



Chromaflo™ column 400-1000

Operating Instructions

Original instructions



Table of Contents

1	Introduction	4
1.1	Important user information	5
1.2	About this manual	6
1.3	Associated documentation	7
2	Safety instructions	8
2.1	Safety precautions	9
2.2	Potentially explosive atmospheres	20
2.3	Labels and symbols	21
2.4	Emergency procedures	24
3	System description	26
3.1	Overview of Chromaflow™ columns and associated equipment	27
3.2	Chromaflow™ nozzles	29
3.3	Basic principles of nozzle operation	31
4	Installation	37
4.1	Site requirements	38
4.2	Transportation	39
4.3	Unpack the column from the crate	42
4.4	Set up associated equipment	44
4.5	Set up a column	48
5	Operation	50
5.1	Overview of column operation	52
5.2	Prepare the column	55
5.2.1	<i>Level the column</i>	57
5.2.2	<i>Connect a Chromaflow™ Packing Station to the column</i>	60
5.2.3	<i>Empty the column</i>	65
5.2.4	<i>Cleaning the column</i>	67
5.2.5	<i>Prime the column and bed supports</i>	72
5.2.6	<i>Prepare and position the adapter</i>	76
5.2.7	<i>Perform a leakage test</i>	85
5.3	Pack the column	87
5.3.1	<i>Factors for resin preparations</i>	88
5.3.2	<i>Prepare slurry</i>	91
5.3.3	<i>Prime slurry lines</i>	92
5.3.4	<i>Pack the column with resin</i>	94
5.3.5	<i>Rinse slurry lines</i>	97
5.4	Test the packed bed	98
5.5	Unpack the column	99
5.5.1	<i>Loosen the packed bed</i>	100
5.5.2	<i>Empty the column of slurry</i>	103
5.5.3	<i>Clean and prime the column after unpacking</i>	105
6	Maintenance	106
6.1	Service	108
6.2	Cleaning	109

6.3	Cleaning stainless steel bed supports	112
6.4	Maintenance schedule	113
6.5	Maintenance procedures	114
6.5.1	Remove the adapter	116
6.5.2	Remove automatic nozzles and exchange O-rings	122
6.5.3	Remove manual nozzles and exchange O-rings	129
6.5.4	Exchange adapter O-rings	134
6.5.5	Exchange plastic bed supports	135
6.5.6	Exchange the adapter stainless steel bed support	142
6.5.7	Exchange the bottom unit O-rings and plastic bed support	149
6.5.8	Exchange the bottom unit O-rings and stainless steel bed support	152
6.5.9	Refit the adapter	156
6.6	Storage	158
7	Troubleshooting	167
8	Reference information	174
8.1	Specifications	175
8.2	Chemical resistance	176
8.3	Recycling information	181
8.4	Regulatory information	182
8.4.1	Contact information	183
8.4.2	European Union and European Economic Area	184
8.4.3	Other regulations and standards	185
8.5	Health and Safety Declaration Form	186
Index		188

1 Introduction

About this chapter

This chapter contains important user information, safety notices, regulatory information, the intended use of Chromaflow™ columns, information about additional equipment and documentation.

In this chapter

Section	See page
1.1 Important user information	5
1.2 About this manual	6
1.3 Associated documentation	7

1.1 Important user information

Read this before operating the product



All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not install, operate, or perform maintenance on the product in any other way than described in the user documentation. If you do, you may be exposed or expose others to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use of Chromaflow™ columns

Chromaflow™ columns are designed for use in laboratory and industrial chromatography. Chromaflow™ columns are intended for purification only and should not be used for diagnostic purposes or in any clinical or *in vitro* procedures.



WARNING

Do not operate the product in any other way than described in the user documentation.

Prerequisites

In order to operate Chromaflow™ columns safely and according to the intended purpose the following prerequisites must be met:

- All users must read and understand the *Safety Instructions* chapter in these *Operating Instructions*.
- All equipment shall be installed according to the instructions in [Chapter 4 Installation, on page 37](#).
- All users shall be acquainted with the use of process equipment and the handling of biological materials.
- All operations shall be performed by qualified personnel who are adequately trained.

1.2 About this manual

Purpose of this manual

The Operating Instructions manual provides information needed to install, operate and maintain the product in a safe way.

Scope of this manual

This Operating Instruction manual describes the basic components and general principles of operation of Chromaflow™ columns and associated equipment. The instructions provided assume that the column is equipped with automatic nozzles and that it is operated with a Chromaflow™ Packing Station. Other configurations are possible.

For detailed information about individual products refer to the relevant information in the documentation package provided with each product. Contact your local Cytiva representative for more information, if needed.

Images in this document

The Chromaflow™ column family encompasses a range of different column sizes and materials. The images depicted in this manual are included for general illustrative purposes and may not be representative of all column sizes and types. For detailed information about individual columns always refer to the relevant information in the documentation package provided with your column.

Typographical conventions

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

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

In electronic format, references in *italics* are clickable hyperlinks.

Notes and tips

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

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

1.3 Associated documentation

Introduction

The documentation package supplied with Chromaflow™ products includes detailed specifications, drawings and traceability documents for the Chromaflow™ column.

Chromaflow™ instruction manuals

The following table lists examples of the user instructions that are provided with Chromaflow™ columns and the Chromaflow™ Packing Station, in addition to these *Operating Instructions*.

Application notes are also available from Cytiva.

Product documentation package provides technical information about the Chromaflow™ column and is delivered with the column.

User documentation	Manual ID number	Description
Chromaflow™ Packing Station 50, 10, 200, and 400 Operating instructions	29046228	For use with Chromaflow™ Packing Station 50, 10, 200, and 400
Chromaflow™ Packing Station Unpacking Instructions	29424265	For use with Chromaflow™ columns 400-1000 Packing Stations

Component documentation

Documentation for components produced both by Cytiva and by a third-party are, if available, also included in the document package.

2 Safety instructions

About this chapter

This chapter contains safety precautions, descriptions of safety labels, emergency procedures and decommissioning information for Chromaflow™ columns.

In this chapter

Section	See page
2.1 Safety precautions	9
2.2 Potentially explosive atmospheres	20
2.3 Labels and symbols	21
2.4 Emergency procedures	24

Important



WARNING

Before installing, operating or maintaining the product, all users must read and understand the entire contents of this chapter to become aware of the hazards involved.

2.1 Safety precautions

Introduction

The safety precautions in this section are grouped into the following categories:

- General precautions
- Flammable liquids and explosive environments
- Personal protection
- Installing and moving the equipment
- Operation
- Maintenance

Note: *Some of the safety precautions in this chapter may concern components or situations described in other Chromaflow™ column product documents.*

Definitions

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. 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 product or other equipment.

2 Safety instructions

2.1 Safety precautions

Safety assessment

Chromaflow™ columns have been designed and manufactured to provide a high level of personal safety. However, the residual risk is highly dependent on the application and environment in which the column is operated. In order to determine the safe operation of the equipment a safety assessment must be made. This safety assessment, in combination with local regulations and policies, will result in specific safety instructions for installation, operation and maintenance, use of proper personal protective equipment, or other arrangements that are needed to operate your process safely.

General precautions



WARNING

Only properly trained personnel may operate and maintain the product.



WARNING

Do not operate the product in any other way than described in the user documentation.



WARNING

Do not use any accessories not supplied or recommended by Cytiva.



WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.



WARNING

Pay extra attention when moving the adapter as there is a risk of crushing your hands.

**WARNING**

Power failure of connected systems. During a power failure of a connected system, or if the **EMERGENCY STOP** button is pressed on connected equipment, the equipment may remain pressurized. Opening a line or vessel at this point could result in the release of potentially hazardous process or cleaning fluid, and cause bodily harm.

When recovering from a power failure or emergency shutdown of a connected system, make sure all lines and vessels are depressurized before opening.

**WARNING**

There should **NEVER** be air or gas under pressure in the column.

**CAUTION**

When working with 20% v/v 1-propanol solutions follow these instructions:

- Work in a well-ventilated area.
- Wear appropriate personal protective equipment.
- Keep storage containers tightly closed.

Flammable liquids and explosive environments

**WARNING**

Flammable liquids. This product is **not approved** to handle liquids under flammable conditions.

**WARNING**

Explosion hazard: If cleaning the column in a potentially explosive atmosphere, prevent static electric charges building up. Do not rub excessively using a dry cloth and over large areas at a time. Instead, clean the column using a damp cloth with slow movements across small areas at a time.

2 Safety instructions

2.1 Safety precautions



WARNING

When the Chromaflow™ column is operated or maintained in a potentially explosive atmosphere, it **MUST be properly grounded** to avoid static discharge.



WARNING

Explosion Hazard. When connecting the grounding cable, make sure that there is no explosive atmosphere present. Static electricity discharge could occur when connecting the grounding cable.



WARNING

Some of the chemicals used with Chromaflow™ columns may be flammable under certain conditions. Make sure to use chemicals only under conditions where they are not flammable. Refer to local and/or national classifications of flammable liquids.



WARNING

Risk assessment! Perform a risk assessment for any risks due to the process or process environment. Evaluate the effects the use of the equipment and operational processes have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.

Personal protection



WARNING

For personal safety during the transportation, installation, operation, maintenance and service of columns, use protective glasses and other personal protective equipment appropriate for the current application at all times. The following personal protective equipment should always be available:

- Protective glasses
- Working gloves to protect against sharp edges
- Protective footwear, preferably with steel toe-cap
- Disposable gloves

Always use clean disposable gloves when manually handling parts.



WARNING

Hazardous substances and biological agents. When using hazardous chemical and biological agents, 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 this product.

Installing and moving the equipment



WARNING

Heavy object. Use suitable lifting equipment when moving the unit. All lifting and moving must be performed in accordance with local regulations.



WARNING

Packing crates and columns shall only be moved by personnel with appropriate training, and in adherence with local regulations. Even if the instructions in the *Operating Instructions* are followed, it is the responsibility of the customer to guarantee the safety of personnel while working with the column.

2 Safety instructions

2.1 Safety precautions



WARNING

Move transport crates and column. Make sure that the forklift has capacity to safely lift the crate and column weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.



WARNING

Move column. Make sure that the center of gravity of the column is well balanced over the lifting arms of the forklift. Otherwise the column may tip off the forklift.



WARNING



Note that crates may not be marked with a center of gravity symbol. Make sure that the crates are properly balanced and centered over the forks of the lifting equipment so that they will not accidentally tip when moved.



WARNING

The lifting eyebolts of the column are intended for lifting specific column components only. The lifting eyebolts are not intended to be used for lifting the entire column. Non-ferritic lifting slings with sufficient lifting capacity to safely lift the weight of the column may be wrapped around the adapter flange to lift the entire column with a crane or hoist. Use sling protectors to protect lifting slings from sharp edges.



WARNING

Heavy object. Because of the significant weight of the column, great care must be taken not to cause squeezing or crushing injuries during movement.

**WARNING**

Pressure safety equipment, such as a pressure relief valve or rupture disc must be installed at both of the mobile phases, before the column is used.

**CAUTION**

To prevent bacterial growth, the product may be filled with denatured alcohol (18% C₂H₅OH (ethanol), 2% C₃H₇OH (isopropanol) and 80% H₂O (water)) at delivery.

The denatured alcohol mixture can be hazardous to humans if consumed.

Flush out the denatured alcohol before assembling, testing or integrating the product into the intended process context.

**NOTICE**

Do not turn on the air supply before all connections have been made and checked.

Operation

**WARNING**

High pressure. Make sure that flow rates are not so high as to affect the packed medium or cause pressure to exceed the specified column maximum pressure.

**WARNING**

If the column is equipped with caster wheels, the wheel brakes must be activated at all times during operation or storage.

**WARNING**

Do not adjust the feet more than 160 mm out from the base of the column stand to the floor. There is a risk of the column tipping over if the feet are screwed out too far.

2 Safety instructions

2.1 Safety precautions



WARNING

Never use compressed air to raise the adapter.



WARNING

When reactivation is performed, pumps that were in use when the **EMERGENCY STOP** on the connected equipment button was pressed may start at the same setting.

If a pump setting was previously on maximum, reduce the pump setting prior to reactivation to avoid an immediate high pressure increase.



WARNING

Pressure may remain in the column and tubing for an extended period after shutdown. Make sure all lines and chambers are depressurized before opening or disconnecting.



WARNING

Do not reset after an emergency shutdown if the column or piping is suspected to be damaged or otherwise defective. Damaged or defective columns or piping might leak or rupture.



WARNING

When unpacking the chromatography resin from the column, check the integrity of all connections and make sure that the collection vessels can accommodate the volumes involved.



CAUTION

If a column is moved from one temperature environment to another, allow the column to equilibrate to the new temperature environment and then recheck bolt tensions and adjust torque settings as necessary.

**CAUTION**

Do not strain nozzle connections. Connect tubing carefully. Failure to do so may damage nozzle components and cause leakage.

**CAUTION**

Make sure that safety valve connecting tubing has an unrestricted flow path.

**CAUTION**

Open flushing ports with care. Jets of priming solution may spray from the flushing ports. Before opening the flushing ports make sure that the ends of the flushing port tubing is directed away from any people or equipment in the vicinity of the column. Wear appropriate personal protection and safety goggles at all times.

Note: *Be aware that when using a manual nozzle, the nozzle positions must be adjusted by manually turning the nozzle to open or close the port. When using an automatic nozzle, the nozzle ports are opened and closed using the Packing Station controls.*

Maintenance

**WARNING**

Only properly trained personnel, or personnel with equivalent knowledge of similar equipment, are allowed to perform maintenance work on the column. Only personnel authorized by Cytiva are allowed to perform service on the Chromaflow™ column.

**WARNING**

When unpacking the chromatography resin from the column, check the integrity of all connections and make sure that the collection vessels can accommodate the volumes involved.

2 Safety instructions

2.1 Safety precautions



WARNING

Do not perform any type of maintenance work on the column while the column is pressurized. Note that the column can be pressurized even when the system is closed down.



WARNING

LOCK OUT / TAG OUT (LOTO)! Before any maintenance or decommissioning work is performed, make sure that:

- the equipment is empty and depressurized.
- the equipment is disconnected from process feed, electrical power and pneumatic supply.
- the equipment is prevented from accidentally becoming re-energized during maintenance.
- the equipment is clearly tagged as taken out of operation.
- all process wetted areas are clean and decontaminated.



WARNING

Decontaminate before maintenance. To avoid personnel being exposed to potentially hazardous substances, make sure that the column is properly decontaminated and sanitized before maintenance or service.



WARNING

To remove the adapter or rebuild the equipment, a hoist with a minimum lifting capacity to safely lift the weight of the column components is required.



