the **Loop wash** instruction was executed.* ***Note:*** *An instruction issued when a **Loop wash** is in progress will not be executed until the wash is completely finished and the valves have turned back to the previous positions.* ***Note:*** ***Loop wash** cannot be executed when the system is in state **HOLD**.*

Instruction name	Description
System wash	***System wash*** is used to fill the system with the selected buffer composition. The flow can be directed to the waste position of either the injection valve or the outlet valve. The flow is directed to the end of the flow path if outlet valve is not present. ***Note:*** *Pressing **End** during **System wash** will terminate both the wash and the run immediately.* *Pressing **Continue** during **System wash** will terminate the wash and the run will continue from the point at which the **System wash** instruction was executed.* ***Note:*** *If **System wash** is performed during a **Gradient** operation, the current component B concentration is maintained during the wash.* ***Note:*** *An instruction issued during a system wash operation cannot be executed until the wash is completely finished and all valves have turned back to the previous positions.* ***Note:*** ***System wash** cannot be executed when the system is in state **HOLD**.* ***Note:*** *Adjust the flow rate during the system wash so that the system pressure does not exceed 2 MPa.*

9.9.3 Manual instructions - Flow path

The table below describes the flow path related manual instructions available for ÄKTA pure.

Instruction name	Description
Inlet A	***Inlet A*** turns Inlet valve A to the selected position. ***Note:*** *Positions* **A1** - **A7** *are available, if Inlet valve* **V9-IA** *or* **V9H-IA** *(7-ports) is used. Only positions* **A1** *and* **A2** *are available, if Inlet valve* **V9-IAB** *or* **V9H-IAB** *(2-ports) is used.*
Inlet B	***Inlet B*** turns the Inlet valve B to the selected position. ***Note:*** *Positions* **B1** - **B7** *are available, if Inlet valve* **V9-IB** *or* **V9H-IB** *(7-ports) is used. Only positions* **B1** *and* **B2** *are available, if Inlet valve* **V9-IAB** *or* **V9H-IAB** *(2-ports) is used.*
Sample inlet	***Sample inlet*** turns the Sample inlet valve to the selected position.
Injection valve	***Injection valve*** sets the Injection valve to the selected position. The instruction gives an injection mark in the chromatogram when the inlet valve switches to ***Inject*** or ***Direct Inject***. ***Note:*** *Sample flow refers to the flow that enters the injection valve via* **SaP** *port.*
Column position	***Column position*** turns the Column valve to the position specified in the parameter ***Position***.

Instruction name	Description
pH valve	***pH valve*** sets the pH cell and the 0.2 MPa restrictor in positions inline or offline. The pH valve also has a calibration position. This position is only available when performing calibration of the pH monitor (In ***System control*** select ***System*** →***Calibrate***).The calibration position can also be used to fill the pH cell with storage solution since the pH valve is in open position. ***Note:*** *It is not possible to turn the pH valve during any type of fractionation as it affects the delay volume.* ***Note:*** *The **pH valve** instruction can be given during the delay volume of the different stop fractionation instructions, but it is executed only after the set delay volume has been collected.*
Outlet valve	***Outlet valve*** turns the Outlet valve to the selected position. The instruction gives a mark in the chromatogram when the valve is switched to the selected position.
Loop valve	***Loop valve*** turns the Loop valve to the selected position.
Mixer valve	***Mixer valve*** turns the Mixer valve to the selected position. ***Note:*** *Setting Mixer valve in **by-pass** position makes it possible to use System pump A for direct loading of the sample onto the column.*
Versatile valve X	***Versatile valve X*** turns the versatile valve number X to the selected position. ***Note:*** *Four sets of positions are available. In positions **1-3** and **2-4** only a single flow channel can be used. In positions **1-4 and 2-3** and **1-2 and 3-4** the flow can be directed through two channels simultaneously.*
Inlet valve (X1)	Instruction ***Inlet valve X1*** turns the extra valve to the selected position. The extra valve is a basic 8 port valve, without air sensor, to be used for general applications.
Inlet valve (X2)	Instruction ***Inlet valve X2*** turns the extra valve to the selected position. The extra valve is a basic 8 port valve, without air sensor, to be used for general applications.

Instruction name	Description
Injection mark	***Injection mark*** sets an injection mark in the chromatogram at the point where this instruction is executed. ***Note:*** *The instruction is useful when the sample is loaded onto the column by the system pump.*

9.9.4 Manual instructions - Monitors

The table below describes the monitor related manual instructions available for ÄKTA pure.

Instruction name	Description
Auto zero UV	***Auto zero UV*** sets the UV signals from **U9-M**, **U9-T**, or **U9-L** to 0 mAU.
Auto zero UV 2nd	***Auto zero UV 2nd*** sets the UV signal from **U9-T**, 2nd or **U9-L**, 2nd to 0 mAU.
Wavelength	***Wavelength*** sets the wavelengths for UV monitor **U9-M** or **U9-T**. The corresponding curves in the chromatogram are marked with the set wavelengths. ***Note:*** *The instruction is available when UV monitor* **U9-M** *or* **U9-T** *is selected in the component list. For best performance when using UV monitor* **U9-M**, *do not use more wavelengths than necessary for the application.* ***Note:*** ***Wavelength*** *for* **U9-T** *can be set to 280 nm, or 280 nm and 260 nm. The default system setting is 280 nm.* ***Note:*** *At low wavelengths, the eluent can have absorbance of its own.*
Wavelength 2nd	***Wavelength 2nd*** sets the wavelengths for UV monitor **U9-T**, 2nd. The corresponding curves in the chromatogram are marked with the set wavelength. ***Note:*** ***Wavelength 2nd*** *can be set to 280 nm, or 280 nm and 260 nm. The default system setting is 280 nm.*
Noise reduction UV	***Noise reduction UV*** filters the noise in the UV signal from UV monitor **U9-M**, **U9-T**, or **U9-L**. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.
Noise reduction UV 2nd	***Noise reduction UV 2nd*** filters the noise in the UV monitor signal from **U9-T**, 2nd or **U9-L**, 2nd. A column-specific averaging time is set automatically when a column is defined in a method run and ***Averaging time*** is set as a variable.

Instruction name	Description
UV lamp	Sets the UV lamp ***ON*** or ***OFF***. Default is ***ON***. The UV lamp is turned ***ON*** when the system changes state to ***RUN***, ***HOLD***, or ***WASH***.
UV lamp 2nd	Sets the UV lamp 2nd ***ON*** or ***OFF***. Default is ***ON***. The UV lamp 2nd is turned ***ON*** when the system changes state to ***RUN***, ***HOLD***, or ***WASH***.
Relative scale cond	***Relative scale cond*** facilitates monitoring of a gradient, for which the user sets the conductivity values for 0% and 100%. The ***Relative scale cond*** can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity). ***Note:*** *The **Relative scale cond** in descending manner is especially useful for conductivity visualization in RPC and HIC, where the conductivity curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).*
Relative scale cond 2nd	***Relative scale cond 2nd*** facilitates monitoring of a gradient with the secondary conductivity monitor, for which the user sets the conductivity values for 0% and 100%. The ***Relative scale cond 2nd*** can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity). ***Note:*** *The **Relative scale cond 2nd** in descending manner is especially useful for conductivity visualization in RPC and HIC, where the conductivity curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).*
Reset auto zero UV	Resets the UV signal to the actual measured value by removing the offset that was generated when ***Auto zero*** was performed.
Reset auto zero UV 2nd	Resets the UV signal of the secondary UV monitor to the actual measured value by removing the offset that was generated when ***Auto zero*** was performed.

Instruction name	Description
Set baseline	***Set baseline*** is used for the calculation of the ***Breakthrough UV curve***. ***Set baseline*** use the current value of the primary wavelength of the primary UV monitor, or a value entered by the user.

9.9.5 Manual instructions - Alarms

The table below describes the alarm related manual instructions available for ÄKTA pure.

Instruction name	Description
Alarm system pressure	***Alarm system pressure*** sets the alarm limits for the system pressure. When enabled and the system pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. Default values for the alarm limits are set by the values in the column list when a column is selected in the method and ***Alarm system pressure*** is set as a variable. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously and then falls below the ***Low alarm*** limit. ***Note:*** *Setting the **Low alarm** or the system flow rate to 0 deactivates the low pressure alarm.*
Alarm sample pressure	***Alarm sample pressure*** sets the alarm limits for the sample pressure. When enabled and the pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously then falls below the ***Low alarm*** limit. ***Note:*** *Setting the **Low alarm** to 0 deactivates the low pressure alarm.*
Alarm delta-column pressure	***Alarm delta column pressure*** sets the alarm limits for the delta-column pressure (pre-column pressure minus post-column pressure). When enabled and the delta-column pressure falls outside the set pressure limits, an alarm will be triggered and the method will be paused. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously then falls below the ***Low alarm*** limit. ***Note:*** *Setting the **Low alarm** to 0 deactivates the low pressure alarm.* ***Note:*** *Instruction **Alarm delta column pressure** is available only when Column valve **V9-C** or **V9H-C** (5-columns) is selected in the component list.*

Instruction name	Description
Alarm pre-column pressure	***Alarm pre column pressure*** sets the alarm limits for the pre-column pressure. When enabled and the pre-column pressure falls outside the set pressure limits, an alarm is issued and the method will be paused. Default values for the alarm limits are set by the values in the column list when a column is selected in the method and ***Alarm pre column pressure*** is set as a variable. ***Low alarm*** is only triggered if the pressure first exceeds the ***Low alarm*** limit for ten seconds continuously then falls below the ***Low alarm*** limit. ***Note:*** *Setting the **Low alarm** to 0 deactivates the low pressure alarm.*
Alarm UV1	***Alarm UV 1*** enables or disables the alarm for the UV 1 signal from UV monitor **U9-M**. When enabled, it sets the alarm limits for the UV 1 signal from UV monitor **U9-M**. When the UV signal falls outside the set limits, an alarm is issued and the method will be paused. ***Note:*** *It is not possible to set an alarm signal for the UV 2 or UV 3 signals from UV monitor* **U9-M**.
Alarm UV 1_280	***Alarm UV 1_280*** enables or disables the alarm for the 280 nm UV signal from UV monitor **U9-T**. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-T**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 2_260	***Alarm UV 2_260*** enables or disables the alarm for the 260 nm UV signal from UV monitor **U9-T**. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-T**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 1_280 2nd	***Alarm UV 1_280 2nd*** enables or disables the alarm for the 280 nm UV signal from UV monitor **U9-T**, 2nd. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-T**, 2nd. When the signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 2_260 2nd	***Alarm UV 2_260 2nd*** enables or disables the alarm for the 260 nm UV signal from UV monitor **U9-T**, 2nd. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-T**, 2nd. When the signal falls outside the set limits, an alarm will be triggered and the method will be paused.