WARNING

During removal of the adapter, or any other movement performed through applying hydrostatic pressure, a pressure gauge must be connected to the system so that the internal column pressure can be monitored.



CAUTION

Be careful when handling the distribution assembly as it is heavy.



CAUTION

The storage solution may evaporate over time when the column is stored. This may create an increase in pressure in the column. Ensure that all valves are closed before removing the TC-blind caps from the bottom slurry inlet and carefully open the valve to release the pressure.



CAUTION

Make sure that the piping system is completely leakage free before performing any Cleaning-In-Place (CIP) or Sanitation-In-Place (SIP) on the column.



CAUTION

Do not use chemicals at temperatures above the specified limits.

2.2 Potentially explosive atmospheres

Introduction

This section outlines important information that must be considered if the column is to be placed in an area with a potentially explosive atmosphere.

Hazardous area classification



WARNING

Risk assessment! Perform a risk assessment for any risks due to the process or process environment. Evaluate the effects the use of the equipment and operational processes have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.

The column can be placed in an area with a potentially explosive atmosphere corresponding to EU ATEX workplace directive, Zone 2.

Definition of Zone 2 according to the directive: A place in which an explosive atmosphere consisting of a mixture with air of flammable substances in the form of gas, vapour or mist is not likely to occur in normal operation but, if it does occur, will persist for a short period only

The column does not fall under the EU ATEX equipment directive, and as a result it is not EX-marked, but it fulfills the explosion safety requirements in the directive's harmonized standards, for example EN 13463-1 or EN ISO 80079-36.

This is valid for the column in its standard execution when grounded with its grounding cable, see Ground connections, on page 62.

Any additional accessory or equipment connected to the column in an area with a potentially explosive atmosphere must carry EX-marking or be assessed to be in conformity with the EU ATEX workplace directive or equivalent equipment classification for hazardous locations.

2.3 Labels and symbols

Introduction

This section describes the product nameplate (label) and other safety and regulatory labels attached to the product.

Column labels

The column is equipped with a nameplate. The nameplate shows the specific manufacturing and performance data for the individual column.

The column nameplate information is explained in the following table.

Label text	Description
	Warning! Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
Dimension	Column inner diameter and minimum/maximum bed height in mm.
Code No	Number indicating that the column is a Chromaflow™ column.
Serial No	Number of the specific instrument.
Year of manufacturing	Date of Manufacturing - year and month (YYYY-MM) the equipment was manufactured.
Tare mass	The weight of the column when empty.
Operating temperature TS	Permitted operating temperature range.
Max column volume V	Maximum volume of column content (resin and fluid).
Design pressure PS	Maximum pressure the instrument has been designed to tolerate
Test pressure PT	Pressure the instrument has been tested to tolerate.
Maximum filling mass	Maximum weight of column content (resin and fluid).
PED fluid group/cat	Pressure Equipment Directive fluid group and category classification.

Safety labels

The table below describes various safety labels that may be found on Chromaflow™ columns or connected equipment.

Note: The labels shown in the following table are examples only.

Symbol/text	Description
	Warning! Read the user documentation before using the equipment. Do not open any covers or replace parts unless specifically stated in the user documentation.
	WARNING! Max operating pressure 3 bar g (44 psi g)
	WARNING! When using corrosive solutions containing e.g. chlorides, the column must be rinsed thoroughly with more than 5 column volumes of pure water. Failure to do this could result in corrosion of the stainless steel components.
	EMERGENCY STOP label, yellow with black text. (EMERGENCY STOP button is red). Stops connected equipment. See Section 2.4 Emergency procedures, on page 24 for further information regarding the emergency stop.
	IMPORTANT! Before service/maintenance or return to Cytiva, clean the equipment and accompany it with a decontamination statement, specifying substances with which it has been in contact during use and the method of cleaning.
	CAUTION! Do not use any solvent solution when cleaning transparent plastic items.

Symbol/text	Description
 <p>CAUTION! Pressure control valve shall be set to 5.5 - 7 bar g for instrument air supply</p>	<p>CAUTION! Pressure control valve shall be set to 5.5 to 7 bar g for instrument air supply.</p>
	<p>Symbol indicating the position of the column frame grounding point.</p>

2.4 Emergency procedures

Introduction

This section describes how to shut down the Chromaflow™ column and associated equipment in an emergency situation, and the procedure for restarting the equipment. The section also describes the result in the event of power failure.

Precautions



WARNING

Pressure may remain in the column and tubing for an extended period after shutdown. Make sure all lines and chambers are depressurized before opening or disconnecting.



WARNING

Do not reset after an emergency shutdown if the column or piping is suspected to be damaged or otherwise defective. Damaged or defective columns or piping might leak or rupture.

Emergency stop buttons

As the operation of Chromaflow™ columns is controlled by the associated equipment, the column itself does not have an emergency stop button. To stop any operation you must use the equivalent button on the connected equipment.

If the column is connected to a packing station, pressing the **EMERGENCY STOP** button on the packing station will stop the packing station pumps and cut the supply of compressed air to the column. This will in turn stop adapter movement and will stop the packing or unpacking operation.

If the column is connected to a process system to perform a run, pressing the **EMERGENCY STOP** button on the process system will stop the run.

In an emergency situation

Press the **EMERGENCY STOP** button on the equipment being used with the column (packing station or process system). Call medical help or apply first aid if the emergency situation has caused injury.

For the rest of the procedures in an emergency situation, refer to the user documentation of the equipment being used with the column (packing station or process system).

Restart

Follow the instruction below to restart after an emergency shut down.

- | Step | Action |
|------|--|
| 1 | Make sure that the condition that was the root cause of the emergency stop has been corrected. |
| 2 |  <p>Turn the EMERGENCY STOP button on the equipment that was used to perform the emergency stop clockwise. This will reset the EMERGENCY STOP button.</p> |
| 3 | Restart the process by pressing the appropriate reset button. <ol style="list-style-type: none">If a Chromaflow™ column is used with a packing station, then press START/RESET to restart the process.If a Chromaflow™ column is used with a process system, then refer to the user documentation for the system to restart the process. |



WARNING

When reactivation is performed, pumps that were in use when the **EMERGENCY STOP** on the connected equipment button was pressed may start at the same setting.

If a pump setting was previously on maximum, reduce the pump setting prior to reactivation to avoid an immediate high pressure increase.

3 System description

About this chapter

This chapter provides a general description of Chromaflow™ columns and the basic operational principles of the nozzle. It also briefly describes packing stations and how these are used together with Chromaflow™ columns.

In this chapter

Section		See page
3.1	Overview of Chromaflow™ columns and associated equipment	27
3.2	Chromaflow™ nozzles	29
3.3	Basic principles of nozzle operation	31

3.1 Overview of Chromaflow™ columns and associated equipment

Introduction

This section describes the main components of Chromaflow™ columns and the associated equipment required for operation.

Column design

Chromaflow™ columns from Cytiva are designed for convenient and cost-efficient large scale chromatography. The patented Chromaflow™ nozzle enables columns to be packed, run, cleaned-in-place, and unpacked without disassembling the column.

Chromaflow™ columns are variable bed height columns with a fixed bottom-end piece and a position variable top adapter assembly. The column tube is manufactured in cast acrylic or stainless steel. Both materials are precision-bored and polished.

Chromaflow™ columns are equipped with manual or automatic nozzles, and with stainless steel or polyethylene bed supports.

Column setup

The normal setup includes the following:

- A Chromaflow™ column
- A Chromaflow™ Packing Station
- A slurry vessel
- A packing buffer / water vessel

An example of a setup with Chromaflow™ column and a Chromaflow™ packing station is shown below.

3 System description

3.1 Overview of Chromaflow™ columns and associated equipment



Part	Description
1	Packing station lower panel
2	Emergency stop
3	Packing station upper panel
4	Adapter flange
5	Slurry and buffer hose connected to top nozzle
6	Top nozzle
7	Adapter plate
8	Adapter O-ring(s)
9	Column tube
10	Bottom distributor
11	Bottom backing plate
12	Bottom nozzle
13	Adjustable foot
14	Column label

3.2 Chromaflow™ nozzles

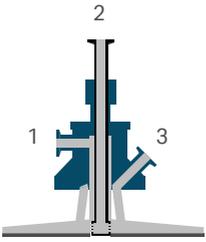
Introduction

Chromaflow™ columns are equipped with one nozzle at the top of the column and one at the bottom. The top and bottom nozzles are identical.

The nozzles are multifunctional, allowing for packing, running, unpacking and cleaning, all without dismantling the column. Nozzles on a column can either be manually or automatically operated. In the automatic mode they are operated from Chromaflow™ Packing Station.

Note: *Be aware that when using a manual nozzle, the nozzle positions must be adjusted by manually turning the nozzle to open or close the port. When using an automatic nozzle, the nozzle ports are opened and closed using Chromaflow™ Packing Station controls.*

A nozzle has three ports.

Part	Function	Illustration
1	Slurry outlet	
2	Slurry inlet	
3	Mobile phase port	

Slurry outlets

The slurry outlets are ports used for emptying during unpacking, or for venting depending on the position of the nozzle and the procedure undertaken. The slurry outlet ports are labeled **SOT** (Slurry Outlet Top) and **SOB** (Slurry Outlet Bottom).

Slurry inlets

The slurry inlets ports are used to either introduce slurry during packing or water during priming or cleaning. The slurry inlet is completely isolated from the mobile phase.

The slurry inlet ports are labeled **SIT** (Slurry Inlet Top) and **SIB** (Slurry Inlet Bottom).

Mobile phase ports

The mobile phase ports, set at an angle, are the inlet or outlet ports for the mobile phase, allowing flow into and out of the column via the distributors and bed supports. The mobile phase pathway is isolated from the other nozzle pathways during normal chromatographic operations.

The mobile phase ports are labeled **MPT** (Mobile Phase Top) and **MPB** (Mobile Phase Bottom).

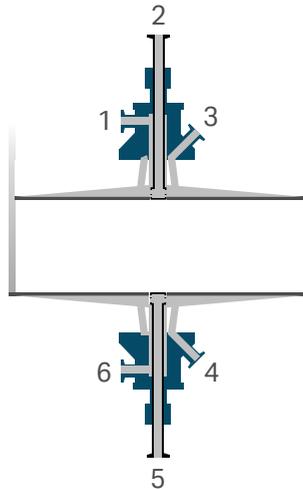
3.3 Basic principles of nozzle operation

Introduction

The nozzles, adapter and bed support are crucial parts of Chromaflow™ columns. This section provides a general overview of how the nozzles function.

Nozzle illustration

The following illustration shows an overview of the Chromaflow™ nozzle ports.



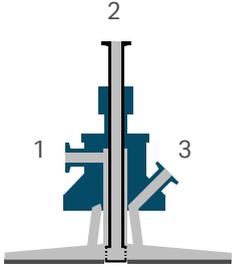
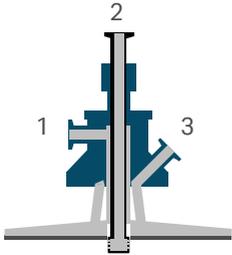
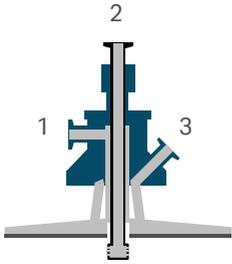
Port	Acronym	Function
1	SOT	Slurry Outlet Top on the top nozzle is the connection point for slurry or water exiting the column or for venting.
2	SIT	Slurry Inlet Top on the top nozzle is the connection point for slurry or water entering the column.
3	MPT	Mobile Phase Top on the top nozzle is the connection point for the mobile phase inlet.
4	MPB	Mobile Phase Bottom on the bottom nozzle is the connection point for the mobile phase outlet.
5	SIB	Slurry Inlet Bottom on the bottom nozzle is the connection point for slurry or water entering the column.
6	SOB	Slurry Outlet Bottom on the bottom nozzle is the connection point for slurry or water exiting the column.

3 System description

3.3 Basic principles of nozzle operation

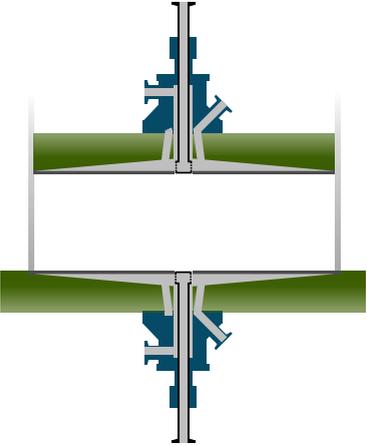
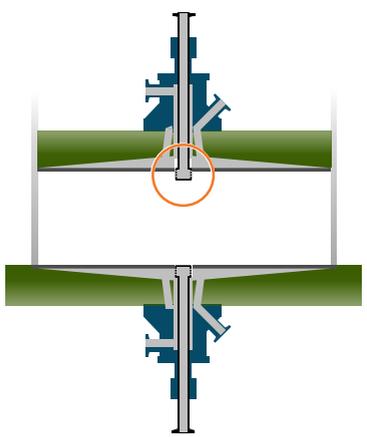
Nozzle positions

The Chromaflow™ nozzle positions are illustrated in the following table.

Nozzle position	Description	Illustration
RUN	<p>The nozzle is fully retracted.</p> <ul style="list-style-type: none">Liquids can only enter into the column and exit the column via the mobile phase ports MPB and MPT (3 in the illustration to the right).Liquids can, without entering the column, pass via the slurry inlet ports SIT and SIB (2) and exit through the slurry outlet ports SOT and SOB (1).	
PACK	<p>The nozzle is halfway inserted into the column.</p> <ul style="list-style-type: none">Liquids can enter the column via the slurry inlet ports SIT and SIB (2).Liquids can enter and exit the column via the mobile phase ports MPB and MPT (3).	
UNPACK	<p>The nozzle is fully inserted into the column.</p> <ul style="list-style-type: none">Liquids can enter the column via the slurry inlet ports SIT and SIB (2).Liquids can exit the column via the slurry outlet ports SOT and SOB (1).Liquids can enter and exit the column via the mobile phase ports MPT and MPB (3).	

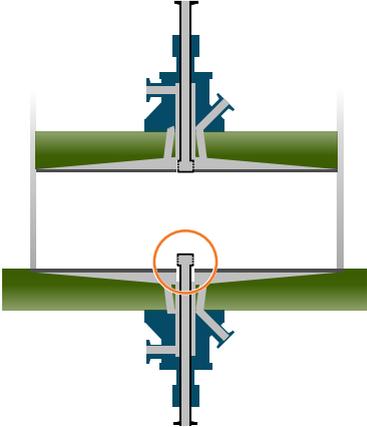
Nozzle function

The top and bottom nozzles of Chromaflow™ columns are controlled separately and can be set in one of three different positions.