Instruction name	Description
Alarm UV	***Alarm UV*** enables or disables the alarm for the UV signal from UV monitor **U9-L**. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-L**. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm UV 2nd	***Alarm UV 2nd*** enables or disables the alarm for the UV signal from UV monitor **U9-L**, 2nd. When enabled, it sets the alarm limits for the UV signal from UV monitor **U9-L**, 2nd. When the UV signal falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm conduc-tivity	***Alarm conductivity*** enables or disables the conductivity alarm. When enabled, it sets the alarm limits for the conductivity signal. When the conductivity falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm conduc-tivity 2nd	***Alarm conductivity 2nd*** enables or disables the conductivity alarm for the secondary conductivity monitor. When enabled, it sets the alarm limits for the conductivity signal. When the conduc-tivity falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm pH	***Alarm pH*** enables or disables the pH alarm. When enabled, it sets the alarm limits for the pH signal. When the pH falls outside the set limits, an alarm will be triggered and the method will be paused.
Alarm inlet A air sensor	***Alarm inlet A air sensor*** enables or disables the air sensor alarm for the built-in air sensor at inlet A. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm inlet B air sensor	***Alarm inlet B air sensor*** enables or disables the air sensor alarm for the built-in air sensor at inlet B. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm sample inlet air sensor	***Alarm sample inlet air sensor*** enables or disables the air sensor alarm for the built-in air sensor at the sample inlet. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm external air sensor X	***Alarm external air sensor X*** enables or disables the alarm for the optional air sensor number X. If the alarm is enabled and air is detected, an alarm will be triggered and the method will be paused.
Alarm analog in X	***Alarm analog in X*** enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the method will be paused.

Instruction name	Description
Alarm digital in X	***Alarm digital in X*** enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in '**Value**' occurs, an alarm will be triggered and the method will be paused.

9.9.6 Manual instructions - Fraction collector F9-R

The table below describe the Fraction collector F9-R manual instructions available forÄKTA pure. When referring to Fraction collector F9-R as a second fraction collector, the instruction name is denoted with ***frac 2*** in the software.

Instruction name	Description
Fractionation	***Fractionation*** is used to set the fractions size when collecting fractions with a Fraction collector. The fractions size can be set in time or in volume.
Stop fractionation	***Stop fractionation*** ends the fractionation after the set delay volume (specified in ***System Settings*** →***Fraction collection*** →***Delay volume***) has been collected. The outlet valve is then turned to position ***Waste***. ***Note:*** *If* ***Stop fractionation*** *is issued when both* ***Fractionation*** *and* ***Peak fractionation*** *are active, fractionation is stopped after the set delay volume has been collected. The outlet valve remains in position* **Frac** *and peak fractionation continues.*
Peak fractionation	***Peak fractionation*** enables collection of only those peaks that fulfill the conditions set in the ***Peak fractionation parameters UV*** instruction described below.
Stop peak fractionation	***Stop peak fractionation*** ends the peak fractionation after the set delay volume (specified in ***System Settings*** →***Fraction collection*** →***Delay volume***) has been collected. The outlet valve is then turned to position **Waste**.
Reset frac number	Sets fraction numbers to restart from 1. The restart occurs when the instruction is issued. The instruction overrides the continuous numbering mode if ***Fractionation numbering mode*** is set to ***Continue*** in ***System Settings***.
Feed tube	***Feed tube*** moves the tube rack forward one tube after the set delay volume has been collected and a fraction mark is set. When fractionation or peak fractionation is not ongoing, ***Feed tube*** moves the rack instantly and no fraction mark is set.

9.9.7 Manual instructions - Fraction collector F9-C

The table below describes the fraction collection related manual instructions available for ÄKTA pure.

Instruction name	Description
Fractionation	***Fractionation*** is used when collecting fractions with a fraction collector.
Stop fractionation	***Stop fractionation*** ends the fractionation after the set delay volume (specified in ***System Settings →Tubing and Delay volumes***) has been collected. The outlet valve is then turned to position ***Waste***. ***Note:*** *If* ***Stop fractionation*** *is issued when both* ***Fractionation*** *and* ***Peak fractionation*** *are active, fractionation is stopped after the set delay volume has been collected. The outlet valve remains in position* **Frac** *and peak fractionation continues.*
Peak fractionation	***Peak fractionation*** enables collection of only those peaks that fulfill the conditions set in the ***Peak fractionation parameters*** instruction.
Stop peak fractionation	***Stop peak fractionation*** ends the peak fractionation after the set delay volume (specified in ***System Settings →Tubing and Delay volumes***) has been collected. The outlet valve is then turned to position **Waste**.
Last tube filled	***Last tube filled*** sets the action to perform after the fraction collector fills the last tube of the run: pause the fractionation, direct the flow to one of the outlet ports of the outlet valve or direct the flow to waste.
Feed tube	***Feed tube*** moves the fractionation arm to the position specified by the parameter ***Start position***, after the set delay volume has been collected. A fraction mark is given in the chromatogram.
Fraction collector wash	***Fraction collector wash*** is used to wash the fraction collector with the current solution present in the system. The wash flow rate is set in the instruction ***Wash settings →Fraction collector wash settings*** and the current inlet positions are used. After the wash, the flow rate and the valve positions automatically go back to their previous settings. ***Note:*** ***Fraction collector wash*** *cannot be executed during any type of fractionation.*

Instruction name	Description
Reset tube type	***Reset tube type*** resets all the tube types in the fraction collector. ***Note:*** *It is not allowed to execute the* ***Reset tube type*** *instruction during fractionation.*
Frac cleaning position	***Frac cleaning position*** enables manual cleaning of the dispenser head. The system is paused and the fractionation arm is moved to the middle front of the interior of the fraction collector. It is then possible to open the door of the fraction collector and manually clean the dispenser head. ***Note:*** *The* ***Frac cleaning position*** *instruction cannot be executed during fractionation.*

9.9.8 Manual instructions - Fraction collector F9-T

The tables below describe the Fraction collector F9-T manual instructions available for ÄKTA pure.

Instruction name	Description
Fractionation	This instruction sets the fractionation parameters when collecting fractions with the Fraction collector. ***Plate type*** sets the type of plates or tubes used. ***Fraction size*** sets the size of the fraction in each well/tube. The fractionation size can be set in time or in volume. ***Start position*** sets at which position fractionation shall start. The options for ***Start position*** are: ***Set A.1;*** resets the plate position and fractionation starts in A1 in the first plate of the chosen type.
Peak fractionation	This instruction enables collection of only those peaks that fulfill the conditions set in the ***Peak fractionation parameters UV*** instruction described below. Fraction marks are set in the chromatogram for each collected peak. The settings are the same as for ***Fractionation***.
Combined fractionation	This instruction combines fractionation and peak fractionation. This enables a more dynamic fractionation where a fixed fractionation is performed between detected peaks. When a peak start is detected, peak fractionation starts and continues until peak end is detected. At peak end, the fixed fractionation is resumed until a new peak start is detected. Fraction marks are set in the chromatogram for each new fraction collected. The settings are the same as for ***Fractionation***.
Stop fractionation	This instruction ends the fractionation after the set delay volume has been collected. The outlet valve is then turned to position ***Waste***.
Next position	The fractionation goes to the next position after the delay volume has been collected. When no fractionation is ongoing, ***Next position*** moves the fractionation arm instantly. When issuing ***Next position*** while the flow is directed to Waste or Out 1, the fractionation arm will move over the positions. These positions will be marked in the chromatogram, but no fractions are collected in these positions.

Instruction name	Description
Next plate	The fractionation goes to the next plate after the set delay volume has been collected. Next plate can only be issued when plate 1 and plate 2 are of the same type.
Go home	This instruction makes the fractionation arm move to its home position. Using this command is only possible when fractionation is not ongoing, e.g. Pause during ongoing fractionation.
Go to right	This instruction makes the fractionation arm move as far right as possible. Using this command is only possible when fractionation is not ongoing, e.g. Pause during ongoing fractionation.
Reset plate	This instruction resets all positions in plates or racks and sets them as available for fractionation. The command cannot be performed for the current plate/rack when fractionation is ongoing.
Flow when moving from plate 1 to plate 2	This instruction sets what happens when the fractionation arm is moving between plates.
Frac behavior when ending a run	This instruction sets what happens after the run has ended.
Delay volume: Monitor to frac F9-T	This instruction is used to define the delay volume between the monitor and the Fraction collector. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The instruction is available only when the Fraction collector is selected in the component list.

9.9.9 Manual instructions - Fraction collector Outlet

The table below describes the manual instructions available for the outlet valve during fraction collection.

Instruction name	Description
Fractionation in outlet valve	**Fractionation in outlet valve** enables fractionation via the outlet valve. When the set fraction size (outlet) has been collected, the outlet valve turns to the next position. A fraction mark is set in the chromatogram for each new outlet position. If using Outlet valve (1-outlet), only one fraction can be collected via Outlet 1 position.
Stop frac in outlet valve	***Stop frac in outlet valve*** ends the fractionation in outlet valve after the set delay volume (specified in ***System Settings →Tubing and Delay volumes***) has been collected. The outlet valve is then turned to position **Waste**.
Peak frac in outlet valve	***Peak frac in outlet valve*** enables collection of only those peaks that fulfill the conditions set in ***Peak fractionation parameters***. When the set fraction size/outlet has been collected, the outlet valve turns to the next position. A fraction mark is set in the chromatogram for each new outlet position. If Outlet valve (1-outlet) is used, only one peak can be collected via Outlet 1 position.
Stop peak frac in outlet valve	***Stop peak frac in outlet valve*** ends the peak fractionation in outlet valve after the set delay volume (specified in ***System Settings →Tubing and Delay volumes***) has been collected. The outlet valve is then turned to position **Waste**.

9.9.10 Manual instructions - Peak fractionation parameters

The table below describes the manual instructions available for the peak fractionation parameters during fraction collection.

Instruction name	Description
Peak fractionation parameters	***Peak fractionation parameters*** sets the detection parameters for peak collection, i.e. the monitor which is used as ***Signal source*** and when a peak starts and ends. This information is used by the instructions ***Peak fractionation***, ***Peak fractionation frac 2***, and ***Peak frac in outlet valve*** in order to start or end the peak collection.

9.9.11 Manual instructions - I/O-box

The table below describes the I/O-box related manual instructions available for ÄKTA pure.

Instruction name	Description
Auto zero analog in X	***Auto zero analog in X*** sets the value of the analog signal in the analog port number X to 0 mV.
Reset auto zero analog in X	***Reset auto zero analog in X*** sets the signal in analog port number X to its current value, i.e. the actual voltage in the analog port number X.
Noise reduction analog in X	***Noise reduction analog in X*** filters the noise in the analog signal in port number X.
Digital out X	***Digital out X*** sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Pulse digital out X	***Pulse digital out X*** generates a pulsed signal in digital port number X. The signal changes from the initial state (0 or 1) to the opposite state and returns to the initial state after the defined length of time.
Configure analog out X	***Configure analog out X*** enables the user to send one of the pre-defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

9.9.12 Manual instructions - Wash settings

The table below describes the wash related manual instructions available for ÄKTA pure.