Nozzle position	Description	Illustration
<p>RUN</p>	<p>When the nozzle tip is fully retracted and level with the bed support the nozzle is in the RUN position. There is no liquid pathway to the column open through the nozzle.</p> <p>The RUN nozzle position is illustrated by both nozzles in the image to the right.</p> <p>Buffers or other process fluids can enter and exit the column via the mobile phase ports. However you can use the RUN position to clean the slurry lines as the pathway, in this nozzle position, is closed off from the column.</p>	
<p>PACK</p>	<p>When the nozzle tip is halfway inserted it is in the PACK position.</p> <p>With the nozzle in this position a passage to the slurry inlet is open, and it is possible to pack by pumping slurry into the column.</p> <p>Liquids can enter and exit the column via the mobile phase ports MPB and MPT.</p> <p>Positioning the top nozzle in the PACK position is used for packing the column using downflow.</p> <p>Positioning the bottom nozzle in the PACK position is used for packing the column using upflow.</p>	

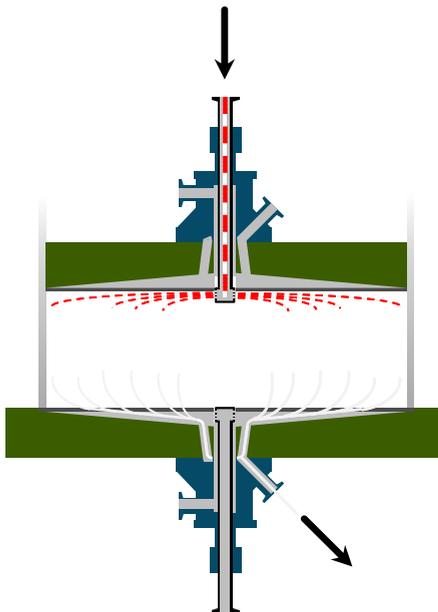
3 System description

3.3 Basic principles of nozzle operation

Nozzle position	Description	Illustration
UNPACK	<p>When the nozzle tip is fully inserted it is in the UNPACK position. This opens a passage behind the nozzle tip which connects to the slurry outlet port. The unpacking position is illustrated by the bottom nozzle in the image.</p> <p>Positioning the bottom nozzle in the UNPACK position is mostly used for unpacking the column.</p> <p>Positioning the top nozzle in the UNPACK position is mainly used for venting purposes.</p>	

Packing

The packing phase of the process is illustrated below. The top nozzle is in the **PACK** position, which is halfway inserted into the column.



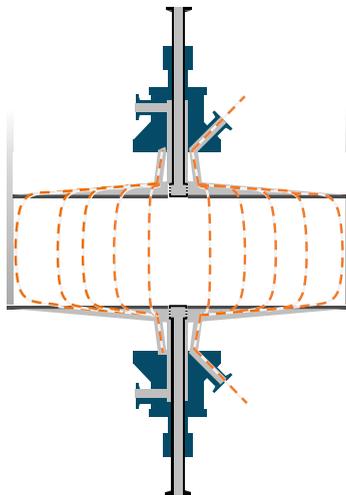
When packing from the top the slurry is pumped in via the top nozzle (**SIT**), as shown in the illustration above. The nozzle sprays the slurry directly into the column. The bottom nozzle is set in the **RUN** position, thus fully retracted. The resin beads cannot pass through the bed support. Therefore the resin is trapped in the column and the bed is built up with excess fluid (water or buffer) flowing out through the bottom mobile phase port (**MPB**).

The column is filled from the top and resin is retained by the lower bed support.

The bed builds from the bottom up. When sufficient resin has been pumped into the column the pump is stopped and the top nozzle (**SIT**) is set to the **RUN** position.

Running (chromatographic phase)

The running phase (the chromatographic phase) of the process is illustrated below. Both top and bottom nozzles are set in the **RUN** position, which is fully retracted.



Depending on the direction of the flow selected, the mobile phase is pumped in via **MPB** or **MPT**.

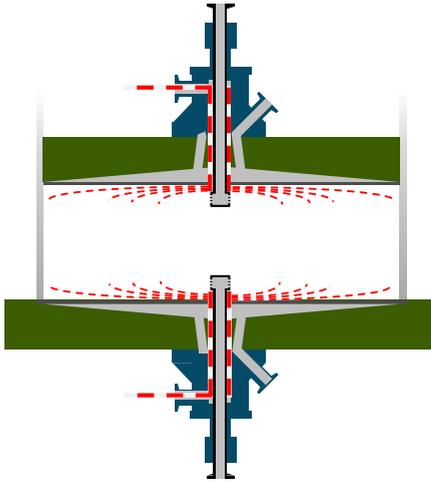
The sample passes through the stationary phase and purification takes place. The mobile phase passes through either **MPT** or **MPB** depending on the flow direction.

Unpacking

During the unpacking phase the nozzles are fully extended in the **UNPACK** position, as shown in the illustration below.

3 System description

3.3 Basic principles of nozzle operation



Resin can be rinsed out of the column when the nozzles are in their unpacking positions. The slurry will flow out of the column through **SOT** or **SOB**, depending on the direction of flow.

4 Installation

In this chapter

Section	See page
4.1	Site requirements 38
4.2	Transportation 39
4.3	Unpack the column from the crate 42
4.4	Set up associated equipment 44
4.5	Set up a column 48

About this chapter

This chapter contains information about Chromaflow™ column installation, site requirements, how to set up Chromaflow™ 400-1000 columns and how to make compressed air connections to the Chromaflow™ Packing Station that is used to operate the column.

Precautions



WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to [Section 2.1 Safety precautions, on page 9](#).

4.1 Site requirements

Introduction

This section provides a brief description of the site requirements for the installation of Chromaflow™ columns.

Space and floor load

For space and floor load requirements, refer to the dimensions and weight information provided in the documentation package.

Note: *Make sure that the floor can handle the weight of Chromaflow™ column at fully loaded conditions. Observe that for the weight to be equally distributed, the floor must be level and without irregularities.*

Note: *In order to allow convenient working conditions for the operator, sufficient space should be provided on all sides of Chromaflow™ column when installed at the intended production location.*

Ambient environment

The production location must have adequate ventilation.

The following should be avoided:

- Direct sources of heat, such as sunlight
- Vibrations
- Corrosive gas
- Dust

4.2 Transportation

Introduction

This section outlines important information that must be considered when transporting Chromaflow™ 400-1000 columns.

Equipment requirements



WARNING

Heavy object. Use suitable lifting equipment when moving the unit. All lifting and moving must be performed in accordance with local regulations.

When transporting and maneuvering Chromaflow™ columns, the following equipment is recommended:

- Suitable lifting equipment, such as a forklift with an adequate capacity to safely lift the weight of the crate and column.
- Buffering material/sufficient padding to protect the column from damage from the lifting forks during lifting and maneuvering.

Transport in crate

Use a forklift or equivalent lifting equipment with a minimum capacity to match the weight of the column filled with storage solution plus the transport crate. Refer to [General technical specifications, on page 175](#) for information about column weight and dimensions and approximate crate weights and dimensions.



WARNING

Packing crates and columns shall only be moved by personnel with appropriate training, and in adherence with local regulations. Even if the instructions in the *Operating Instructions* are followed, it is the responsibility of the customer to guarantee the safety of personnel while working with the column.

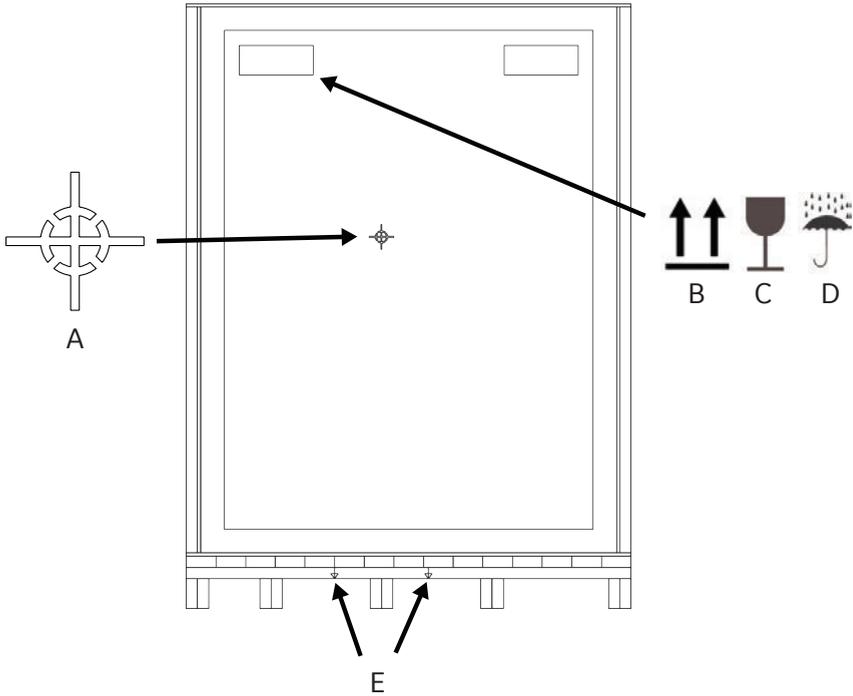
Make sure that intended openings and apertures are large enough to allow passage of the transport crate when lifted from the floor for transport.



WARNING

Move transport crates and column. Make sure that the forklift has capacity to safely lift the crate and column weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.

The illustration below shows how the crate should be moved.



Symbol	Description
A	Center of gravity
B	This side up
C	Fragile (glass)
D	Do not expose to water
E	Arrows show where to place the lifting arms of the forklift/ pallet lifter



WARNING



Note that crates may not be marked with a center of gravity symbol. Make sure that the crates are properly balanced and centered over the forks of the lifting equipment so that they will not accidentally tip when moved.

Transport after removal from the crate



WARNING

Move column. Make sure that the center of gravity of the column is well balanced over the lifting arms of the forklift. Otherwise the column may tip off the forklift.



WARNING

The lifting eyebolts of the column are intended for lifting specific column components only. The lifting eyebolts are not intended to be used for lifting the entire column. Non-ferritic lifting slings with sufficient lifting capacity to safely lift the weight of the column may be wrapped around the adapter flange to lift the entire column with a crane or hoist. Use sling protectors to protect lifting slings from sharp edges.

- Make sure that intended openings and apertures are large enough to allow passage of the column.
- Make sure that there is sufficient clearance space to allow passage of the column when lifted from the floor.
- Use wooden blocks between the column and the forks of the forklift or pallet jack during lifting to protect the bottom of the column.
- Do not lift the column any higher than necessary for clearance.

4.3 Unpack the column from the crate

Introduction

This section provides a brief instruction for taking the Chromaflow™ column out of the transport crate.

Precautions



WARNING

Heavy object. Because of the significant weight of the column, great care must be taken not to cause squeezing or crushing injuries during movement.



CAUTION

To prevent bacterial growth, the product may be filled with denatured alcohol (18% C₂H₅OH (ethanol), 2% C₃H₇OH (isopropanol) and 80% H₂O (water)) at delivery.

The denatured alcohol mixture can be hazardous to humans if consumed.

Flush out the denatured alcohol before assembling, testing or integrating the product into the intended process context.



NOTICE

Chromaflow™ columns are usually prepared and installed by Cytiva personnel. Contact Cytiva if you require reinstallation at a new site.



NOTICE

The column must be unpacked from the crate, emptied and cleaned as soon as possible after delivery, preferably within 3 months. Storage temperature must be 4°C to 24°C.

Procedure

Step	Action
------	--------

1	Place the transport crate on an even floor surface.
---	---

Step	Action
2	Remove the side of the transport crate that has been fastened with screws marked with black paint.
3	Remove the top of the transport crate.
4	Remove the wooden supports that have been used to keep the column steady in the crate.
5	Remove the column from the transport crate using a forklift or hoist and slings. Note: <i>Use wooden blocks between the column and the forks of the forklift or pallet jack during lifting to protect the bottom of the column.</i>
6	Use a pallet truck to transport the column to its working location.
7	Level the column as instructed in Section 5.2.1 Level the column, on page 57 .
8	Carefully clean the column surface from particles that might have ended up on the column following transportation and unpacking to prevent any damage.

Visual inspection

When the column is unpacked, carefully check for signs of damage that may have occurred during transportation. Report any damage immediately to your local Cytiva office and to the Transport Company concerned.

- Check that the packing list and the contents delivered are consistent.
- Check that the labels correspond to those in the documentation package.

4.4 Set up associated equipment

Introduction

Chromaflow™ column requires connection to associated equipment for packing, unpacking, and control of the automatic nozzles, if fitted. Refer to the user documentation for detailed setup instructions of the following equipment.

- Chromaflow™ Packing Station
- Slurry vessel
- Water vessel



NOTICE

Do not expose hard piping to mechanical stress. Placing nozzle connections under strain may result in damage to column ports. Use flexible couplings and/or supports to avoid strain stress.



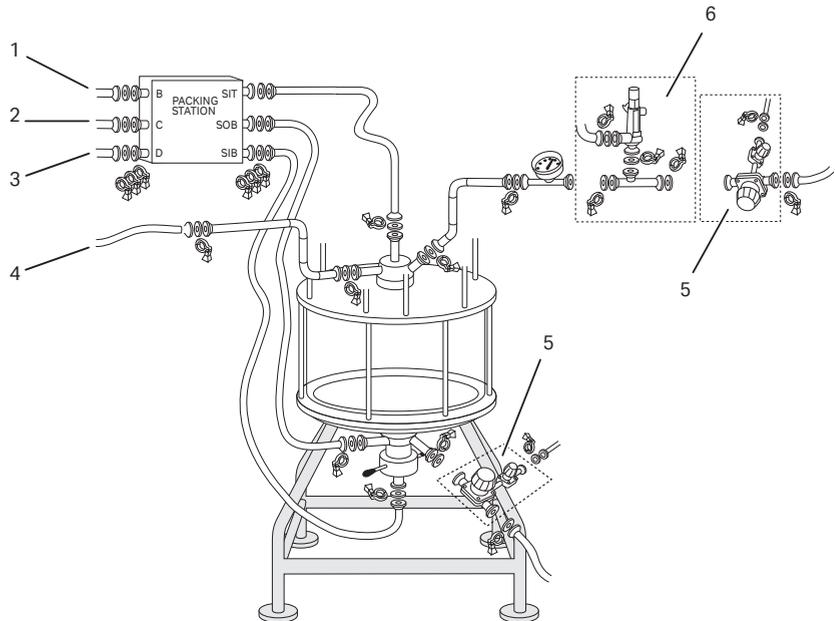
NOTICE

Resin supply connections must be arranged so that piping dimensions, piping lengths, valves and height differences do not obstruct processing.