Instruction name	Description
System wash settings	***System wash settings*** sets the flow rate used for ***System wash***. ***Note:*** *The volume for system wash is set in the* ***System wash*** *instruction.* ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (I.D. 0.75 mm) is used.* ***Note:*** *Adjust the flow rate during the system wash so that the system pressure does not exceed 2 MPa.*
System pump wash settings	***System pump wash settings*** sets the flow rate and wash volume used during system pump washes. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (I.D. 0.75 mm) is used.*
Sample pump wash settings	***Sample pump wash settings*** sets the flow rate and wash volume used during sample pump washes.
Fraction collector wash settings (F9-C only)	***Fraction collector wash settings*** sets the flow rate during ***Fraction collector wash.***
Mixer by-pass wash settings	***Mixer by-pass wash settings*** sets the flow rate used during ***Mixer by-pass wash*** and defines wash volumes for mixer by-pass wash options. ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (I.D. 0.75 mm) is used.*

Instruction name	Description
Loop wash settings	***Loop wash settings*** sets the flow rate used during ***Loop wash***. ***Note:*** *The volume for system wash is set in the* ***Loop wash*** *instruction.* ***Note:*** *The flow rate must not exceed 10 mL/min if narrow inlet tubing (I.D. 0.75 mm) is used.*

9.9.13 Manual instructions - Watch parameters

The table below describes the watch parameter instructions available for ÄKTA pure.

Instruction name	Description
Watch UV parameters	***Watch UV parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the UV signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch UV 2nd parameters	***Watch UV 2nd parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the UV 2nd signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV1/UV2 parameters (Only for **U9-M**)	***Watch UV 2nd parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the UV 2nd signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV 260/280 parameters (Only for **U9-T**)	Sets the ***Delta peak*** and ***Accepted fluctuation*** limit of the ratio UV 260/280 signal for some of the ***Test*** parameters in the ***Watch*** and ***Hold until*** instructions.
Watch ratio UV 260/280 2nd parameters (Only for **U9-T**, 2nd)	Sets the ***Delta peak*** and ***Accepted fluctuation*** limit of the ratio UV 260/280 signal from UV monitor **U9-T**, 2nd for some of the ***Test*** parameters in the ***Watch*** and ***Hold until*** instructions.
Watch conductivity parameters	***Watch conductivity parameters*** sets the accepted fluctuation and ***Delta peak*** limit of the conductivity signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch 2nd conductivity parameters	***Watch 2nd conductivity parameters*** sets the accepted fluctuation and ***Delta peak*** limit of the conductivity signal from the secondary conductivity monitor for some of the tests in the ***Watch*** and ***Hold until*** instructions.
Watch flow parameters	***Watch flow parameters*** sets the value for the accepted fluctuation of the flow rate signal used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until*** with signal ***System flow***.
Watch pH parameters	***Watch pH parameters*** sets the value for the accepted fluctuation of the pH signal used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until***.

Instruction name	Description
Watch pressure parameters	***Watch pressure parameters*** sets the value for the accepted fluctuation of the pressure signals used for the test ***Stable signal*** in the instructions ***Watch*** and ***Hold until***.
Watch analog in parameters	***Watch analog in parameters*** sets the accepted signal fluctuation and ***Delta peak*** limit of the analog signal for some of the tests in the ***Watch*** and ***Hold until*** instructions.

9.9.14 Manual instructions - Advanced

The table below describes the advanced manual instructions available for ÄKTA pure.

Instruction name	Description
Pressure control parameters	By using ***Pressure control*** the method can be run with the set flow rate without the risk of method stop due to pressure alarm. Pressure control is enabled in the instruction ***System flow*** or ***Sample flow***. ***Pressure control parameters*** provides the P and I factors used in the regulator and can be adjusted for different columns. Pressure control min flow rate can be set either as volumetric or as linear flow. A column type must be selected before using linear flow.
Constant pressure flow	This instruction enables column packing at constant pressure. The system pump automatically adjusts the flow rate within the specified ***Minimum allowed flow rate*** – ***Maximum allowed flow rate*** range. The goal is to reach and keep the set ***Pressure*** at the selected ***Pressure sensor*** using the P and I factors set in the ***Constant pressure flow parameters*** instruction. The total volume is continuously updated using the actual flow rate. Both pressure control flow rates can be set either as volumetric or as linear flow. A column type must be selected before using linear flow. ***Note:*** *When* ***Constant pressure flow*** *is used, the P and I factors set in the* ***Constant pressure flow parameters*** *instruction are used to control the pressure, instead of the P and I values set in the* ***Pressure control parameters*** *instruction.* ***Note:*** *Pressure sensor* ***Delta column pressure*** *is available only when column valve* **V9-C** *or* **V9H-C** *(5-columns) is selected in the component list.*
Constant pressure flow parameters	This instruction sets the values for the P and I factors needed to keep a constant pressure by varying the flow rate. The signal used for pressure control is set in the instruction ***Constant pressure flow***.

Instruction name	Description
Column packing flow	This instruction is used to set flow rates over 25 mL/min and 150 mL/min for ÄKTA pure 25 and ÄKTA pure 150, respectively. Both A and B pumps are used to generate the flow, making it possible to set flow rates up to 50 mL/min and 300 mL/min for ÄKTA pure 25 and ÄKTA pure 150, respectively. Flow rate can be set either as volumetric or as linear flow. A column type must be selected before using linear flow. Before executing the ***Column packing flow*** instruction it is important to: • Immerse inlet tubing **A1** and **B1** in the same buffer • Disconnect the column outlet tubing from the Column valve and place the tubing in a waste vessel ***Note:*** *When running **Column packing flow** only isocratic runs can be performed, gradients cannot be generated.*
Delay volume: Monitor to outlet valve	This instruction is used to define the delay volume between the monitor and the Outlet valve. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The delay volume must be changed when changing tubing to another i.d. or length or when removing or adding components.
Delay volume: Monitor to frac	This instruction is used to define the delay volume between the monitor and the Fraction collector. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The instruction is available only when the Fraction collector is selected in the component list.
Start volume count	This instruction starts the volume counter function. The counted volume is saved into a memory. This instruction is best used in combination with ***Watch*** instructions.
Stop volume count	This instruction stops the volume counter function. The counted volume is stored in the memory and can be recalled with the instruction ***Hold counted volume***. The counted volume can also be recalled in following runs and is stored until a new ***Stop volume count*** instruction is issued. This instruction is best used in combination with ***Watch*** instructions.
Method progressing flow	This instruction defines the flow from which the volume base is calculated. When set to ***Automatic***, the position of the injection valve determines if the system flow or the sample flow is used.

Instruction name	Description
Set trigger [1-12]	This instruction sets the value of trigger [1-12] to either 0 or 1. The default value is 0. When used together with the ***Watch*** instruction, with trigger [1-12] selected as signal, changing the value of trigger [1-12] can be used to trigger a user-defined action. For more information, see the help for the ***Watch*** instruction.
Set counter	This instruction sets the value of the counter 1-4 to a value between -10000 and +10000. The default value is 0. To facilitate scouting runs, the counter mode can be set to On or Off. The default value is On. Setting the mode to Off does not disable ***Update counter***. When used together with the ***Watch*** instruction, with counter 1-4 selected as signal, changing the value of counter 1-4 can be used to trigger a user-defined action. For more information, see the help for the ***Watch*** instruction. The counter value is stored and can be recalled in following runs. The value is kept until a new counter value is set from the instructions ***Set counter*** or ***Update counter***.
Update counter	This instruction updates the value of counter 1-4 with the values -1, 0, or +1. The default value is +1. When used together with the ***Watch*** instruction, with counter 1-4 selected as signal, changing the value of counter 1-4 can be used to trigger a user-defined action. For more information, see the help for the ***Watch*** instruction. The counter value is stored and can be recalled in following runs. The value is kept until a new counter value is set from the instructions ***Set counter*** or ***Update counter***.

9.9.15 Manual instructions - Other

The table below describes the other manual instructions available for ÄKTA pure.

Instruction name	Description
Set mark	***Set mark*** inserts a mark into the current chromatogram with the text entered for the parameter ***Mark text*** .
Timer	***Timer*** sets the system to pause or end after a set volume or time has passed. ***Select base*** sets the base to either accumulated time or accumulated volume. ***Timeout*** sets the volume or time. ***Action*** sets the action to perform (pause or end).

9.10 Available Run data

The table below lists all available ***Run data*** for ÄKTA pure.

Run Data	Range/Unit	Description
System state	N/A	Status of connection and run.
Acc. Volume	mL	Total accumulated volume in the current method or manual run.
Block volume	mL	Accumulated volume in the current block (method run only).
Acc. Time	min	Total accumulated time in the current method or manual run.
Block time	min	Accumulated time in the current block (method run only).
Scouting no	N/A	The current scouting number in the scouting scheme.
System flow	0.001 – 50.000 mL/min (ÄKTA pure 25) 0.01 – 300.00 mL/min (ÄKTA pure 150)	The set flow rate of the system pumps.
System linear flow	cm/h	The set flow velocity of the system pumps. Only available if a column is selected.
Sample flow	0.001 – 50.000 mL/min (ÄKTA pure 25) 0.01 – 300.00 mL/min (ÄKTA pure 150)	The set flow rate of the sample pump.
Sample linear flow	cm/h	The set flow velocity of the sample pump. Only available if a column is selected.
Inlet A	A1 - A7	The set position of the inlet valve A.
Inlet B	B1 - B7	The set position of the inlet valve B.
Inlet S	S1 - S7, buff	The set position of the sample inlet valve.
Conc B	0.0 – 100.0%B	The set concentration B or the current value during a gradient.
Mixer valve	N/A	The set position of the Mixer valve.

Run Data	Range/Unit	Description
Injection	N/A	The set position of the Injection valve.
Loop position	N/A	The set position of the Loop valve.
Column position	N/A	The set position of the Column valve.
Column flow direction	N/A	The set flow direction position of the Column valve **V9-C**, **V9H-C**, **V9-Cm**, **V9-Cs**, and **V9H-Cs**.
System pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	The system pressure signal (at the system pumps).
Sample pressure	-1.00 – 10.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	The sample pressure signal (at the sample pump).
PreC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	The pre-column pressure signal.
DeltaC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	The delta-column pressure signal.
PostC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	The post-column pressure signal.
UV 1	-6000.000 – 6000.000 mAU	The first UV/Vis absorbance signal of UV monitor **U9-M**.
UV 2	-6000.000 – 6000.000 mAU	The 2nd UV/Vis absorbance signal of UV monitor **U9-M**.
UV 3	-6000.000 – 6000.000 mAU	The 3rd UV/Vis absorbance signal of UV monitor **U9-M**.
Ratio UV2/UV1	-1000 to 1000	The ratio between UV absorbance at wavelengths 2 and 1 of UV monitor **U9-M**.

Run Data	Range/Unit	Description
UV 1_280	-6000.000 – 6000.000 mAu	The UV absorbance signal at 280 nm of UV monitor **U9-T**.
UV 2_260	-6000.000 – 6000.000 mAu	The UV absorbance signal at 260 nm of UV monitor **U9-T**.
UV 1_280 2nd	-6000.000 – 6000.000 mAu	The UV absorbance signal at 280 nm of UV monitor **U9-T**, 2nd.
UV 2_260 2nd	-6000.000 – 6000.000 mAu	The UV absorbance signal at 260 nm of UV monitor **U9-T**, 2nd.
Ratio UV 260/280	-1000 to 1000	The ratio between UV absorbance at wavelengths 260 nm and 280 nm of UV monitor **U9-T**.
Ratio UV 260/280 2nd	-1000 to 1000	The ratio between UV absorbance at wavelengths 260 nm and 280 nm of UV monitor **U9-T**, 2nd.
UV	-6000.000 – 6000.000 mAu	The UV absorbance signal of UV monitor **U9-L**.
UV 2nd	-6000.000 – 6000.000 mAU	The UV absorbance signal of UV monitor **U9-L**, 2nd.
Breakthrough UV	0 – 100%	Two UV signals are used to show column saturation levels when the column is close to sample breakthrough.
Cond	0.00 – 999.99 mS/cm	The conductivity signal.
Cond 2nd	0.00 – 999.99 mS/cm	The conductivity signal of the secondary conductivity monitor.
% Cond	0.0 – 100.0%	The conductivity signal as a percentage of a set range.
% Cond 2nd	0.0 – 100.0%	The conductivity signal of the secondary conductivity monitor as a percentage of a set range.
Cond temp	0.0 – 99.0°C	The temperature signal (in the conductivity flow cell).
Cond temp 2nd	0.0 – 99.0°C	The temperature signal (in the flow cell of the secondary conductivity monitor).
pH	0.00 – 14.00	The pH signal.

Run Data	Range/Unit	Description
pH valve	N/A	The set position of the pH valve.
Outlet	N/A	The set position of the outlet valve.
Frac position	N/A	The current tube position of the fraction collector.
Frac 2 position	N/A	The current tube position of the fraction collector 2.
Inlet valve X1	N/A	The set position of the X1 valve.
Inlet valve X2	N/A	The set position of the X2 valve.
Versatile valve	N/A	The set position of the versatile valve.
Versatile valve 2	N/A	The set position of the versatile valve 2.
Versatile valve 3	N/A	The set position of the versatile valve 3.
Versatile valve 4	N/A	The set position of the versatile valve 4.
Air inlet A	No air, Air	The current state of the air alarm for the integrated air sensor in inlet valve A.
Air inlet B	No air, Air	The current state of the air alarm for the integrated air sensor in inlet valve B.
Air inlet S	No air, Air	The current state of the air alarm for the integrated air sensor in inlet valve IS.
Ext. air sensor to ***Ext. air sensor 4***	No air, Air	The current state of the air alarm for the external air sensors.
Analog in 1 to ***Analog in 4***	-2000.0 – 2000.0 mV	The I/O-box analog input signals.
Digital in 1 to ***Digital in 8***	0, 1	The I/O-box digital input signals.

Run Data	Range/Unit	Description
Digital out 1 to ***Digital out 8***	0, 1	The set value of the I/O-box digital output signals.
Counter 1 to ***Counter 4***	N/A	The current value of the counters.
Counted volume	N/A	The calculated volume between ***Start volume count*** and ***Stop volume count***.

9.11 Available Curves

The table below lists all available ***Curves*** for ÄKTA pure.

Curve	Range	Sampling frequency	Description
UV 1	-6000.000 – 6000.000 mAU	10 Hz	The first UV/Vis absorbance signal of the **U9-M** monitor.
UV 2	-6000.000 – 6000.000 mAU	2 Hz	The 2nd UV/Vis absorbance signal of the **U9-M** monitor.
UV 3	-6000.000 – 6000.000 mAU	2 Hz	The 3rd UV/Vis absorbance signal of the **U9-M** monitor.
Ratio UV2/UV1	-1000 to 1000	10 Hz	The ratio between UV absorbance at wavelengths 2 and 1 of the **U9-M** monitor.
UV 1_280	-6000.000 – 6000.000 mAu	10 Hz (5 Hz if UV2_260 is also enabled)	The UV absorbance signal at 280 nm of UV monitor **U9-T**.
UV 2_260	-6000.000 – 6000.000 mAu	5 Hz	The UV absorbance signal at 260 nm of UV monitor **U9-T**.
UV 1_280 2nd	-6000.000 – 6000.000 mAu	10 Hz (5 Hz if UV2_260 2nd is also enabled)	The UV absorbance signal at 280 nm of UV monitor **U9-T**, 2nd.
UV 2_260 2nd	-6000.000 – 6000.000 mAu	5 Hz	The UV absorbance signal at 260 nm of UV monitor **U9-T**, 2nd.
Ratio UV 260/280	-1000 to 1000	5 Hz	The ratio between UV absorbance at wavelengths 260 nm and 280 nm of UV monitor **U9-T**.

Curve	Range	Sampling frequency	Description
Ratio UV 260/280 2nd	-1000 to 1000	5 Hz	The ratio between UV absorbance at wavelengths 260 nm and 280 nm of UV monitor **U9-T**, 2nd.
UV	-6000.000 – 6000.000 mAU	10 Hz	The UV absorbance signal of UV monitor **U9-L** monitor.
UV 2nd	-6000.000 – 6000.000 mAU	10 Hz	The UV absorbance signal of the second UV monitor **U9-L**.
Break-through UV	0 – 100%	10 Hz	Two UV signals are used to show column saturation levels when the column is close to sample breakthrough.
Cond	0.00 – 999.99 mS/cm	5 Hz	The conductivity signal.
Cond 2nd	0.00 – 999.99 mS/cm	5 Hz	The conductivity signal of the secondary conductivity monitor.
% Cond	0.0 – 100.0%	1 Hz	The conductivity signal as a percentage of a set range.
% Cond 2nd	0.0 – 100.0%	1 Hz	The conductivity signal of the secondary conductivity monitor as a percentage of a set range.
Cond temp	0.0 – 99.0°C	0.5 Hz	The temperature signal (in the conductivity flow cell).
Cond temp 2nd	0.0 – 99.0°C	0.5 Hz	The temperature signal (in the flow cell of the secondary conductivity monitor).
Conc B	0.0 – 100.0%	1 Hz	The set concentration B or the current value during a gradient.

Curve	Range	Sampling frequency	Description
System flow	0.001 – 50.000 mL/min (ÄKTA pure 25) 0.01 – 300.00 mL/min (ÄKTA pure 150)	1 Hz	The set flow rate of the system pumps.
System linear flow	cm/h	1 Hz	The set flow velocity of the system pumps. Only available if a column is selected.
Sample flow	0.001 – 50.000 mL/min (ÄKTA pure 25) 0.01 – 300.00 mL/min (ÄKTA pure 150)	1 Hz	The set flow rate of the sample pump.
Sample linear flow	cm/h	1 Hz	The set flow velocity of the sample pump. Only available if a column is selected.
pH	0.00 – 14.00	1 Hz	The pH signal.
Counted volume	0 – 1 000 000 mL	10 Hz	The calculated volume between ***Start volume count*** and ***Stop volume count***.
PreC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	1 Hz	The pre-column pressure signal.
DeltaC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	1 Hz	The delta-column pressure signal.
System pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	10 Hz	The system pressure signal (at the system pumps).

Curve	Range	Sampling frequency	Description
Sample pressure	-1.00 – 10.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	10 Hz	The sample pressure signal (at the sample pump).
PostC pressure	-1.00 – 20.00 MPa (ÄKTA pure 25) -1.00 – 5.00 MPa (ÄKTA pure 150)	1 Hz	The post-column pressure signal.
UV cell path length	0.04, 0.2, 0.5 cm (**U9-T**, or **U9-L**) 0.05, 0.2, 1.0 cm (**U9-M**)	1 Hz	The nominal cell path length of the UV monitor.
UV cell path length 2nd	0.04, 0.2, 0.5 cm	1 Hz	The nominal cell path length of UV monitor **U9-T**, 2nd or **U9-L**, 2nd.
Analog in 1 to ***Analog in 4***	-2000.0 – 2000.0 mV	10 Hz	The I/O-box analog input signals.
Digital in 1 to ***Digital in 8***	0, 1	10 Hz	The I/O-box digital input signals.
Digital out 1 to ***Digital out 8***	0, 1	10 Hz	The I/O-box digital output signals.

9.12 Injection volumes and peak broadening

The width of peaks at the fraction collector is influenced by the following parameters.

- The properties of the column.
- The dimensions of the tubing.
- The dimensions of the modules in the flow path.
- The fluid dynamics.

Initial sample volume affects the peak width in size exclusion chromatography (SEC) and other isocratic techniques. A sample zone is broadened during passage through a SEC column so that the sample is diluted and the resolution decreases with increasing sample volume. Sample volume does not however affect the resolution in adsorption chromatography techniques such as affinity chromatography (AC), ion exchange chromatography (IEX), and hydrophobic interaction chromatography (HIC) if the retention factor k is high.

The effect of peak broadening in the system from sample injection to peak detection (including dilution on the column) is apparent in the chromatogram from the UV monitor, but broadening from the UV monitor to fraction collection is not visible in the chromatogram. This "hidden" effect is more pronounced for smaller peak volumes.

Narrow and short tubing reduces peak broadening. However, narrow tubing also increases the back pressure. See for recommendations on tubing dimensions for prepacked columns.

9.13 Delay volumes

Introduction

A number of methods, both theoretical and experimental, exist for determining the delay volume of a system. The easiest and recommended method is to perform a theoretical determination. Delay volumes for standard configurations are listed in *Standard delay volumes, on page 636*.

Explanation of delay volume

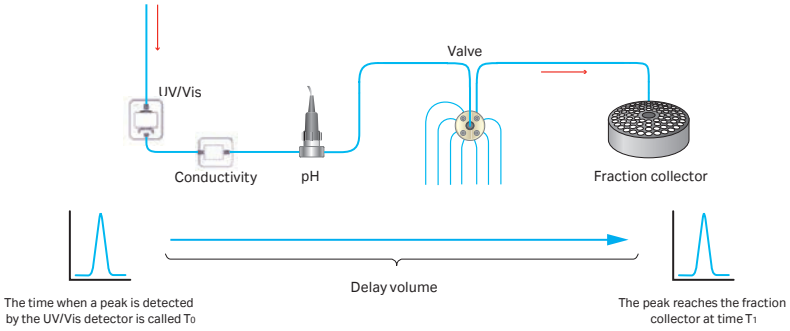
The delay volume is the volume between the detector selected as fractionation source and the fraction collector or outlet that is used.

Adjust the delay volume when:

- A module has been installed after the module that is set as the peak fractionation source in the flow path.
- Another monitor is used as peak fractionation source than the default setting. The default peak fractionation source is the primary wavelength of the primary UV monitor.

See *Set the delay volume, on page 203* for detailed instructions.

The illustration below shows and example of the delay volume between the UV/Vis monitor and the fraction collector.



Theoretical determination of delay volumes

A theoretical determination is performed as described in the steps below:

Step	Action
1	Identify all components in the system flow path that contribute to the delay volume of interest.

Step	Action
2	Determine the internal volumes of all hardware modules and tubing, see *Section 9.14 Component volumes, on page 640* for information about theoretical module volumes and *Section 9.3 Tubing and connectors, on page 536* for information about tubing lengths and dimensions.
3	To obtain the total delay volume, sum up half of the flow cell volume of the monitor used (that is, the UV or UV/Vis monitor) with all volumes of tubing and modules that are located after the monitor in the flow path. ***Note:*** *For pH-valve* **V9-pH** *and* **V9H-pH** *always use the volume for the valve in bypass position. The system automatically adds the volumes for the flow restrictor and the pH flow cell when if they are part of the system.*

See *Set the delay volume, on page 203* for instructions on how to update the delay volume.