For specifications regarding resin supply and connection requirements, refer to the documentation package.

Example illustrations of setups

Packing setup



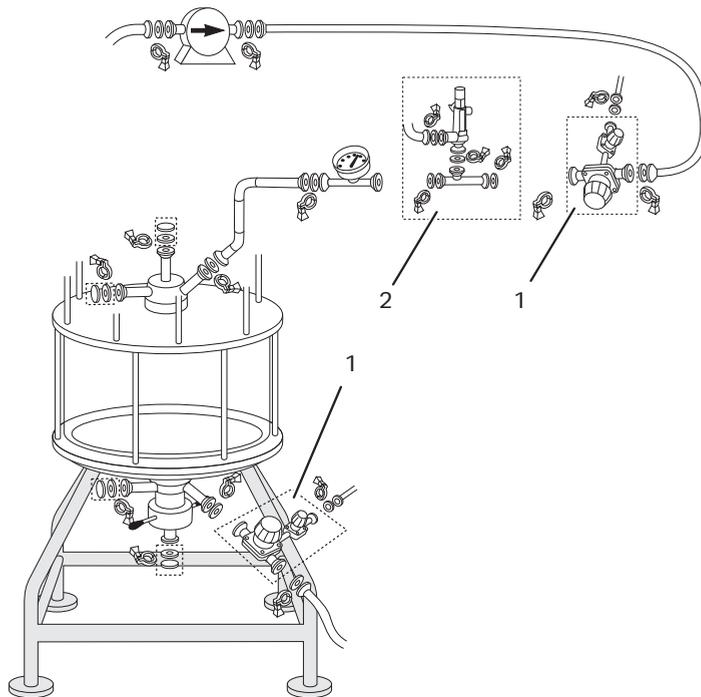
Part	Function
1	Inlet connection to packing buffer tank
2	Inlet connection to slurry tank
3	Inlet connection to collection tank
4	Outlet connection
5	Isolation valve

4 Installation

4.4 Set up associated equipment

Part	Function
6	Pressure relief valve or equivalent pressure safety equipment

Running setup



Part	Function
1	Isolation valves
2	Pressure relief valve or equivalent pressure safety equipment

Procedure

Before connecting to an air supply, check the specific hose connection requirements for your column. This information is stated in the documentation package.



NOTICE

The air supply must conform to the compressed air specifications in the Chromaflow™ Packing Station. Refer to the relevant user documentation.

Step	Action
1	Position the associated equipment so that it is easy to operate and access.
2	Always use tubing with the same inner diameters and the same clamp sizes as the connection ports of the nozzle, valves and pump or Chromaflow™ Packing Station. Gaskets must have the correct bore diameter to avoid cavitation or flow restriction. Choose the largest dimension for best flow.

4.5 Set up a column

Introduction

This section explains how to set up Chromaflow™ columns.

Grounding connections



WARNING

Explosion hazard: The column **MUST be properly grounded** to avoid static discharge.



WARNING

Explosion Hazard. When connecting the grounding cable, make sure that there is no explosive atmosphere present. Static electricity discharge could occur when connecting the grounding cable.

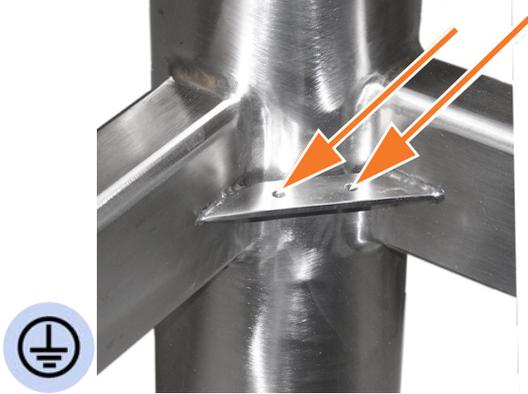


WARNING

Explosion hazard Verify that the product is properly grounded after installation and maintenance before taking the product into operation in a potentially explosive environment.

The following instruction describes how to ground a column.

Step	Action
1	Connect one end of a grounding cable to a grounding terminal in the plant grounding network and the other end to the grounding terminal located on the inside of the column leg (marked with a grounding symbol).



| 2 | Connect one end of a second grounding cable to the grounding terminal on the inside of the column leg and the other end to the grounding terminal on the associated equipment. |

Attach Mobile Phase connections



WARNING

Pressure safety equipment, such as a pressure relief valve or rupture disc must be installed at both of the mobile phases, before the column is used.

It is recommended to attach a T-connector with a pressure sensor and one valve on **MPT** and a second valve on **MPB** to isolate the column after packing.

Note: For packing upflow attach the pressure sensor on **MPB**.

5 Operation

About this chapter

This chapter provides general instructions for working with Chromaflow™ columns. Specific information about individual columns can be found in the documentation package provided with your column.

In this chapter

Section		See page
5.1	Overview of column operation	52
5.2	Prepare the column	55
5.3	Pack the column	87
5.4	Test the packed bed	98
5.5	Unpack the column	99

Precautions



WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to [Chapter 2 Safety instructions, on page 8](#).



WARNING

Only properly trained personnel may operate and maintain the product.



WARNING

Do not operate the product in any other way than described in the user documentation.



WARNING

There should **NEVER** be air or gas under pressure in the column.

5.1 Overview of column operation

Introduction

This section presents a general overview of normal operation using a Chromaflow™ column once it has been removed from the transport crate and installed according to installation instructions provided in [Chapter 4 Installation, on page 37](#).

Important

It is essential to read through the complete sequence of this chapter before starting to operate Chromaflow™ columns.

Work flow example

The sequence of operations described below is the typical work flow for normal column chromatography. The work flow described assumes that the column is a new Chromaflow™ column or that it is a column that has been in storage. Make sure the following prerequisites are fulfilled:

- The column is fitted with an adapter.
- The column is filled with storage solution.
- The column is empty of resin.



NOTICE

It is recommended to replace plastic bed supports at each maintenance.

New plastic bed supports must be wetted before operation.

Phase	Function	Actions
1	<p>Preparing</p> <p>See Section 5.2 Prepare the column, on page 55 for instructions.</p>	<ol style="list-style-type: none"> 1. Level the column. 2. Connect the column to the packing station (if applicable), and the required services (water, buffer, slurry, and cleaning solution). 3. Empty the column of storage solution and clean it. 4. Prime the column (including priming stainless steel bed supports or wetting the plastic bed supports). 5. Position the adapter (if applicable). 6. Perform a leakage test.
2	<p>Packing</p> <p>See Section 5.3 Pack the column, on page 87 for instructions.</p>	<ol style="list-style-type: none"> 1. Prepare the resin. 2. Prime the slurry lines. 3. Pack the column. 4. Rinse the slurry and packing lines.
3	<p>Testing</p> <p>See Section 5.4 Test the packed bed, on page 98 for a brief overview. Refer to the resin documentation for details.</p>	<p>Equilibrate and test the packed bed.</p>
4	<p>Running</p> <p>Refer to the user documentation for the chromatography system used.</p>	<p>Connect the column to a process system and run chromatography.</p> <p>Then proceed to one of the following steps:</p> <p>Note:</p> <p><i>The next step is optional:</i></p> <ul style="list-style-type: none"> • <i>To test the packed bed, go to step 3.</i> • <i>To run chromatography again, go to step 4.</i> • <i>To clean the column with the packed bed (CIP, Cleaning-In-Place), go to step 5.</i> • <i>To unpack and clean the column, go to step 6.</i>

5 Operation

5.1 Overview of column operation

Phase	Function	Actions
5	Cleaning (CIP) Refer to the user documentation for the chromatography system used.	Clean a column with a packed bed for further production. Note: <i>The next step is optional:</i> <ul style="list-style-type: none">• To test the packed bed, go to step 3.• To run chromatography again, go to step 4.
6	Unpacking and cleaning See Section 5.5 Unpack the column, on page 99 for instructions.	Unpack the bed and clean the column. Note: <i>The next step is optional:</i> <ul style="list-style-type: none">• To prepare the column for packing, go to step 1.• To perform maintenance on the column, go to step 7.• To store the column, go to step 8.
7	Maintenance See Section 6.5 Maintenance procedures, on page 114 for instructions.	Perform maintenance and change all O-rings, change plastic bed supports or clean stainless steel bed supports. Note: <i>The next step is optional:</i> <ul style="list-style-type: none">• To prepare the column for packing, go to step 1.• To store the column, go to step 8.
8	Storage See Section 6.6 Storage, on page 158 for instructions.	Prepare the column for storage. After storage, go to step 1.

5.2 Prepare the column

Introduction

This section describes the how to prepare a Chromaflow™ column to make sure it is ready for packing resin.

In this section

Section	See page
5.2.1 Level the column	57
5.2.2 Connect a Chromaflow™ Packing Station to the column	60
5.2.3 Empty the column	65
5.2.4 Cleaning the column	67
5.2.5 Prime the column and bed supports	72
5.2.6 Prepare and position the adapter	76
5.2.7 Perform a leakage test	85

Precautions



WARNING

If the column is equipped with caster wheels, the wheel brakes must be activated at all times during operation or storage.



CAUTION

If a column is moved from one temperature environment to another, allow the column to equilibrate to the new temperature environment and then recheck bolt tensions and adjust torque settings as necessary.

5 Operation

5.2 Prepare the column



NOTICE

Throughout the following instructions, mobile phase top (**MPT**) should be closed unless otherwise stated.



NOTICE

Before using the column, make sure that the adapter and adapter O-rings are clean and absolutely free of all dust and particulate matter. Such matter can scratch and damage the tube.

Note: *When using automatic nozzles and a packing station **NOZZLE CONTROL** should always be set to **0** when the nozzle control function is not used.*

5.2.1 Level the column

Introduction

This section describes how to level a column after it has been moved to the process location.

Note: *The column must be leveled before use so that any discrepancy from the horizontal does not exceed 5 mm.*

Tip: *If the column is moved regularly, but has one dedicated position for operation, it is recommended to mark its exact position on the floor at the dedicated place of operation after leveling. Then it is easy to return it to the exact same position, and re-leveling is not necessary.*

Level the column

Follow the instructions to level the Chromaflow™ columns.



WARNING

Do not adjust the feet more than 160 mm out from the base of the column stand to the floor. There is a risk of the column tipping over if the feet are screwed out too far.



NOTICE

Relieve the pressure on the column feet by raising the column using jacks or similar lifting and supporting equipment while adjusting the feet. Screw threads may be damaged if uneven pressure is placed on the column feet.



NOTICE

It is very important that the adjustment is done without the weight of the column resting on the leg being adjusted. Failure to do so *will* result in damage to the threads on that leg.



NOTICE

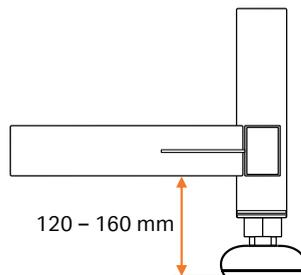
Make sure that the column is level after it has been moved. If the column is not level, column performance may be negatively affected.

5 Operation

5.2 Prepare the column

5.2.1 Level the column

Step	Action
1	Place two spirit levels diagonally across the top of the column flange.
2	Place jacks or similar suitable lifting equipment under the column stand.
3	Use the jacks or the lifting equipment, to carefully relieve the pressure on the column feet. Make sure that uneven load is not placed on the feet.
	Note: <i>Do not lift the column more than is absolutely necessary to avoid risk of tipping.</i>
4	Move the spirit level to different places around the column flange, changing the angle measured by 90 degrees each time, to make sure that the column is level in all directions.
5	Loosen the locking nuts on the column feet.
6	Adjust the level of the column by screwing the column feet away from the column stand as required until the feet touch the floor and the column stands level on the floor. Measure to make sure that the feet are extended between 120 to 160 mm from the floor and the base of the stand.



- 7 Move the spirit level around the column flange as described in step 4 to make sure that the column is level in all directions.

Step	Action
8	When the column is level and all four feet are resting on the floor, finger-tighten the locking nuts on the feet and then tighten the nuts to a torque of 70 Nm using a calibrated wrench.



9	Remove the jacks or the lifting equipment.
---	--

Tip: *To make sure that the column is level before beginning priming or packing procedures, the column can be filled with water and the water level measured around the circumference of the column tube. If the distance between the surface of the water and the top of the column tube deviates by more than 5 mm the column must be leveled.*

5 Operation

5.2 Prepare the column

5.2.2 Connect a Chromaflow™ Packing Station to the column

5.2.2 Connect a Chromaflow™ Packing Station to the column

Introduction

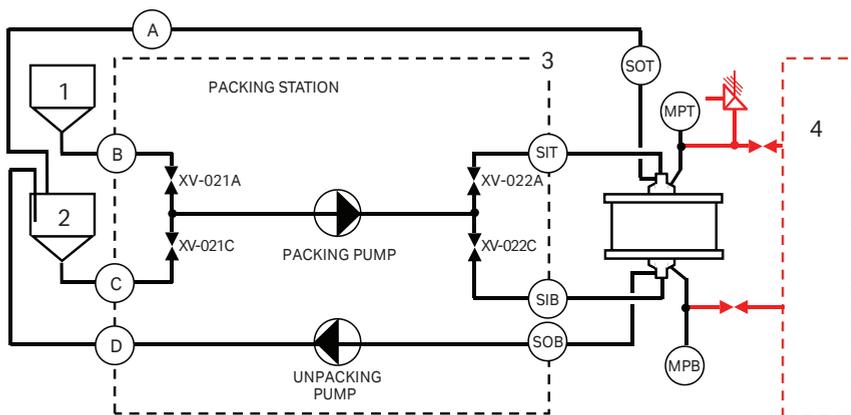
The Chromaflow™ Packing Station is used to pump liquids in and out of the column and to control the automatic nozzels. This section describes how to connect the column to a Chromaflow™ Packing Station.

Note: Using other types of packing equipment is possible but not recommended.

Note: Be aware that when using a manual nozzle, the nozzle positions must be adjusted by manually turning the nozzle to open or close the port. When using an automatic nozzle, the nozzle ports are opened and closed using the Chromaflow™ Packing Station controls.

Connections between column and Chromaflow™ Packing Station

The illustration below shows the connections between a Chromaflow™ column, a Chromaflow™ Packing Station and vessels.