Standard delay volumes

The table below lists the delay volumes for standard configurations using the available tubing kits with and without a pH valve and using tubing of standard length from the outlet valve to the fraction collector. The values are based on the flow cells for UV monitor **U9-M**. When using **U9-T** or **U9-L**, add 3 µL for the 0.4 mm flow cell, 9 µL for the 2 mm flow cell, and 4 µL for the 5 mm flow cell. The values for delay volume Monitor to frac F9-C assume that the internal tubing of Fraction collector F9-C has the same inner diameter as the tubing kit.

Note: *Always set the delay volume from the monitor selected as peak fractionation source to the point of collection of eluent. Depending on the system configuration, this will be* ***Delay volume →Monitor to outlet valve*** *or* ***Delay volume →Monitor to frac*** *in the system settings.*

Delay volumes ÄKTA pure 25

Tubing kit	pH valve V9-pH	pH valve restrictor	Monitor to outlet valve[1]	Monitor to frac F9-R[1]	Monitor to frac F9-C[1]	Monitor to frac F9-T[1]
0.25	No	N/A	66 µL	86 µL	214 µL	96 µL
0.25	Yes	25 µL	77 µL	96 µL	225 µL	106 µL
0.5 (standard)	No	N/A	125 µL	205 µL	435 µL	215 µL
0.5 (standard)	Yes	48 µL	152 µL	231 µL	462 µL	241 µL

Tubing kit	pH valve V9-pH	pH valve restrictor	Monitor to outlet valve[1]	Monitor to frac F9-R[1]	Monitor to frac F9-C[1]	Monitor to frac F9-T[1]
0.75	No	N/A	223 µL	400 µL	803 µL	410 µL
0.75	Yes	88 µL	277 µL	454 µL	857 µL	464 µL
1.0	No	N/A	361 µL	675 µL	1319 µL	685 µL
1.0	Yes	143 µL	452 µL	766 µL	1410 µL	776 µL

[1] Delay volume UV monitor to point of collection of eluent

Delay volumes ÄKTA pure micro/ Micro kit

Tubing kit	Monitor to outlet valve[1]	Monitor to frac F9-R/F9-T with micro nozzle[1]
Red 0.13 mm	12 µL	18 µL
Blue 0.25 mm	38 µL	60 µL

[1] Delay volume UV monitor to point of collection of eluent

Delay volumes ÄKTA pure 150

Tubing kit	pH valve V9H-pH	pH valve restrictor	Monitor to outlet valve[1]	Monitor to frac F9-R[1]	Monitor to frac F9-C[1]
0.5	No	N/A	198 µL	278 µL	508 µL
0.5	Yes	60 µL	245 µL	324 µL	555 µL
0.75 (standard)	No	N/A	296 µL	473 µL	876 µL
0.75 (standard)	Yes	100 µL	370 µL	547 µL	950 µL
1.0	No	N/A	434 µL	748 µL	1392 µL
1.0	Yes	155 µL	545 µL	859 µL	1503 µL

[1] Delay volume UV monitor to point of collection of eluent

Note: *If the pH valve is mounted, make sure to set the value for the delay volume of the pH valve restrictor. The system automatically adds the volumes for the flow restrictor and the pH flow cell if they are part of the flow path.*

Experimental method for determination of delay volumes

Delay volumes can be determined experimentally for Fraction collector F9-R, and F9-T, but not for Fraction collector F9-C. Instructions for measuring the delay volume using the UV monitor **U9-M**, **U9-T**, or **U9-L** are provided below.

Two volumes are used to perform experimental delay volume measurements:

Parameter	Description
V1	The volume between the Injection valve and the UV monitor used
V2	The volume between the Injection valve and the fractionation collector tubing tip

Follow the steps below to measure the delay volume.

Step	Action
1	Check that a flow rate of 1 mL/min is delivered by the pump. ***Note:*** *If the flow rate is not correct, check if air bubbles may have entered the pump. Make sure to correct any problems before continuing.*
2	Fill a small sample loop, for example 20 µL, with a 1% acetone solution.
3	Set all components between the monitor and the fraction collector to by-pass mode.
4	Fill the system with water. Run the pump at a flow rate of 1 mL/min and inject the acetone solution as a sample.
5	Write down the volume from the point of injection to the maximum of the peak in the chromatogram. This volume is volume measurement V1.
6	Reconfigure the system as follows to include the fraction collector in the flow path before the monitor: **a.** Disconnect the two tubing segments from the UV monitor flow cell and connect these with a low-dead-volume connector, a 1/16" female to a 1/16" female union connector. **b.** Mount the fractionation collector tubing tip on the top of the UV monitor flow cell. Connect tubing to the bottom of the UV monitor flow cell and lead this tubing to waste. It is preferable if the waste tubing gives some back pressure as the flow restrictor now is placed before the UV flow cell.
7	Set the ***Frac*** size to a large volume, for example 100 mL, so that the Outlet valve is in the ***Frac*** position during the entire run.
8	Start the pump at a flow rate of 1 mL/min and inject the acetone solution.

Step	Action
9	Write down the volume from the point of injection to the maximum of the peak in the chromatogram. This volume is volume measurement V2.
10	To obtain the delay volume: **a.** Subtract V1 from V2. **b.** Add half of the volume of the UV flow cell.

Use of different monitors for peak fractionation in the same method

If different monitors or detectors are used for peak fractionation in different parts of the same method, the delay volumes have to be set as method instructions for each of the method parts. For example, both an external fluorescence detector and the UV monitor module can be used for peak fractionation.

9.14 Component volumes

The table below shows the component volumes of ÄKTA pure.

Component	Volume (µL)
Inlet valve **V9-IA**, **V9-IB**	88
Inlet valve **V9H-IA**, **V9H-IB**	212
Inlet valve **V9-IAB**	95
Inlet valve **V9H-IAB**	116
Sample inlet valve **V9-IS**	88
Sample inlet valve **V9H-IS**	212
Inlet valve **V9-IX**	88
Inlet valve **V9H-IX**	212
Air sensor **L9-1.2**	20
Air sensor **L9-1.5**	35
Pump P9 (**P9-A**, and **P9-B**) (total volume for two heads including T-connector and check valves)	549
Pump P9H (**P9H A**, **P9H B**, **P9H S**) (total volume for two heads including T-connector and check valves)	2163
System pump flow restrictor	30
Sample pump flow restrictor	30
System pump pressure monitor **R9**	45
Sample pump pressure monitor **R9**	45
Sample pump **S9** (**P9-S** total volume for two heads including T-connector and check valves)	1392
Online filter	20
Mixer, 0.6 mL	600
Mixer, 1.4 mL	1400
Mixer, 5 mL	5000
Mixer, 15 mL	15000

Component	Volume (µL)
Mixer valve **V9-M**	14
Mixer valve **V9H-M**	31
Loop valve **V9-L**	17
Loop valve **V9H-L**	76
Versatile valve **V9-V**	14
Versatile valve **V9H-V**	31
Injection valve **V9-Inj**	10
Injection valve **V9H-Inj**	23
Injection valve **V9M-J**	0.8
Column valve **V9-C**	110
Column valve **V9H-C**	190
Column valve **V9-C2**	110
Column valve **V9H-C2**	190
Column valve **V9-Cm**	17
Column valve **V9-Cs**	14
Column valve **V9H-Cs**	31
UV monitor **U9-M**: Flow cell 0.5 mm	10
UV monitor **U9-M**: Flow cell 2 mm Micro	2.6
UV monitor **U9-M**: Flow cell 2 mm	11
UV monitor **U9-M**: Flow cell 10 mm	12
UV monitor **U9-T**: Flow cell 0.4 mm	17
UV monitor **U9-T**: Flow cell 2 mm	30
UV monitor **U9-T**: Flow cell 5 mm	20
UV monitor **U9-L**: Flow cell 0.4 mm	17
UV monitor **U9-L**: Flow cell 2 mm	30
UV monitor **U9-L**: Flow cell 5 mm	20
Conductivity cell **C9**	22
Conductivity cell **C9M**	5.4

Component	Volume (µL)
Flow restrictor **FR-902**	10
pH valve **V9-pH**, in ***By-pass*** position	15
pH valve **V9H-pH**, in ***By-pass*** position	35
pH flow cell mounted in pH valve **V9-pH**	76
pH flow cell mounted in pH valve **V9H-pH**	129
Flow restrictor **FR-902** and tubing when mounted on pH valve **V9-pH**	48
Outlet valve **V9-O**	9
Outlet valve **V9H-O**	82
Outlet valve **V9-Os**	9
Outlet valve **V9H-Os**	28
Outlet valve **V9M-Os**	0.7
Fraction collector **F9-C** internal tubing (0.5 mm i.d.)	147
Fraction collector **F9-C** Dispenser head	94
F9-R Micro nozzle	1
F9-R Tubing holder	0
F9-T Standard nozzle	10
F9-T Micro nozzle	1
F9-T Tubing nozzle	0

Note: *The given values for the component volumes of the valves are average values. Depending on the chosen flow path the actual component volume may differ somewhat.*

9.15 Pressure control

Introduction

By using the function ***Pressure control*** to regulate the run, the method can be run with the set flow rate without the risk of method stop due to pressure alarm. If the pressure approaches the pressure limit, for example if the sample has higher viscosity than the buffer, the flow rate is automatically lowered. Pressure control is enabled in the manual instructions ***Pumps*** →***System flow*** or ***Pumps*** →***Sample flow***. The default setting for Pressure Control is ***Off***. To enable the function, set what pressure signal to use. It is recommended to use the pre-column pressure. The instruction ***Advanced*** →***Pressure control parameters*** provides the P and I factors used in the regulator and can be adjusted for different columns, see information further down.

In the Method editor, pressure control is enabled by selecting ***Control the flow to avoid overpressure*** in the predefined phase ***Method settings***.

Pressure control parameters

The table below describes the factors used for pressure regulation.

Parameter	Description
P factor	Proportional component in PI pressure regulation. Reduces the error between actual and requested target pressure, but may leave a permanent error.
I factor	Integrating component in PI pressure regulation. Eliminates the stationary error from the P factor, but introduces a slight instability that may lead to oscillation in the pressure and the actual flow rate. Set I = 0 to disable the I factor. As a general guide use a small I factor for high pressure columns and a large I factor for low pressure columns, see *Recommended pressure control parameters, on page 644* for more recommendations.
Target value for pressure control	Sets the target value for the PI pressure regulation as a percentage of the pressure limit. If the target pressure is too close to the pressure limit there is a risk that a short pressure spike will trigger the pressure alarm. The pressure limit is set in the ***Alarm pressure*** instruction. The ***Alarm pressure*** used for pressure control depends on the settings in the ***System flow*** instruction.
Pressure control min flow rate	If the flow rate is reduced below the value set in ***Pressure control min flow rate***, the method is paused and the system is set to state ***ALARMS AND ERRORS***. ***Pressure control min flow rate*** can be set either as volumetric or as linear flow. A column type must be selected before using linear flow.

Recommended pressure control parameters

The table below contains the recommended values for P and I parameters for different resin types.

Column/Resin	Recommended *P factor*	Recommended *I factor*	Additional information
Default	8	40	N/A
Small soft resin columns	8	40	N/A
Large soft resin columns[1]	8	300 - 600	A higher I value than the default value is needed to speed up pressure ramp-up times.
Small rigid resin columns[2]	8	15	A lower I value than the default value is needed to avoid large fluctuations in pressure or flow rate. As an alternative, try increasing P.
	20	40	

[1] Soft resin is defined as all Cytiva separation resin, except silica and MonoBeads.
[2] Rigid resin is defined as Cytiva separation resin that is based on silica and MonoBeads.

Back pressure

Using narrow tubing between components will improve resolution but will lead to increased back pressure in the system. Narrow tubing after the column will increase the pressure in the column at a given flow rate. Make sure that the pressure sensor limits in the system are set so that the maximum pressure for the column used is not exceeded.

Additional instructions for avoiding pressure alarms

The instruction ***Max flow during valve turn*** sets the maximum flow rate used during the turning of the Injection valve and Outlet valve in order to avoid high pressure alarms. If the flow rate passing through the Injection valve or the Outlet valve is higher than the set max flow rate, the valves will only turn after decreasing the flow to the specified flow rate. After the valves have turned, the previous flow rate will be restored. The instruction is found in ***System Control*** →***System settings*** →***Advanced***.

9.16 Node IDs

Node ID for core modules

The table below lists the Node ID for the core modules.

Core module	Label	Node ID
System pump A	**P9 A / P9H A**	0
System pump B	**P9 B / P9H B**	1
Pressure monitor	**R9**	0
Mixer	**M9**	0
Injection valve	**V9-Inj / V9H-Inj**	4

Node ID for optional modules

The table below lists the Node ID for the optional modules.

Note: *The Node ID of the Sample pump* **S9** *and the Fraction collector F9-C cannot be changed by the user.*

Note: *The Node ID for the UV monitor* **U9-M** *and the UV detector* **U9-D** *must only be changed by service personnel.*

Module	Label	Node ID
Injection valve V9M-J	**V9M-J**	25
Inlet valve A	**V9-IA / V9H-IA**	0
Inlet valve B	**V9-IB / V9H-IB**	1
Inlet valve AB	**V9-IAB / V9H-IAB**	3
Inlet valve X1	**V9-IX / V9H-IX**	15
Inlet valve X2	**V9-IX / V9H-IX**	16
Mixer valve	**V9-M / V9H-M**	22
Sample inlet valve	**V9-IS / V9H-IS**	2
Loop valve	**V9-L / V9H-L**	17
Column valve (5-columns)	**V9-C / V9H-C**	5
Pre-column pressure monitor	N/A	2

Module	Label	Node ID
Post-column pressure monitor	N/A	3
Second column valve (5-columns)	**V9-C2 / V9H-C2**	6
Pre-column pressure monitor	N/A	4
Post-column pressure monitor	N/A	5
Column valve (3-column)	**V9-Cm**	26
Column valve (1-column)	**V9-Cs / V9H-Cs**	7
pH valve	**V9-pH / V9H-pH**	11
Outlet valve (10-outlets)	**V9-O / V9H-O**	8
Outlet valve (1-outlet)	**V9-Os / V9H-Os /V9M-Os**	19
Versatile valve	**V9-V / V9H-V**	20
Versatile valve 2	**V9-V / V9H-V**	21
Versatile valve 3	**V9-V / V9H-V**	23
Versatile valve 4	**V9-V / V9H-V**	24
UV monitor	**U9-M**	0
UV detector	**U9-D**	0
UV monitor	**U9-T**	0
UV monitor 2nd	**U9-T**	1
UV monitor	**U9-L**	0
UV monitor 2nd	**U9-L**	1
Conductivity monitor	**C9 /C9M**	0
Conductivity monitor 2nd	**C9 /C9M**	1
External air sensor	**L9**	0
External air sensor 2	**L9**	1
External air sensor 3	**L9**	2
External air sensor 4	**L9**	3

Module	Label	Node ID
Fraction collector **F9-C**	**F9-C**	0
Fraction collector **F9-R**	**F9-R**	0
Fraction collector **F9-T**	**F9-T**	0
Fraction collector, 2nd	**F9-R**	1
Sample pump	**P9-S** / **P9H S**	2
I/O-box	**E9**	0
I/O-box , 2nd	**E9**	1

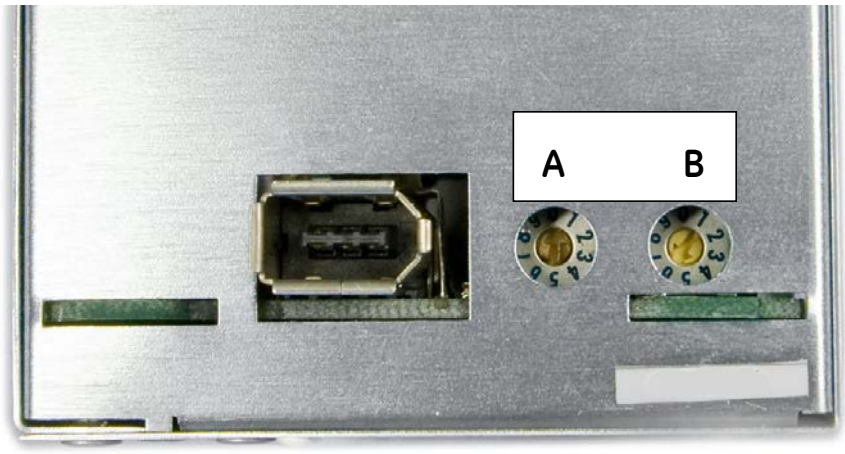
Set the Node ID

Follow the steps below to, set, check, or change the Node ID of a module.

Step	Action
1	Remove the existing module according to the instruction in *Section 2.5 Installation of internal modules, on page 96*.
2	Change the position of an arrow on a rotating switch at the back of the module to set the Node ID. • The first switch, labeled **A**, sets the tens. • The second switch, labeled **B**, sets the units.
3	Check the Node ID and compare it with the listed Node IDs in the tables above.
4	To change the Node ID, use a screw driver to set the arrows of the switches to the desired number.
5	Re-install the module in the instrument, if applicable.

Valve modules have two rotating switches, as shown in the image below.

To set the Node ID to **6** for a valve module, switch **A** is set to **0** and switch **B** is set to **6**.



10 Ordering information

Introduction

This section lists accessories and user replaceable spare parts available for ÄKTA pure.

Cables

Item	Code no.
Jumper 1 IEC 1394 (F-type)	28956489
Jumper D-SUB (D-type)	29011365
External module cable, short (F-type)	29012474
External module cable, long (F-type)	29011366
Cable 2.5 m UniNet-9 D-type	29032425

Conductivity monitor

Item	Code no.
Conductivity monitor **C9**	29011363
Conductivity monitor **C9M**	29298326

External air sensors

Item	Code no.
Air sensor **L9-1.2** mm	28956502
Air sensor **L9-1.5** mm	28956500

Flow restrictor

Item	Code no.
Flow restrictor **FR-902**	18112135

Fraction collector F9-C

Item	Code no.
Fraction collector **F9-C**	29027743
Tubing kit for **F9-C**	29033632
Cassette tray	28954209
Cassette, for deepwell plate (2-pack)	28954212
Deep well plate, 96 x 2 mL	77015200
Deep well plate, 48 x 5 mL	77015500
Deep well plate, 24 x 10 mL	77015102
Cassette, for 50 mL tubes (2-pack)	28956402
Cassette, for 3 mL tubes (2-pack)	28956427
Cassette, for 5 mL tubes (2-pack)	29133422
Cassette, for 8 mL tubes (2-pack)	28956425
Cassette, for 15 mL tubes (2-pack)	28956404
Rack, for 50 mL tubes	28980319
Rack, for 250 mL bottles	28981873
Cable 2.5 m, UniNet-9 D-type	29032425

Fraction collector F9-R

Item	Code no.
Fraction collector **F9-R**	29011362
Tube Rack Complete, 175 x 12 mm	19868403
Tube Rack Complete, 95 x 10-18 mm	18305003
Tube Rack Complete, 40 x 30 mm	18112467
Bowl	18305103
Tube support	18305402
Tube holder	18646401
Tube rack upgrade kit, 175 x 12 mm	19724202
Tube rack upgrade kit, 95 x 18 mm	19868902

Item	Code no.
Tube rack upgrade kit, 40 x 30 mm	18112468
Drive sleeve	19606702
Micro nozzle **F9-R**	29501533
Eppendorf tube holder	18852201

Fraction collector F9-T

Item	Code no.
Fraction collector **F9-T**	29454032
F9-T tunnel	29476924
F9-T standard nozzle	29477967
F9-T tubing nozzle	29510082
F9-T micro nozzle	29501534
Tubing guide for nozzle	29507802
Microplate holder **F9-T**	29476921
Tube rack - 0.5 mL tubes	29491085
Micro kit for **F9-T**	29695762

Holders

Item	Code no.
Adapter for air sensor	28956342
Bottle holder	28956327
Column clamp o.d. 10 to 21 mm	28956319
Multidirectional column clamp	29339864
Column holder	28956282
Column holder rod	28956270
Flexible column holder	28956295
Loop holder	29011350
Multi-purpose holder	29011349

Item	Code no.
Rail extension	29011352
Tube holder (5-pack)	28954329
Tubing holder comb	28956286
Tubing holder spool	28956274
Inlet filter holder kit	11000407
Screw lid GL45 kit	11000410

Miscellaneous

Item	Code no.
I/O box **E9**	29011361
Real-time unit	29285868

Injection valve accessories

Item	Code no.
Sample loop 10 µL	18112039
Sample loop 50 µL	29325047
Sample loop 100 µL	18111398
Sample loop 500 µL (mounted at delivery)	18111399
Sample loop 1 mL	18111401
Sample loop 2 mL	18111402
Sample loop 10 mL	18116124
Superloop 10 mL	19758501
Superloop 50 mL	18111382
Superloop 150 mL	18102385
Injection fill port	18112766
Injection kit	18111089
Connector 1/16" male and Luer female	28985812

Micro kit

Item	Code no.
Micro kit for ÄKTA pure 25	29302910

Mixer

Item	Code no.
Mixer chamber 0.6 mL	28956186
Mixer chamber 1.4 mL (mounted at delivery)	28956225
Mixer chamber 5 mL (included with ÄKTA pure 150)	28956246
Mixer chamber 15 mL	28980309
O-ring 13.1 × 1.6 mm ***Note:*** *For Mixer chamber 0.6, 1.4, and 5 mL.*	28953545
O-ring 13.1 × 1.6 mm (highly resistant) (can be used as an alternative to 28953545)	29011326
O-ring 22.1 × 1.6 mm ***Note:*** *For Mixer chamber 15 mL.*	28981857
Online filter kit	18102711
Online filter (micro)	18111801
Online filter clamp	29658270