Part	Function
1	Buffer vessel
2	Slurry vessel
3	The extent of Chromaflow™ Packing Station
4	The extent of the chromatography system
Packing pump	Packing pump
Unpacking pump	Unpacking pump

Part	Function
A	Slurry outlet line from the top nozzle
B	Packing buffer inlet connection ¹
C	Slurry inlet connection ¹
D	Slurry outlet line from the bottom nozzle
MPT	Mobile Phase Top
SIT	Slurry Inlet Top
SOT	Slurry Outlet Top
MPB	Mobile Phase Bottom
SIB	Slurry Inlet Bottom
SOB	Slurry Outlet Bottom ¹
XV-021A	Valve for water/buffer inlet to Chromaflow™ Packing Station
XV-021C	Valve for slurry inlet to Chromaflow™ Packing Station
XV-022A	Valve for buffer/slurry inlet to column via the top nozzle (SIT)
XV-022C	Valve for buffer/slurry inlet to column via the bottom nozzle (SIB)

¹ Tubes should be reinforced to prevent collapse.

Connect the column to the Chromaflow™ Packing Station

Note: When connecting a Chromaflow™ Packing Station to a Chromaflow™ column refer to the documentation provided with the packing station.



CAUTION

Do not strain nozzle connections. Connect tubing carefully. Failure to do so may damage nozzle components and cause leakage.



NOTICE

Do not turn on the air supply before all connections have been made and checked.

5 Operation

5.2 Prepare the column

5.2.2 Connect a Chromaflow™ Packing Station to the column



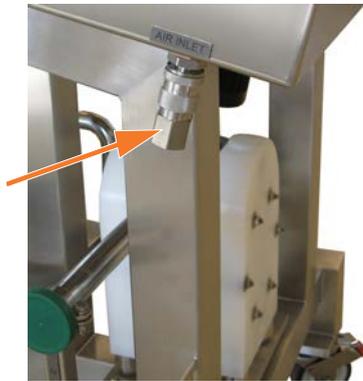
NOTICE

Make sure that pressure and supply volumes of compressed air are adequate before starting any connected equipment.

Follow the steps below to connect Chromaflow™ Packing Station to the column.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Ground the packing station to process earth. Refer to the instructions in <i>Chromaflow™ Packing Station Operating instructions</i> for details. |
| 2 | Connect the compressed air supply to the Chromaflow™ Packing Station air inlet (AIR INLET). |



- | | |
|---|--|
| 3 | Make sure that the pressure regulator for the compressed air located inside the cabinet is set to the correct bar g pressure for your operation. Adjustments are made by turning the knob that is indicated below. |
|---|--|



Step	Action
4	Connect the panel of pneumatic nozzle control hoses to the connection panel on column.
	
5	Connect Chromaflow™ Packing Station outlet SIT to column SIT .
6	Connect Chromaflow™ Packing Station outlet SIB to column SIB .
7	Connect Chromaflow™ Packing Station outlet SOB to column SOB .

Connect vessels to the Chromaflow™ Packing Station

Make the following connections to couple vessels to Chromaflow™ Packing Station.

Step	Action
1	Connect inlet C to the slurry vessel.
2	Connect inlet B to the water/buffer vessel.
3	Connect outlet D either to a collection vessel, drain or slurry vessel. This connection is operation dependent.

Connect the column to vessels

The majority of column connections to vessels are made via the Chromaflow™ Packing Station, as described in the previous section. The only connections to and from column nozzle ports that are not made via Chromaflow™ Packing Station are the line **A (SOT)** direct connection to an appropriate collection vessel, and the **MPT** and **MPB** connections to a process system.

When pathway **A** is connected to **SOT** and led, depending on the application to be performed, to either the water/buffer or slurry vessel, it is essential that the end of the tubing does not become submerged below the surface level of the liquid in the vessel. This is because pathway **A** also perform a venting function in some operations, as air leaves and enters the column via the line A tubing, to prevent a vacuum forming in the column.

5 Operation

5.2 Prepare the column

5.2.2 Connect a Chromaflow™ Packing Station to the column



NOTICE

Make sure that the end of the tube connection **A** remains above the level of the liquid in the collection tank at all times.

Fit pressure safety equipment to column



WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.



WARNING

Pressure safety equipment, such as a pressure relief valve or rupture disc must be installed at both of the mobile phases, before the column is used.

Adequate pressure safety relief must be provided during column operation. Pressure safety relief alternatives are customer specific and are therefore not included in the Chromaflow™ column delivery. Contact your local Cytiva representative if further information is needed.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Fit pressure sensing safety equipment on the inlet mobile phase port (recommended range 0 to 6 bar).
The inlet mobile phase port is normally MPT . |
| 2 | Fit one isolation valve to the inlet mobile phase port, after the pressure sensor on the process system side and mount a second isolation valve on the outlet mobile phase port. The valves should have the same inner diameter as the column inlet/outlet. |
| 3 | Connect tubing to the pressure safety equipment on the MPB and MPT ports and lead the tubing to a suitable drain or collection vessel. |



CAUTION

Make sure that safety valve connecting tubing has an unrestricted flow path.

5.2.3 Empty the column

Introduction

Emptying the column is relevant when the column contains storage liquid. All new columns must be emptied and cleaned before operation.

Empty the column

Follow the instructions to empty the column before use, when appropriate.



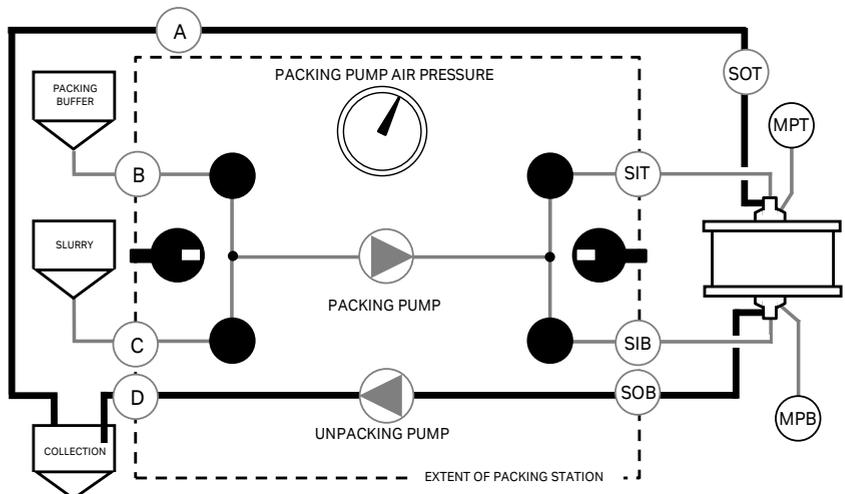
WARNING

When unpacking the chromatography resin from the column, check the integrity of all connections and make sure that the collection vessels can accommodate the volumes involved.



NOTICE

Make sure that the tube connection **A** (see the illustration below) connecting hose to the drain remains above the level of the liquid in the collection tank at all times. This is necessary to allow air to enter the tubing and the column.



5 Operation

5.2 Prepare the column

5.2.3 Empty the column

Step	Action
1	If a Chromaflow™ Packing Station is used, activate the automatic nozzle control by setting the NOZZLE CONTROL switch to I .
2	Set the top and bottom nozzles to UNPACK .
3	Start the unpacking pump. The storage solution is removed from the column via outlet D , and air enters the column via tube connection A as illustrated above.
4	Stop the unpacking pump when the column appears to be empty of storage solution.
5	Proceed immediately to cleaning.

5.2.4 Cleaning the column

Introduction

Before use the assembled column must be cleaned. This section describes the recommended procedures for cleaning the column prior to packing.



NOTICE

Avoid high flow rates and rapid pressure or flow changes that can result in damage to the bed supports. This is especially important to consider when the column is empty and when the bed support is unsupported.

Clean the column

After the column is empty, follow the instructions below to clean it. See [Section 8.2 Chemical resistance, on page 176](#) for suitable cleaning solutions.



WARNING

When unpacking the chromatography resin from the column, check the integrity of all connections and make sure that the collection vessels can accommodate the volumes involved.



NOTICE

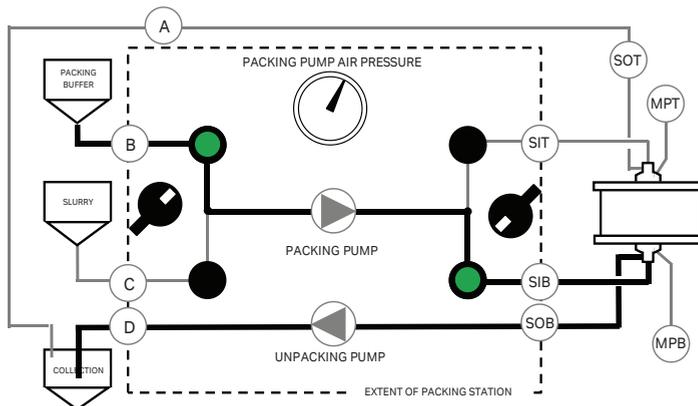
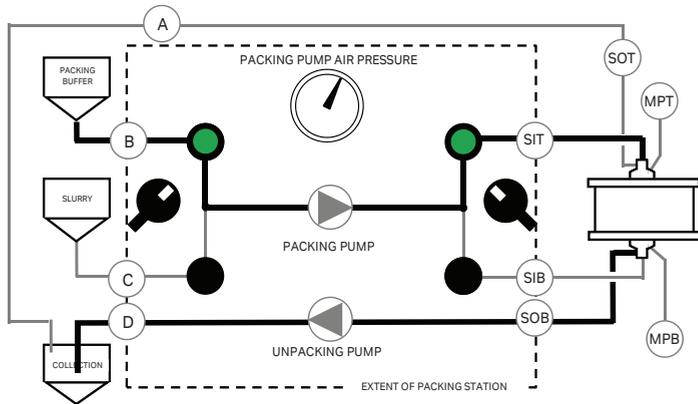
Make sure to clean the column after each unpacking to remove substances that can have a negative effect on column performance.

Step	Action
1	Fill the packing buffer vessel with cleaning solution.
2	Set the top and bottom nozzle controls to RUN . This will prevent spilling in the next step.
3	Connect the pathway D (from SOB) to waste/collection vessel. Connect the pathway A (from SOT) to waste/collection vessel. Make sure that the end of the tubing is not submerged in liquid in the vessel.
4	Set both nozzles to UNPACK .
5	Open the pathway B .

5 Operation
 5.2 Prepare the column
 5.2.4 Cleaning the column

Step Action

- 6 Start the packing pump and toggle between **SIT** and **SIB** with 3 to 5 seconds interval. Adjust the pump to full flow rate so that cleaning fluid reaches the column wall with sufficient force to wash the exposed surface of the O-ring.

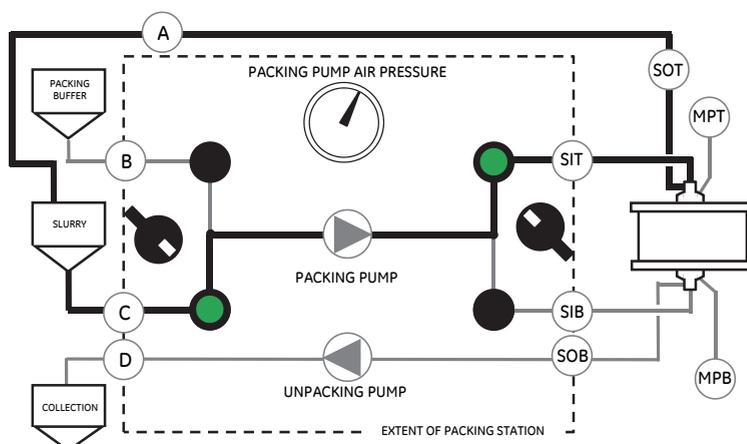


Cleaning solution will spray out from the top and bottom nozzles, respectively, cleaning the column and exit via line **D**.

Continue this procedure for some time, making sure the column is not completely filled.

- 7 Stop the packing pump.
- 8 Start the unpacking pump and pump until all cleaning solution is removed from the column.
- 9 Set the top nozzle to **PACK**.

Step	Action
10	Start both pumps, packing pump and unpacking pump and run simultaneously. Stop the pumps when the top bed support appears to be clean.
11	If cleaning liquid remains in the column, remove this by setting the bottom nozzle to UNPACK and use the unpacking pump to empty the column.
12	Set the top and bottom nozzle to RUN .
13	Open the pathway to SIB .
14	Start the packing pump to rinse the bottom slurry lines.
15	Open the pathway to SIT to rinse the top slurry lines.



16	Stop the pump.
17	Remove any remaining cleaning solution from the packing buffer vessel.
18	Proceed to rinse the column.

Rinse the column

Step	Action
1	Fill the packing buffer vessel with water.
2	Set the top nozzle to UNPACK and the bottom nozzle to PACK .
3	Start the packing pump. Water will flow into the column via the bottom nozzle. Once the column is full, liquid starts to exit via SOT .

5 Operation

5.2 Prepare the column

5.2.4 Cleaning the column

Step	Action
4	Rinse the column with at least 5 column volumes of water.
5	Stop the pump.
6	Proceed to prime and purge the column using clean water. Follow the instructions in Section 5.2.5 Prime the column and bed supports, on page 72 .

Prime the flushing ports

The flushing ports must be primed to lubricate the O-rings. Sterilizing/sanitizing solution is flushed through these valves, by pump or syringe, and into the chamber between the adapter seals when the adapter is lowered into the column. It is recommended to sanitize the barriers by changing the liquid in the chamber at least once every three months, and before each new packing.

As a sanitizing solution 20% ethanol is recommended.



CAUTION

Open flushing ports with care. Jets of priming solution may spray from the flushing ports. Before opening the flushing ports make sure that the ends of the flushing port tubing is directed away from any people or equipment in the vicinity of the column. Wear appropriate personal protection and safety goggles at all times.



NOTICE

Do not use a higher concentration than 20% v/v ethanol in the sanitizing solution as this may damage the acrylic tube.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Follow these instructions to lubricate the O-rings and the chamber.

Step	Action
1	Connect a syringe or a pump to one of the valves and use the other as an outlet valve.
2	Flush and then fill the chamber with 20% ethanol.



3	Close the flushing port valve.
---	--------------------------------

When the chamber is filled with liquid it serves two functions:

- It acts as a sanitary barrier.
- It provides lubrication to the two O-ring seals on the adapter.

5.2.5 Prime the column and bed supports

Introduction

This section describes the recommended methods for removing air from the Chromaflow™ column prior to column packing.



WARNING

High pressure. Make sure that flow rates are not so high as to affect the packed medium or cause pressure to exceed the specified column maximum pressure.