Module components

Item	Code no.
Module Panel	29011364
Multi-module front	29011351
Extension box	29110806

pH monitor

Item	Code no.
pH electrode	28954215
O-ring 5.3 × 2.4 mm	28956497

System Pumps and Sample pump S9H

Item	Code no.
P9 Seal kit 25 mL	28952642
P9 Piston kit 25 mL	28952640
P9H Seal kit 150 mL	28979373
P9H Piston kit 150 mL	28979368
Check valve kit	28979364
Sample pump **S9H**	29050593

Sample Pump S9

Item	Code no.
Sample pump **S9**	29027745
P9-S Seal kit	28960250
P9-S Piston kit	18111213
Check valve kit	28979364
Cable 2.5 m UniNet-9 D-type	29032425

Tubing

Item	Code no.
Reference capillary 1	28950749
Reference capillary 2	28950750
Tubing Kit 0.5 mm standard, ÄKTA pure 25	29011327
Tubing Kit 0.5 mm, ÄKTA pure 150	29051669

Item	Code no.
Tubing Kit 0.25 mm, ÄKTA pure 25	29011328
Tubing Kit 0.75 mm, ÄKTA pure 25	29011329
Tubing Kit 0.75 mm standard, ÄKTA pure 150	29048242
Tubing Kit 1.0 mm	29032426
Tubing kit 10×1.0 m, ETFE ID 1.0 mm OD 1/16	28980995
Tubing kit for sample inlet valve **V9-IS** (7-ports)	29035331
Tubing kit for sample inlet valve **V9H-IS** (7-ports)	29051166
Sample tubing kit for 7 inlets, i.d. 0.75 mm	28957217
Inlet tubing kit 2+2	29011330
V9-pH tubing kit	29011331
V9H-pH tubing kit standard	29051674
Tubing kit for inlet valve **V9-IA** (7 ports)	29011332
Tubing kit for inlet valve **V9H-IA** (7 ports)	29051197
Tubing kit for inlet valve **V9-IB** (7 ports)	29011333
Tubing kit for inlet valve **V9H-IB** (7 ports)	29051189
Tubing kit, Micro	29261880
Outlet tubing kit, ÄKTA pure 25	29011334
Outlet tubing kit, ÄKTA pure 150	29048611
Rinse system tubing	29011348
Union 1/16 male/male, i.d. 0.5 mm (5-pack)	28954326
Tubing cutter	18111246
Inlet filter holder kit	11000407
Inlet filter set	11000414

UV monitor

Item	Code no.
UV monitor **U9-M**	28917580
UV monitor **U9-T**	29710522

Item	Code no.
UV monitor **U9-L**	29011360
UV flow cell **U9-0.5** (0.5 mm for **U9-M**)	28979386
UV flow cell **U9-2** (2 mm for **U9-M**)	28979380
UV flow cell **U9-2M** (micro cell for **U9-M**)	29507801
UV flow cell **U9-10** (10 mm for **U9-M**)	28956378
UV flow cell 0.4 mm for **U9-T** and **U9-L**	29364878
UV flow cell 2 mm for **U9-T** and **U9-L**	29011325
UV flow cell 5 mm for **U9-T** and **U9-L**	18112824

Valves

Item	Code no.
Column valve kit **V9-C**	29011367
Column valve kit **V9H-C**	29050951
Column valve **V9-Cm**	29383526
Column valve **V9-Cs**	29011355
Column valve **V9H-Cs**	29090693
Inlet valve **V9-X1**	28957227
Inlet valve **V9H-X1**	28979326
Inlet valve **V9-X2**	28957234
Inlet valve **V9H-X2**	28979328
Inlet valve kit **V9-IA**	29012263
Inlet valve kit **V9H-IA**	29050945
Inlet valve kit **V9-IB**	29012370
Inlet valve kit **V9H-IB**	29050946
Inlet valve kit **V9-IAB**	29011357
Inlet valve kit **V9H-IAB**	29089652
Injection valve **V9M-J**	29502123
Sample inlet valve kit **V9-IS**	29027746

Item	Code no.
Sample inlet valve kit **V9H-IS**	29050943
Loop valve kit **V9-L**	29011358
Loop valve kit **V9H-L**	29090689
Mixer valve kit **V9-M**	29011354
Mixer valve kit **V9H-M**	29090692
Outlet valve kit **V9-O**	29012261
Outlet valve kit **V9H-O**	29050949
Outlet valve kit **V9-Os**	29011356
Outlet valve kit **V9H-Os**	29090694
Outlet valve **V9M-Os**	29502129
pH valve kit **V9-pH**	29011359
pH valve kit **V9H-pH**	29051684
Versatile valve **V9-V**	29011353
Versatile valve **V9H-V**	29090691

Note: *All valve kits include the necessary tubing.*

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Index

A

Affinity chromatography, *558*, *559*
    predefined method, *558*, *559*
Air sensors, *47*, *107*, *120*, *310*, *312*, *314*, *507*, *515*
    A test, *310*
    air sensor adapter, *107*
    B test, *312*, *314*
    error codes, *515*
    external air sensors, *120*
    inlet valves, *47*
    troubleshooting, *507*
ÄKTA pure , *11*, *16*, *17*, *118*, *339*, *454*
    description, *16*, *118*
    illustrations, *17*
    main features, *11*
    maintenance, *339*
    troubleshooting, *454*
Anion exchange, *560*
    predefined method, *560*

C

Cabinet, *504*
    troubleshooting, *504*
Cassettes, *129*, *130*
    quickrelease function, *130*
Cation exchange, *560*
    predefined method, *560*
Chromatofocusing, *560*
    predefined method, *560*
CIP, *289*, *366*, *377*, *564–566*
    column CIP, *377*
    column maintenance method, *564*
    column predefined phase, *565*
    system CIP, *366*
    system maintenance method, *564*
    system predefined phase, *289*, *566*
Cleaning, *295*, *296*
    column, *296*
    system, *295*
Column, *66*, *103*, *233*, *238*, *296*, *377*
    attach column holder, *233*
    cleaning, *296*
    column CIP, *377*
    column holder, *103*
    column valve, *66*
    connect column, *233*
    pressure alarm, *238*
    storage, *296*
Column CIP, *564*, *565*
    maintenance method, *564*
    predefined phase, *565*
Column performance test, *290*, *564*, *565*
    maintenance method, *564*
    predefined phase, *290*, *565*
Column preparation, *564*, *565*
    maintenance method, *564*
    predefined phase, *565*
Column valve, *66*, *68–70*, *316*
    description, *66*
    flow paths V9-C, *68*
    flow paths V9-Cm, *69*
    flow paths V9-Cs, *70*
    V9-C test, *316*
Column valves, *85*
    pressure monitors, *85*
Column wash, *565*
    predefined phase, *565*
Component volumes, *640*
Conductivity monitor, *92*, *191*, *355*, *395*, *467*, *515*
    calibration, *355*
    clean the conductivity flow cell, *395*
    description, *92*
    error codes, *515*
    installing two monitors, *191*
    troubleshooting, *467*
Connectors, *402*, *537*
    for ÄKTA pure, *537*
    replace connectors, *402*

D

Default drop, *281*

Delay volume, *263*, *635*
reference information, *635*
settings, *263*
Delta-column pressure, *85*
description, *85*
Desalting, *560*
predefined method, *560*
Dispensing, *280*, *281*
Documentation, *13*
Drop position, *281*
Drop sync, *147*

E

Elution, *565*
predefined phase, *565*
Equilibration, *565*
predefined phase, *565*
Extension box, *117*

F

Flow cells, *75*, *87–89*, *92*
conductivity, *92*
pH, *75*
UV, U9-L, *89*
UV, U9-M, *87*
UV, U9-T, *88*
Flow path, *32*, *209*, *537*, *640*
component volumes, *640*
illustration, *32*, *209*, *537*
prepare, *209*
specifications, *32*
Flow restrictor, *94*, *416*, *449*
check function, *449*
FR-902 description, *94*
replace flow restrictor, *416*
Flowpath, *57*
Injection valve, *57*
V9-Inj, *57*
V9H-Inj, *57*
Fraction collector, *123*, *130*, *272*, *318*, *320*, *325*, *404*, *476*, *484*, *486*, *520*, *533*
description, *123*
error codes F9-R, *520*
F9-C test, *318*
F9-R test, *320*, *325*
function, *123*
preparation, *272*
quickrelease function, *130*
replace internal tubing, *404*
specifications, *533*
troubleshooting, *476*, *484*, *486*
Fraction collector F9-C, *381*
cleaning, *381*
Fraction collector F9-C sensor, *383*
cleaning, *383*
Fraction collector F9-R, *385*
cleaning, *385*
Fraction collector F9-T, *386*
cleaning, *386*
Fraction collector F9-T drop sync, *388*
cleaning, *388*
Fraction collector F9-C, *129*, *132–134*
Cassettes and racks, *129*
connect tubing, *134*
requirements on deep well plates, *133*
requirements on tubes, *132*
Fractionation, *140*, *261*, *263*, *264*, *272*
assemble tube rack, *140*
delay volume, *263*
fixed volume, *263*
fraction marking, *263*, *264*
fractionation types, *261*
peak fractionation, *264*
pre-fractionation volume, *263*
prepare, *272*
Fractionation settings, *282*
F9-C, *282*
F9-T, *282*
F9-R, *282*

H

Hardware installation, *96*
of a module, *96*
Holders, *101–107*, *117*, *651*, *652*
air sensor adapter, *107*
bottle holder, *107*
column clamp, *104*
column holder, *103*
column holder rod, *104*
Extension box, *117*
flexible column holder, *105*
loop holder, *102*
multi-purpose holder, *101*

ordering information, *651*, *652*
rail extension, *101*
tubing holder comb, *106*
tubing holder spool, *106*
Hydrophobic interaction chromatography (HIC), *561*
predefined method, *561*

I

I/O-box E9, *158*, *159*, *508*, *522*, *535*
connectors, *159*
description, *158*
error codes, *522*
Fraction collector F9-C
installation, *158*
specifications, *535*
troubleshooting, *508*
ICU, *505*
troubleshooting, *505*
Illustration, *57*, *61*
Injection valve, *57*
V9-Inj, *57*
V9H-Inj, *57*
V9M-J, *61*
flowpath, *61*
port, *61*
Injection valve, *57*, *61*
description, *57*
flow paths, *57*
V9M-J, *61*
flowpath, *61*
Injection valves, *179*, *180*
configuration, *179*
Inlet tubing, *213*, *220*, *221*
prepare, *213*
prime inlet tubing B, *221*
prime inlets, *220*
inlet valves, *310*, *312*, *314*
V9-IA test, *310*
V9-IB test, *314*
V9-IB test, *312*
Inlet valves, *47*, *474*
air sensors, *47*
description, *47*
inlet valve A, *47*
inlet valve AB, *47*
inlet valve B, *47*
inlet valve X1, *47*
inlet valve X2, *47*
troubleshooting, *474*
Installation, *214*
prepare waste tubing, *214*
Instrument control panel, *34*, *35*, *510*, *522*
error codes, *522*
function, *34*
location, *34*
status indications, *35*
troubleshooting, *510*
Instrument control unit, *514*
error codes, *514*
Instrument overview, *17*, *19*, *20*, *137*
Fraction collector F9-R, *137*
Instrument configurations, *17*
main parts, *17*
module labels, *20*
modules, *20*
wet side, *19*
Ion exchange, *560*
predefined method, *560*

L

LED light, *147*
Log on, *217*
UNICORN, *217*
Loop valve, *63*
description, *63*
flow paths, *63*

M

Maintenance, *340*, *345*, *348*, *364*, *365*, *401*
calibration procedures, *348*
clean the instrument, *365*
cleaning, *364*
Maintenance Manager, *340*
maintenance program, *345*
replacement procedures, *401*
Maintenance manager, *341*
handling maintenance notifications, *341*
Maintenance methods, *564*
column CIP, *564*
column performance test, *564*
column preparation, *564*
system CIP, *564*

system preparation, *564*
Manual instructions, *594*
Manual loop fill, *565*
predefined phase, *565*
Manual Loop Fill, *561*
predefined method, *561*
Method settings, *565*
predefined phase, *565*
Methods, *285*, *558–564*
affinity chromatography, *558*, *559*
anion exchange, *560*
cation exchange, *560*
chromatofocusing, *560*
create method, *285*
desalting, *560*
hydrophobic interaction chromatography (HIC), *561*
maintenance methods, *285*
manual loop fill, *561*
NHS-coupling, *562*
predefined maintenance methods, *564*
predefined methods, *285*, *558*
purification methods, *285*
reverse phase chromatography (RPC), *563*
size exclusion chromatography, *561*
Miscellaneous, *565*
predefined phase, *565*
Mixer, *44*, *54*, *211*, *408*, *409*, *417*, *500*, *515*, *528*
choose mixer chamber, *211*
description, *44*
error codes, *515*
mixer valve, *54*
replace mixer, *408*
replace the O-ring, *409*
replace the online filter, *417*
specifications, *528*
troubleshooting, *500*
Mixer valve, *54*, *55*
description, *54*
flow paths, *55*
Module, *499*
troubleshooting, *499*
Module panel, *116*, *502*
description, *116*
troubleshooting, *502*
Module Panel Assembly, *172*
Modules, *40*, *44*, *47*, *54*, *57*, *63*, *66*, *74*, *75*, *80*, *84*, *87*, *92*, *94*, *152*, *171*
column valve, *66*
conductivity monitor, *92*
flow restrictor, *94*
injection valve, *57*
inlet valves, *47*
loop valve, *63*
mixer, *44*
mixer valve, *54*
outlet valve, *80*
pH valve, *75*
pressure monitor, *84*
pumps, *40*, *152*
recommended positions, *171*
UV monitor, *87*
versatile valve, *74*
Monitors, *78*, *84*, *87*, *92*, *120*, *530–532*
conductivity, *92*
external air sensors, *120*
pH, *78*
pressure monitor, *84*
specifications, *530–532*
UV, *87*

N

NHS-coupling, *562*
predefined method, *562*
Node ID, *96*, *645*, *647*
change Node ID, *647*
check Node ID, *647*
description, *96*
list, *645*
Notes and tips, *10*

O

Offset, *281*
Optional configurations, *96*
install optional modules, *96*
Ordering information, *649–657*
cables, *649*
conductivity monitor, *649*
dummy module, *653*
external air sensors, *649*
flow restrictor, *649*
fraction collector, *651*
fraction collector F9-R, *650*, *651*
holders, *651*, *652*

I/O-box E9, *652*
mixer, *653*
pH monitor, *654*
pump, *653*, *654*
tubing, *654*, *655*
UV monitor, *650*, *652*, *655*, *656*
valves, *656*, *657*
Outlet tubing, *213*
prepare, *213*
Outlet valve, *80–82*
description, *80*
V9-O ports, *81*
V9-Os ports, *82*

P

Performance tests, *306*, *310*, *312*, *314*, *316*, *318*, *320*, *325*, *327*, *332*
air sensor A and inlet valve V9-IA, *310*
air sensor B and inlet valve V9-IB, *314*
air sensor B and inlet valve V9-IB, *312*
column valve V9-C, *316*
fraction collector F9-C, *318*
fraction collector F9-R, *320*, *325*
overview, *306*
UV U9-L system, *332*
UV U9-M system, *327*
UV U9-T system, *332*
pH monitor, *78*, *296*, *349*, *389*, *407*, *451*, *515*
calibration, *349*
clean the pH electrode, *389*
description, *78*
error codes, *515*
replace the pH electrode, *407*
storage of the pH electrode, *296*, *451*
pH monitors, *470*
troubleshooting, *470*
pH valve, *75*, *76*, *470*
description, *75*
flow paths, *76*
troubleshooting, *470*
Phases, *289*, *290*, *565*, *566*
column CIP, *565*
column performance test, *290*, *565*
column preparation, *565*
column wash, *565*
elution, *565*
equilibration, *565*
manual loop fill, *565*
method settings, *565*
miscellaneous, *565*
predefined phases, *565*
sample application, *566*
system CIP, *289*, *566*
system preparation, *566*
Plate, *150*
Plates, *149*, *278*
Prepare, *278*
Power, *505*
troubleshooting, *505*
Power save, *205*
Pre-fractionation volume, *263*
Prepare the system, *209*, *211–214*, *217*, *220*, *233*, *243*, *272*
connect column, *233*
flow path, *209*
fraction collector, *272*
inlet tubing, *213*
mixer, *211*
outlet tubing, *213*
prime inlets, *220*
purge system pumps, *220*
sample application, *243*
Start UNICORN, *217*
UV monitor U9-L, *212*
UV monitor U9-M, *212*
UV monitor U9-T, *212*
waste tubing, *214*
Pressure alarm, *238*
set, *238*
Pressure control, *294*
system configuration, *294*
Pressure monitor, *514*
error codes, *514*
Pressure monitors, *84*, *351*, *472*
check the pressure monitors, *351*
description, *84*
troubleshooting, *472*
Pressure sensor, *351*
calibration, *351*
Prime inlet tubing B, *221*
Process picture, *293*
actions in the process picture, *293*

Pump, *491*
troubleshooting, *491*
Pump piston rinsing system, *42*, *43*, *154*, *441*, *444–447*
flow path, *43*
illustration, *42*, *154*, *441*, *444*, *446*
prime, *445*, *447*
Pumps, *40*, *152*, *221*, *228*, *397*, *419*, *422*, *431*, *439*, *440*, *444*, *516*, *526*
change pump rinsing solution, *444*
clean check valves, *397*
description, *40*
description, sample pump, *152*
error codes, *516*
purge sample pump, *228*
purge system pumps, *221*
replace check valves, *419*
replace pump piston seals of pump P9, *422*
replace pump piston seals of Pump P9-S, *431*
replace pump pistons, *439*
replace pump rinsing system tubing, *440*
specifications, *526*

Q

QuickRelease function, *130*

R

Rack, *151*
reference information, *634*
injection volumes and peak broadening, *634*
Reference information, *523*, *526*, *549*, *635*, *643*, *644*
back pressure, *644*
chemical resistance guide, *549*
delay volumes, *635*
module specifications, *526*
pressure regulation, *643*
Reverse phase chromatography (RPC), *563*
predefined method, *563*
Run, *291–293*, *295*, *625*, *630*
after run procedures, *295*
available curves, *630*
available run data, *625*
monitor run, *293*
perform manual run, *292*
perform run, *291*
prepare run, *291*

S

Safety notices, *10*
Sample application, *57*, *243*, *249*, *256*, *566*
flow paths, *57*
injection valve, *57*
predefined phase, *566*
sample loading, *243*
sample loop, *256*
superloop, *249*
Sample loop, *256*
sample loading, *256*
Sample pump, *152*
description, *152*
Size exclusion chromatography, *561*
predefined method, *561*
Software overview, *12*
software modules, *12*
Storage, *295*, *296*
column, *296*
pH electrode, *296*
system, *295*
Superloop, *249*, *503*
sample loading, *249*
troubleshooting, *503*
System CIP, *289*, *564*, *566*
maintenance method, *564*
predefined phase, *289*, *566*
System cleaning, *295*
System configuration, *96*, *171*, *175*, *176*, *294*
control pressure, *294*
installation of modules, *96*
modules and positions, *171*
overview, *171*
system properties, *175*
system settings, *176*
System Control module, *594*
manual instructions, *594*
System preparation, *207*, *564*, *566*
before preparation, *207*
maintenance method, *564*
predefined phase, *566*

System pumps, *40*
operating ranges, *40*
System settings, *201*, *202*
blue kit, *202*
red kit, *201*
System storage, *295*
System test, *327*, *332*
UV U9-L, *332*
UV U9-M, *327*
UV U9-T, *332*

T

Technical specifications, *526*
module specifications, *526*
Trays, *129*
Troubleshooting, *476*, *484*, *486*, *511*, *514*
error codes, *514*
F9-C, *476*
F9-R, *484*
F9-T, *486*
Fraction collector, *476*, *484*, *486*
instrument communication, *511*
Tube position F9-T, *150*
Tube position F9-T, *151*
Tube rack, *140*
assembly, *140*
Tubes, *149*, *278*, *279*
50 mL, *279*
Prepare, *278*, *279*
small, *278*
tube holders F9-T, *149*
tube position F9-T, *149*
Tubing, *55*, *82*, *90*, *93*, *112–114*, *402*, *404*, *536*
Blue, *114*
Conductivity monitor, *93*
connect, *55*
for ÄKTA pure, *536*
mixer valve, *55*
outlet valve, *82*
pre-injection, *112*
Red, *113*
replace tubing, *402*, *404*
UV monitors, *90*
Tubing kit, *112*
installation, *112*
Typographical conventions, *9*

U

UNICORN, *217*, *218*
connect to system, *218*
Log on, *217*
Start, *217*
User information, *9*
UV detector U9-D, *517*
error codes, *517*
UV monitor, *87*, *189*, *212*, *360*, *392*, *411*, *414*
calibration, *212*, *360*
clean UV flow cell, *392*
description, *87*
installing two monitors, *189*
replace UV flow cell, *411*, *414*
UV monitor U9-L, *89*, *212*, *332*, *463*, *518*
description, *89*, *212*
error codes, *518*
system test, *332*
troubleshooting, *463*
UV monitor U9-M, *87*, *212*, *327*, *459*, *516*
description, *87*, *212*
error codes, *516*
system test, *327*
troubleshooting, *459*
UV monitor U9-T, *88*, *212*, *332*, *463*, *517*
description, *88*, *212*
error codes, *517*
system test, *332*
troubleshooting, *463*

V

Valve, *474*
troubleshooting, *474*
Valves, *46*, *47*, *54*, *57*, *63*, *66*, *67*, *74*, *75*, *80*, *514*, *527*
column valve, *66*
error codes, *514*
extra inlet valves, *47*
injection valve, *57*
inlet valves, *47*
loop valve, *63*
mixer valve, *54*
outlet valve, *80*
overview, *46*
pH valve, *75*
specifications, *527*

two Column valve, *67*
versatile valve, *74*
Versatile valve, *74*
description, *74*
flow paths, *74*

W

Waste tubing, *214*
prepare, *214*

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29119969 AF V:21 09/2022