NOTICE

Avoid high flow rates and rapid pressure or flow changes that can result in damage to the bed supports. This is especially important to consider when the column is empty and when the bed support is unsupported.

Wet bed supports

Always wet plastic bed supports before column priming and operation:

- Always wet plastic bed supports when plastic bed supports are new or have been replaced. See [Wet the new bed support, on page 135](#) for instructions.
- Always prime plastic bed supports when the column has contained little or no liquid for any period of time.
- Always prime plastic bed supports when you suspect that the plastic bed supports are not hydrophilic.

Note: *The buffer recommended for wetting plastic bed supports is 20% 1-propanol.*

Water or 20% v/v ethanol can be used for columns with stainless steel bed supports.



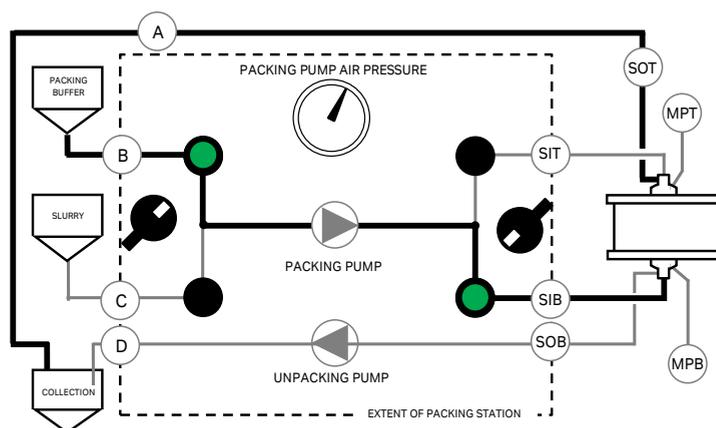
CAUTION

When working with 20% v/v 1-propanol solutions follow these instructions:

- Work in a well-ventilated area.
- Wear appropriate personal protective equipment.
- Keep storage containers tightly closed.

Priming and purging procedure

Step	Action
1	Set the bottom nozzle to PACK position and the top nozzle to UNPACK .
2	Open the pathway B from the packing buffer and open the pathway to SIB . Make sure SOT is open and that the line A is not submerged in the vessel.



- 3 Start the packing pump to allow liquid to enter the column via **SIB** and air exit through **SOT**. Fill the column slowly until the column is completely filled with liquid.
- 4 Stop the pump and switch the top nozzle to **RUN**.
- 5 Start the packing pump once more and slowly increase the column pressure.

Note:

Use the **FINE ADJUSTMENT** knob on the packing station to increase pressure.

- 6 When the column pressure is about 90% of the pressure rating, close the pathway to the column. On the Chromaflow™ Packing Station this is done by switching the **SIT/SIB** control to neutral (the mid position). See the illustration below.

Note:

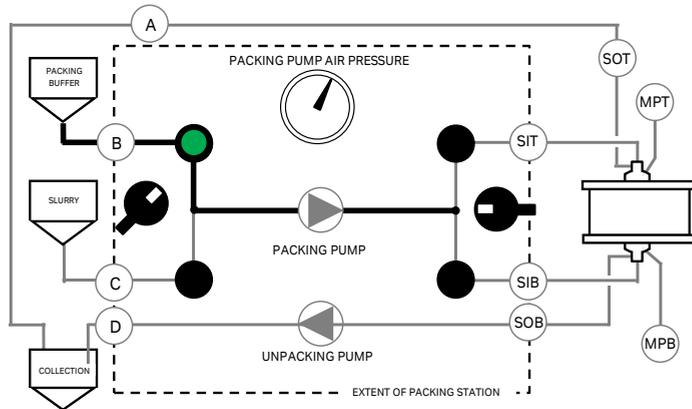
Do not touch the **FINE ADJUSTMENT** knob after shifting the **SIT/SIB** switch to neutral.

5 Operation

5.2 Prepare the column

5.2.5 Prime the column and bed supports

Step	Action
------	--------



- 7 Quickly open the bottom mobile phase port **MPB** to drain.
- 8 Once the pressure decreases to 0.3 bar g, close **MPB**. It is normal for air bubbles to rise from the bottom screen during this procedure.
- 9 Open the pathway to **SIB** and allow the pressure to rise again.

Note:

*Do not touch the **FINE ADJUSTMENT** knob. The pressure will adjust to the same value as before.*

- 10 Repeat steps 7-9 (the pressurization and purging procedure) 4 to 5 times until no more bubbles rise from the bottom screen.
- 11 Stop the packing pump.
- 12 Set the top nozzle to **UNPACK**.
- 13 Start the packing pump and continue to fill the column.
- 14 When the column is completely filled, set the top nozzle to **RUN**.
- 15 Allow the pressure to rise again. When the column pressure is about 90% of the pressure rating, set the column controller switch (**SIT/SIB**) on the Chromaflow™ Packing Station to close (the mid position).
- 16 Quickly open the top mobile phase port **MPT**. Liquid will exit through the bed support and air will escape through the port.
- 17 Once the pressure reaches 0.3 bar g, close the **MPT** port and allow for the pressure to rise again.
- 18 Set the column switch back to **SIB** and allow the pressure to rise again.

Step	Action
19	Repeat steps 16-18 (the pressurization and purging procedure) 4 to 5 times.
20	Quickly open the top mobile phase port MPT and let the pressure drop to 0.3 bar g, then close the MPT port.
21	The column and bed supports are now primed.

**NOTICE**

Do not allow the bed supports to dry out before priming the column. This may damage the bed supports and impair column performance.

5 Operation

5.2 Prepare the column

5.2.6 Prepare and position the adapter

5.2.6 Prepare and position the adapter

Introduction

This section describes how to prepare and position the adapter of a Chromaflow™ column to the correct bed height before packing.

Precautions



WARNING

Never use compressed air to raise the adapter.



WARNING

Pay extra attention when moving the adapter as there is a risk of crushing your hands.



WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.



NOTICE

The adapter is moved up or down by pressurizing the column with liquid, usually water. **DO NOT** raise/lower the column by hand as this may damage the threads on the adapter locking rods.

Lubricate the adapter

Before using or adjusting the adapter, it is crucial to make sure that all contact surfaces are clean, free from particles and lubricated. To lubricate the contact surfaces:

Step	Action
-------------	---------------

- | | |
|---|--|
| 1 | Moisten the column tube top inside with 20% ethanol. |
| 2 | Moisten the top O-ring with 20% ethanol. |

Note: Refer to [Section 8.2 Chemical resistance, on page 176](#) for alternative lubrication liquids.

Level the adapter

Follow the instructions to make sure the adapter is level before moving it.

Step	Action
1	The column must be primed as described in Section 5.2.5 Prime the column and bed supports, on page 72 and the flushing port must be primed to lubricate the seals as described in Prime the flushing ports, on page 70 .
2	Measure the distance between the column flange and the adapter flange at three points in the same position as the wing nuts using a vernier or steel rule. If the adapter is level go to the last step in this instruction.



- | | |
|---|--|
| 3 | Determine the lowest point of the adapter flange. Secure the wing nut and the nut under the flange at this position. |
| 4 | Loosen all other nuts and position them some distance away from the flange to allow the adapter to move downwards. |
| 5 | Open the MPT to waste. |
| 6 | Use the wing nuts to slowly lower the adapter until it is level. |
| 7 | Secure all nuts. |

5 Operation

5.2 Prepare the column

5.2.6 Prepare and position the adapter

Step	Action
8	Read the bed height indicator. Compare the read height to the target bed height. If there is a difference, this is the height the adapter needs to be adjusted.

Position the adapter

The adapter is moved to the desired bed height by using the Chromaflow™ Packing Station pumps to add or remove liquid from the column. Select the relevant instruction for leveling and lowering or raising the adapter below.



WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.

Lower the adapter

Follow this instruction to lower the adapter down to the desired bed height.

Step	Action
1	Make sure that the adaptor is level before setting the nuts in position.



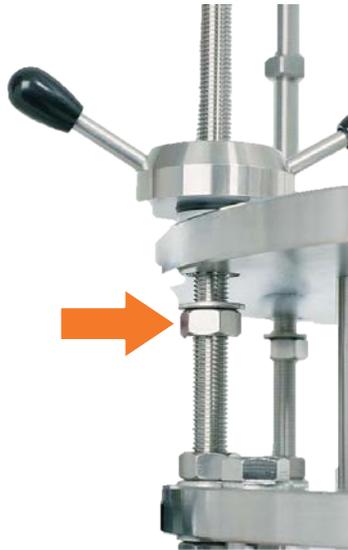
5 Operation

5.2 Prepare the column

5.2.6 Prepare and position the adapter

Step	Action
------	--------

- | | |
|---|--|
| 2 | Adjust the nuts positioned below the flange by the difference between the measured bed height and the target bed height by setting the lower adapter nuts and clamp nuts on the adapter locking rods to the desired position below the flange. |
|---|--|



- | | |
|---|--|
| 3 | Make sure that the nuts are at the same distance from the flange. Use a caliper or steel rule. |
| 4 | Set the bottom nozzle control to UNPACK and the top nozzle control to RUN . |
| 5 | Close MPT and MPB . |
| 6 | Start the unpacking pump at a very low flow rate, thereby removing liquid from the column. The adapter will move down slowly and horizontally. |



NOTICE

If the adapter is not moving horizontally, then stop the pump and re-level, refer to [Re-level the adapter, on page 119](#) before proceeding.

Step	Action
7	Allow the adapter to move down towards the preset level, stopping just above it. <div data-bbox="435 353 1204 525">NOTICE Stop the pump just before the adapter reaches the desired level.</div>
8	Confirm that the adapter flange is still parallel with the column flange. <div data-bbox="435 600 1204 773">NOTICE If the flanges are not level, refer to Re-level the adapter, on page 119 before proceeding.</div>
9	Adjust the remaining distance to the preset level manually.
10	Adjust the adapter nuts and clamp nuts to just above and below the flange until the adapter is secured in position.

Raise the adapter

Follow this instruction to level and to move the adapter up to the desired bed height.

5 Operation

5.2 Prepare the column

5.2.6 Prepare and position the adapter

Step	Action
------	--------

- | | |
|---|---|
| 1 | Adjust the nuts and clamp nuts positioned above the adapter flange by the difference between the measured bed height and the target bed height by setting the adapter nuts and clamp nuts on the adapter locking rods to the desired position above the flange. |
|---|---|



Step	Action
2	Make sure that the nuts are at the same distance from the flange. Use a caliper or steel ruler.



- 3 Set the bottom nozzle to **PACK** and the top nozzle to **RUN**.
- 4 Close **MPT** and **MPB**.
- 5 Start the packing pump at a very low flow rate, thereby filling the column with liquid. The adapter will move up slowly and horizontally.

**NOTICE**

If the adapter is not moving horizontally, then stop the pump and re-level before proceeding. Refer to [Re-level the adapter, on page 119](#) for instructions.

5 Operation

5.2 Prepare the column

5.2.6 Prepare and position the adapter

Step	Action
6	Allow the adapter to move up the preset level, stopping just under it. <div data-bbox="368 323 1134 496" style="border: 1px solid black; padding: 10px;"><p>NOTICE Stop the pump just before the adapter reaches the desired level.</p></div>
7	Confirm that the adapter flange is still parallel with the column flange. If not level, refer to Re-level the adapter, on page 119 before proceeding.
8	With the adapter in the correct and leveled position, make it secure by adjusting the nuts so that they rest tightly on each side of the flange.

5.2.7 Perform a leakage test

Introduction

A leakage test must be performed before the column is packed with chromatography resin. The purpose of the test is to check the column for leakage and verify that the column pressure can be stably maintained over a longer period.



NOTICE

Only liquid should be used in the column during the leakage test. Do not perform a leakage test on a column containing resin.

Leakage test procedure



WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.

Step	Action
1	Make sure that the column has been primed as described in Section 5.2.5 Prime the column and bed supports, on page 72 . Keep the same column set up for the leakage test as is used for priming.
2	Make sure that adequate pressure relief is provided, that a pressure gauge and isolating valve is mounted between the column and the upper MPT port valve, and an isolating valve is mounted on MPB . Note: <i>The pressure in the column during the leakage test is monitored using the MPT port pressure gauge.</i>
3	Set the bottom nozzle to PACK and set the top nozzle to RUN .
4	Isolate the MPT and MPB .
5	Start the packing pump, and use the FINE ADJUSTMENT knob (or equivalent) to build up pressure in the column by slowly pumping water into the column through the SIB .

5 Operation

5.2 Prepare the column

5.2.7 Perform a leakage test

Step	Action
6	<p>Continue pumping water into the column until the pressure reaches between 1.5 bar g and 2.0 bar g. Then stop the pump and isolate the column by setting the bottom nozzle to RUN.</p> <p>Note:</p> <p><i>The pressure used for the leakage test should be set below the range limit for the safety valves/rupture discs that are used.</i></p>
7	<p>Wait for the pressure drop to stabilize before checking the column for leakage. The pressure in the column is likely to fall initially due to settling of seals and flexing of components.</p>
8	<p>Monitor the column pressure for 30 minutes after stabilizing. The pressure should not drop by more than 0.2 bar g over the test period and there should be no visible liquid leakage.</p> <p>Note:</p> <p><i>If the pressure does drop by more than 0.2 bar g and continues to drop after the 30 minutes have passed, there is a substantial risk that the column is leaking.</i></p> <p><i>If no leak is found but doubt remains about if the column is leaking, redo the test before releasing the pressure. Note also that a drop in pressure could be due to a leaking valve and not the column itself.</i></p>
9	<p>Release the column pressure slowly at the end of the test by carefully opening either MPT or MPB port and allowing the pressure in the column to drop completely.</p> <p>Once the pressure has been released, close the isolating valve.</p>

5.3 Pack the column

In this section

Section	See page
5.3.1 Factors for resin preparations	88
5.3.2 Prepare slurry	91
5.3.3 Prime slurry lines	92
5.3.4 Pack the column with resin	94
5.3.5 Rinse slurry lines	97

Introduction

Each resin has clearly defined and documented methods for optimal packing. When packing Chromaflow™ columns with resin it is also important to understand the importance of controlling the following factors:

- Resin volumes
- Slurry concentrations
- Compression factor
- Flow rates

This section explains these factors and describes how to calculate volume, how to prepare slurry, and how to pack the column.

For additional information about methods to meet your specific needs, contact your local Cytiva representative.

5 Operation

5.3 Pack the column

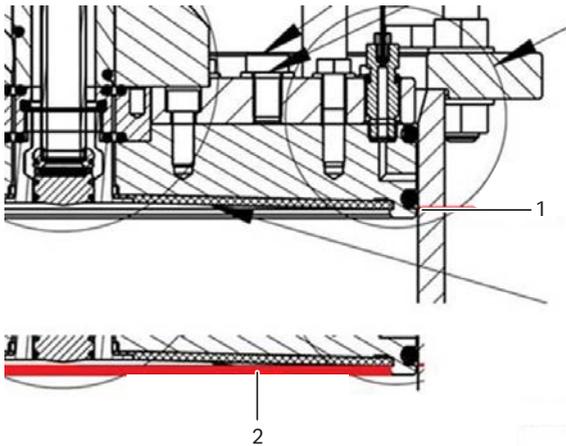
5.3.1 Factors for resin preparations

5.3.1 Factors for resin preparations

Reading bed height

To avoid a risk of suboptimal compression in the packed bed, the bed height for Chromaflow™ columns using plastic bed supports should be read level with the bottom of the lower seal on the adaptor. Reading at this point includes the volume between the end lip on the distributor and the front outer face of the bed support.

The illustration below shows the PE sinter column.



The illustration below shows the SS bed support column.

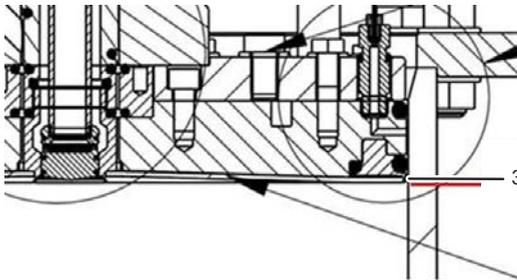


Table 5.1:

Part	Description
1	PE sinter bed supports measure lower edge of O-ring
2	Extra volume pocket
3	SS sinter bed supports measure under distributor adaptor

Reading the bed height beneath the O-ring for a plastic bed support and at the face of the stainless steel bed support columns gives the correct cylindrical volume. The conical volume (from the table above) for the different diameter columns is then added to obtain the true column volume.

Calculating column volumes

The following table illustrates column volumes for corresponding bed heights and Chromaflow™ column diameters, without taking compression into account.

Note: *Volumes indicated are the total column volume and include the volume for the two conical distributor plates in the columns.*

Column diameter (cm)	Cross-section area (cm ²)	Bed height (cm)						
		10	15	20	25	30	40	50
40	1260	13 L	19 L	26 L	32 L	38 L	51 L	63 L
60	2830	30 L	44 L	58 L	72 L	86 L	114 L	143 L
80	5030	53 L	78 L	104 L	129 L	154 L	204 L	254 L
100	7850	84 L	124 L	163 L	202 L	241 L	320 L	398 L

Calculating slurry volumes

The amount of resin required for packing the column, allowing for the compression factor required, can be calculated using the following formula:

$$V = (\pi \times r^2 \times H \times Cf / 1000)$$

Where:

V = volume of resin needed, liter

π = 3.14

r = radius of column (half the diameter), cm

H = bed height in column, cm

Cf = Compression factor of resin

Add a safety amount of additional slurry to the required volume calculation, to allow for slurry volumes in pipes, valves, and pumps.

Note: *As all resin will compress to some extent, and pipes, valves, and pump will contain some amount of slurry, extra slurry must be allowed for and added to the calculated slurry volume.*

Column diameter (cm)	Conical area volume (L)
40	0.46
60	1.4
80	3.0
100	5.8

5 Operation

5.3 Pack the column

5.3.1 Factors for resin preparations

Slurry concentrations

The resin is normally diluted with buffer and mixed into a slurry. The percentage of resin in the slurry, the slurry concentration, is necessary in the calculation of slurry volume required. You should always check the documentation provided with the resin for recommendations. For determination of slurry concentration see Slurry Concentration Kit (art no. 29096100).

Example calculation:

To pack a 400 mm column to bed height of 10 cm with resin with a compression factor of 1.1 and a slurry concentration of 50%:

Settled media required (liters) = packed bed volume (liters) × compression factor
(13 × 1.1)/0.5 = 28.6 L

Result: 28.6 liters of slurry at 50% concentration is required to pack the bed.

Note: *As all resin will compress to some extent, and pipes, valves, and pump will contain some amount of slurry, extra slurry must be allowed for and added to the calculated slurry volume.*

Compression factor

The resin needs to be compressed slightly to maintain a stable packed bed. The multiplication factor to convert packed bed volume to settled resin volume is the compression factor.

compression factor CF = (gravity settled bed height)/(packed bed height)

Depending on the type of media used, a compression factor between 1.1 to 1.25 is recommended. You should always check the documentation provided with the resin for recommendations.

Flow rates

For some packing operations, optimal packing flow rates can be used to create beds suitable for production.

Contact your local Cytiva representative for optimal flow rates for your operations.

5.3.2 Prepare slurry

Calculate slurry volume

Note: Prepare the slurry at least two hours before usage in order for it to properly degas. The slurry can be prepared up to one day ahead.

For determination of slurry concentration see *Slurry Concentration Kit* (art no. 29096100).

Calculate the required volume of resin as follows:

Settled resin required (liters) = (packed bed volume (liters, from the table provided in [Calculating column volumes, on page 89](#)) × compression factor) / slurry concentration

Add a safety factor of slurry to allow for slurry volume of pipes, valves, and pump.



NOTICE

Always prepare an excess of slurry. An excess of slurry will help to prevent air from entering the system.

Prepare slurry

Step	Action
1	Resuspend the resin in the slurry tank, by using the plastic stirrer rod (or the automatic stirrer in the vessel).
2	Pour the slurry into a graded vessel (slurry vessel).
3	Take a sample for slurry concentration determination by transferring 100 mL of the slurry to a graduated cylinder.
4	Keep the slurry as a homogeneous suspension before starting the packing.

Final checks

Check that the actions listed below are completed before continuing with the packing procedure.

- Make sure that the slurry vessel, buffer vessel and column are correctly connected to the Chromaflow™ Packing Station.
- Make sure that the column has been prepared for packing and primed as described [Section 5.2.5 Prime the column and bed supports, on page 72](#).
- Make sure that the slurry is prepared and available in the slurry vessel.
- Stir the slurry, using a plastic stirrer rod or automatic stirrer, until a homogeneous suspension is obtained.

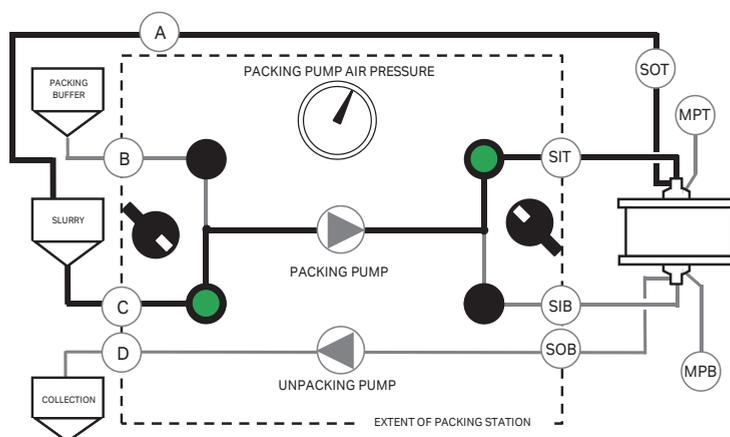
5.3.3 Prime slurry lines

Introduction

The slurry lines must be primed before beginning to pack the column with chromatography resin. This is done to remove the air from the slurry lines in order to minimize the risk of introducing air into the primed column. If the column has a stainless tube, priming the slurry line is essential. This means that the dead volume of the slurry lines is not required for measuring the amount of resin introduced into the column.

Procedure

The illustrations below shows the pathways and upper control panel settings used for top nozzle slurry line tubing priming.



NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

Follow this procedure to prime the **SIT** line.

Step	Action
1	Close MPT and MPB ports.
2	Set the top nozzle and bottom nozzle to RUN .
3	Open the pathway to SIT .

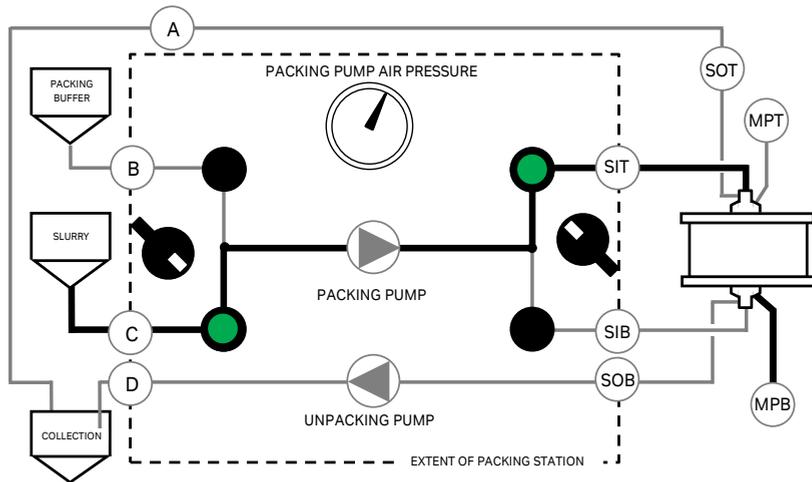
Step	Action
4	Open the pathway C , the slurry vessel valve.
5	Start the packing pump and use the FINE ADJUSTMENT knob (or equivalent) to increase the speed to approximately 50% to pump slurry through SIT line and back to slurry vessel, ensuring homogenous slurry is achieved.
6	Take a slurry sample.
7	Having primed the lines, slow the pump down using the FINE ADJUSTMENT knob, and continue immediately to pack the column without stopping the pump.

Note: *Note, for a stainless column, start measuring the amount of slurry introduced into the column.*

5.3.4 Pack the column with resin

Procedure

The following illustration shows the pathways used for packing the column using downflow.



Follow the instructions to pack the column using downflow.

Step	Action
1	Set the top nozzle to PACK and set bottom nozzle to RUN .
2	Open the pathway C , the slurry vessel valve.
3	Open the pathway to SIT .
4	Open MPB to drain (or collection vessel).
5	Carefully increase the speed of the packing pump to maximum flow rate, or use a defined flow rate dependent on the medium used.

Note:

Use the **FINE ADJUSTMENT** (*fine adjustment*) knob on the packing station to increase pressure.

Result:

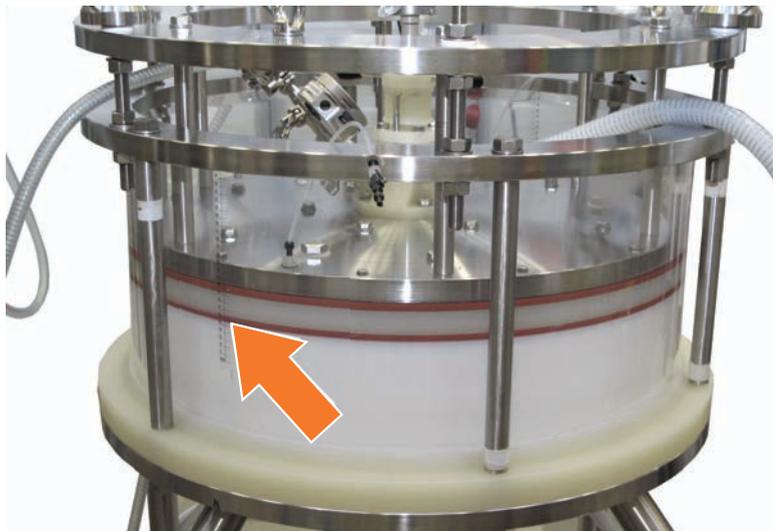
The packed bed starts to build in the column from the bottom, and the internal pressure increases.

Step	Action
6	Monitor the column pressure. If the pressure reaches above 2.75 bar reduce the pump speed to maintain approximately 2.75 bar.

**NOTICE**

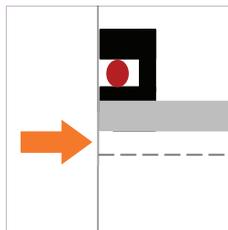
Monitor the column pressure carefully to make sure the column is not subjected to over-pressure.

7	Monitor the level of the packed bed.
---	--------------------------------------



- If the column has a stainless steel bed support then stop the pump when the packed bed reaches 0.5 cm from the top bed support, or when a defined compression factor is reached.

See the illustration below showing the column from the side, where the packed bed level (dotted line) is 0.5 cm below the bed support (grey).



5 Operation

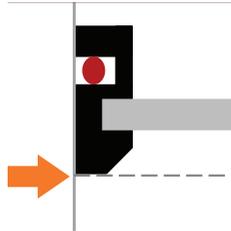
5.3 Pack the column

5.3.4 Pack the column with resin

Step	Action
------	--------

- | | |
|--|---|
| | <p>b. If the column has a plastic bed support then stop the pump when the packed bed reaches the lowest visible part of the adapter, below the bottom O-ring, or when a defined compression factor is reached.</p> |
|--|---|

See the illustration below showing the column from the side, where the packed bed level (dotted line) reaches the adapter (black).



Note:

A badly or loosely packed bed may show as a larger gap between the packed bed and the bed support. This can lead to unsupported flow which can cause damage to the bed support.

Tip:

The defined compression factor can be controlled by measuring the amount of transferred slurry into the column, and correlating it to the slurry concentration.

- | | |
|----|--|
| 8 | Set the top nozzle to RUN . |
| 9 | Close the packing station SIT valve by turning the switch to the mid position. |
| 10 | Close MPB when MPT pressure is lower than 0.1 bar, but just above 0, to avoid draining of the column by siphoning. |

The packed bed with the correct compression will provide adequate support for the bed support under all normal running conditions.

5.4 Test the packed bed

Introduction

After packing the bed it is advisable to test the efficiency of column performance to make sure the packed bed is within the required specifications for performing chromatography separations. This will require a chromatography system and a suitable test protocol. Refer to HETP (Height Equivalent to a Theoretical Plate) testing protocols and pass criteria for the relevant resin.

This section briefly describes the concept of efficiency testing and gives examples of testing criteria.

Tip: For a theoretical background and a brief overview of column efficiency testing an Application note (28937207) is available for download from the Cytiva website.

Testing column efficiency

Efficiency testing is the analysis of the residence time distribution for a tracer substance passing through the column. Typical test signals applied to the column are pulse or step signals.

In order to characterize the chromatography column without interference, tracer substance and elution conditions are selected such that chemical interactions with the resin and disturbances of the fluid flow are avoided.

Testing criteria

Specifications for qualification of optimal column efficiency may be defined as:

- Reduced plate height $h \leq 3$
- Asymmetry factor $0.8 < A_s < 1.8$

These specifications rely on the use of the following optimized test conditions and parameters:

- An inert tracer substance to avoid interaction with the resin
- Optimal liquid velocity enabling highest theoretical efficiency
- Low external volume
- A low sample volume in the pulse test (1% of CV)

Specifications for column qualification as well as test procedures should always be reviewed with regard to specific application needs as well as practical constraints that may not allow for application of optimal test conditions. The specification for reduced plate height and asymmetry may very well be different for different purification steps in the process.

5.5 Unpack the column

In this section

Section	See page
5.5.1 Loosen the packed bed	100
5.5.2 Empty the column of slurry	103
5.5.3 Clean and prime the column after unpacking	105

Introduction

This section outlines a general procedure for unpacking and cleaning a column.

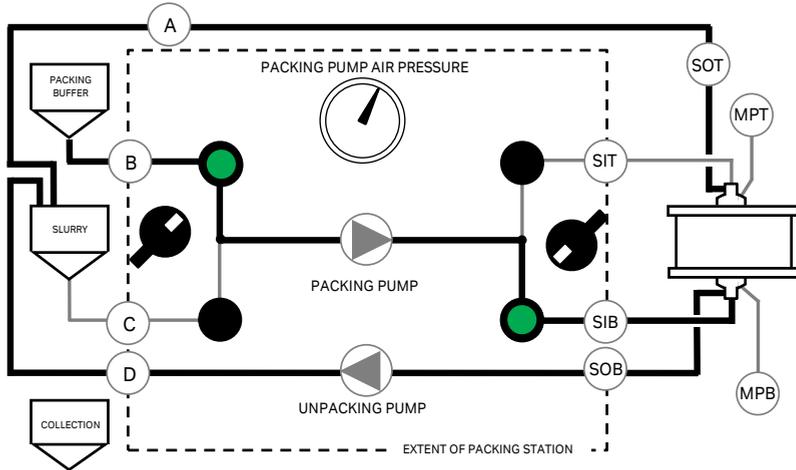
The unpacking procedure consists of the three following phases:

1. Loosening the packed bed, see [Section 5.5.1 Loosen the packed bed, on page 100](#)
2. Emptying the column of slurry, see [Section 5.5.2 Empty the column of slurry, on page 103](#)
3. Cleaning and priming the column after unpacking, see [Section 5.5.3 Clean and prime the column after unpacking, on page 105](#)

5.5.1 Loosen the packed bed

Loosening procedure

The illustration below shows the pathways used when loosening the packed bed.

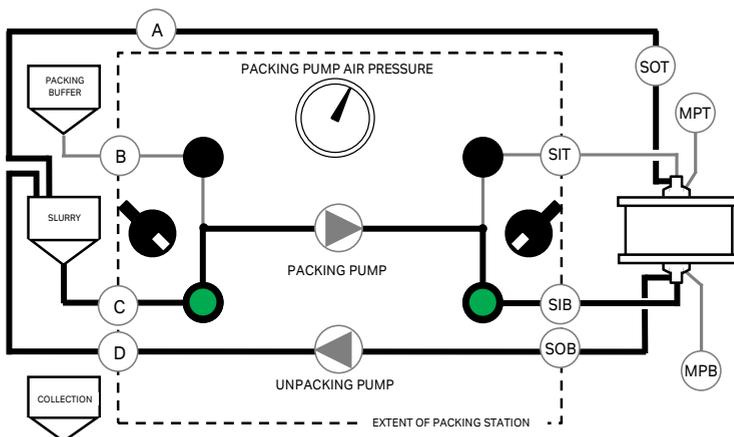


Follow the instructions below to begin to unpack the column by loosening the packed bed.

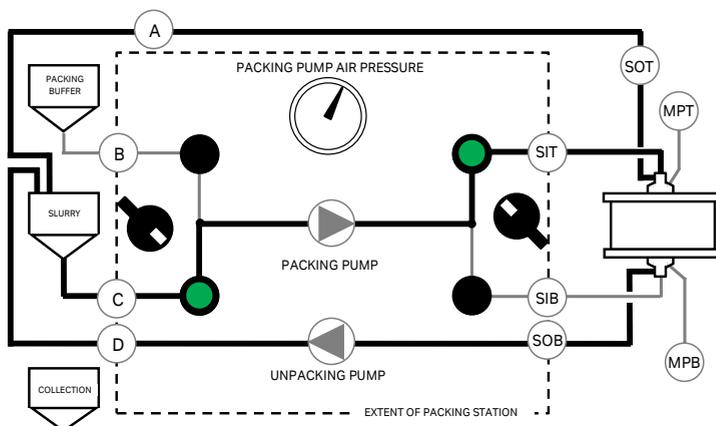
Step	Action
1	Make sure that the slurry vessel, buffer vessel and column are correctly connected to the packing station.
2	Connect outlet A and D to the slurry vessel.
<div data-bbox="396 1233 483 1319" data-label="Image"> </div> <div data-bbox="529 1224 633 1252" data-label="Section-Header"> <p>NOTICE</p> </div> <div data-bbox="529 1263 1076 1385" data-label="Text"> <p>Make sure that the outlet A connecting hose to the slurry tank remains above the level of the liquid in the tank at all times. This is necessary to allow air to enter the tubing and the column.</p> </div>	
3	Fill the buffer tank with at least two times the column volume of buffer for unpacking.
4	Set both top and bottom nozzles to UNPACK .
5	Check that the MPB and MPT ports are closed.
6	Open SIB .



- | Step | Action |
|------|--|
| 7 | Open pathway B (packing buffer or water). |
| 8 | Start the packing pump at maximum flow rate and pump until approximately a half column volume (0.5 CV) of slurry has collected in the slurry vessel. |
| 9 | Open pathway C .
Slurry will now be recycled into the column and back to the slurry vessel. |



- 10 After around 3 to 5 minutes, open **SIT** to redirect the flow entering the column.



- 11 After around 3 to 5 minutes, open **SIB** to redirect the flow entering the column.

5 Operation

5.5 Unpack the column

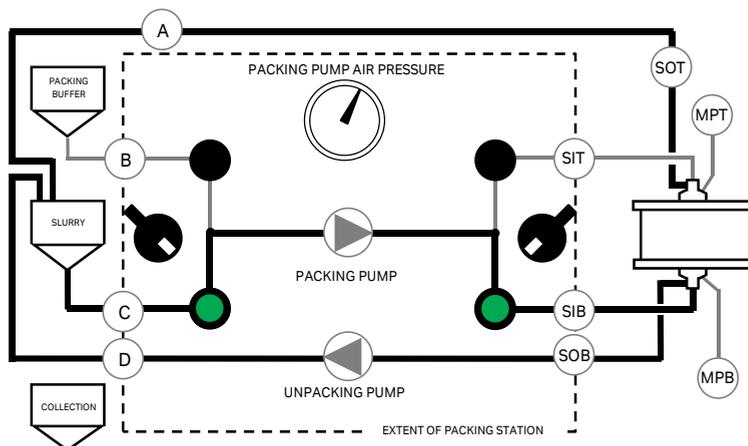
5.5.1 Loosen the packed bed

Step	Action
12	If necessary, repeat steps 9 to 10 to toggle between SIT and SOB until all of the resin is re-suspended in the recycling slurry, then stop the packing pump.
13	Proceed to the next section to empty the column of slurry.

5.5.2 Empty the column of slurry

Emptying procedure

The illustration below shows the pathways used when emptying the column of slurry.



Note: When emptying the column of slurry, the **A** and **D** outlet tubing connections can be directed to the slurry tank as the collection tank, if this is of a suitable size to accommodate the volume to be emptied from the column.

Follow the instructions below to complete the unpacking of the column by emptying the column.

Step	Action
1	Make sure that the packed bed has been loosened according to the instructions above.
2	Set the top nozzle to UNPACK .
3	Start the unpacking pump. Slurry is removed from the column via SOB and the D line, and air enters the column via SOT and line A .



NOTICE

Make sure that the outlet **A** connecting hose to the collection tank or slurry tank remains above the level of the liquid in the tank at all times. This is necessary to allow air to enter the tubing and the column.

5 Operation

5.5 Unpack the column

5.5.2 Empty the column of slurry

Step	Action
------	--------

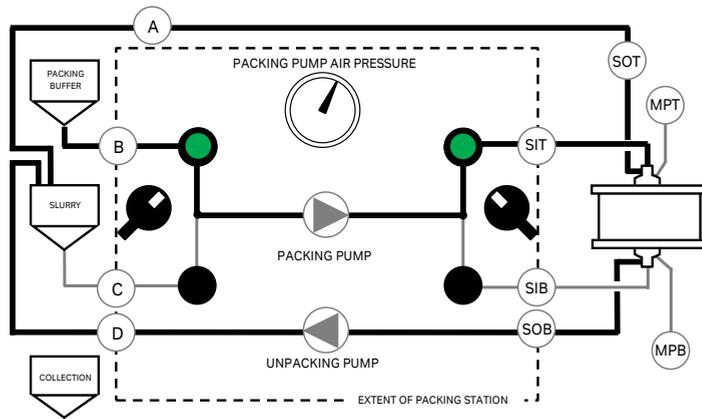
4	Stop the pump when the column is empty of slurry.
---	---

Note:

If the chromatography resin does not fully re-slurry, repeat steps 8 to 10 in the instruction for loosening the packed bed.

5	Change to pathway B and start the packing pump, and toggle between the SOT and SOB to flush out the remaining resin.
---	---

Be careful to limit the amount of unpacking buffer or water used so that slurry dilution is avoided.



6	When the column is empty, stop the pump.
---	--

7	Set the top nozzle to PACK and open the pathway through SIT . Run the packing pump and unpacking pump simultaneously for a final rinse of the bed supports and the complete column.
---	---



NOTICE

Monitor the column pressure to make sure the column is not subjected to over-pressure.

8	Set both nozzles to RUN .
---	----------------------------------

9	Proceed to clean the column and lines.
---	--

5.5.3 Clean and prime the column after unpacking

Cleaning procedure

Step	Action
1	Clean the empty column, see Section 5.2.4 Cleaning the column, on page 67 .
2	Rinse the column after cleaning, see Rinse the column, on page 69 .
3	Clean the sanitary barrier using the flushing ports, see Prime the flushing ports, on page 70 .
4	Prime and purge the column using clean water. Follow the instructions in Section 5.2.5 Prime the column and bed supports, on page 72 .

If required, replace the water with storage solution (for storage) or packing buffer for operation.

6 Maintenance

About this chapter

Regular cleaning and maintenance of the Chromaflow™ columns is essential to maintain the column in a good condition and extend the operational lifetime of column components and to achieve reliable results. It is important that the column is kept free from contamination such as microbial growth and adsorbed proteins in the liquid handling system.

This chapter provides an overview of general cleaning, maintenance, and storage information applicable to Chromaflow™ columns.

In this chapter

Section	See page
6.1 Service	108
6.2 Cleaning	109
6.3 Cleaning stainless steel bed supports	112
6.4 Maintenance schedule	113
6.5 Maintenance procedures	114
6.6 Storage	158

Precautions



WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to [Chapter 2 Safety instructions, on page 8](#).

**WARNING**

LOCK OUT / TAG OUT (LOTO)! Before any maintenance or decommissioning work is performed, make sure that:

- the equipment is empty and depressurized.
- the equipment is disconnected from process feed, electrical power and pneumatic supply.
- the equipment is prevented from accidentally becoming re-energized during maintenance.
- the equipment is clearly tagged as taken out of operation.
- all process wetted areas are clean and decontaminated.

Note: *After any maintenance or service has been performed on the column it is strongly advised that a leakage test is performed to make sure of the column integrity prior to packing media. Refer to instructions in [Section 5.2.7 Perform a leakage test, on page 85](#).*

6.1 Service

Introduction

Regular service and preventive maintenance of the column is necessary to maintain the column in optimal condition and extend the operational lifetime of column components.

Service and preventive maintenance work should be performed according to Cytiva recommendations.



WARNING

Only properly trained personnel, or personnel with equivalent knowledge of similar equipment, are allowed to perform maintenance work on the column. Only personnel authorized by Cytiva are allowed to perform service on the Chromaflow™ column.

Service frequency

The requirements for frequency of service and preventive maintenance depend on the frequency of use of the column and the specific applications performed.

A general recommendation is one service and preventive maintenance visit every two years, but if columns are in continuous operation an annual service and preventive maintenance visit is recommended.

Service agreements

Contact your local Cytiva representative for information about frequency of service requirements to suit individual application needs and for details of the Cytiva service agreement options available for Chromaflow™ columns.

6.2 Cleaning

Introduction

Cleaning and sanitizing are essential elements of any process to ensure that biological contamination is kept within acceptable limits. This section describes when to clean and aspects relevant to cleaning.

During a normal operation cycle cleaning can be required for the following reasons:

1. Before and after servicing the column. Firstly to ensure the vessel is clean to perform work upon, and secondly to ensure the vessel is clean for return to operation.
2. During normal process cycles with a packed column.
3. Before storing a column with or without a packed bed.
4. After storage in preparation for packing and running.
5. Before decommissioning and removal from the process environment.

Cleaning methods

There are two methods for performing cleaning on Chromaflow™ columns. Both methods involve pumping solutions through the column whether it is packed with media or not. One method is performed with a packing station and one method without (CIP).



CAUTION

Make sure that the piping system is completely leakage free before performing any Cleaning-In-Place (CIP) or Sanitation-In-Place (SIP) on the column.

If the column is packed and in use then usually the system attached to the column is sufficient for CIP during standard operation. Most other cases require a packing station to be connected to the column to operate the nozzles and clean the various pathways as well as the interior of the column itself.

For CIP cleaning method using other systems, refer to the relevant user documentation.

For cleaning after unpacking, refer to instructions in [Section 5.5.3 Clean and prime the column after unpacking, on page 105](#).

For cleaning stainless steel bed supports, refer to instructions in [Section 6.3 Cleaning stainless steel bed supports, on page 112](#). For sanitization of the column an Application note (Use of sodium hydroxide for cleaning and sanitization of chromatography media and systems, art no 18112457) is available for download from cytiva.com. Plastic bed supports are not cleaned but exchanged.

Note: *To sanitize the nozzle tip the nozzle must be placed in the UNPACK position.*

**WARNING**

Explosion hazard: If cleaning the column in a potentially explosive atmosphere, prevent static electric charges building up. Do not rub excessively using a dry cloth and over large areas at a time. Instead, clean the column using a damp cloth with slow movements across small areas at a time.

**NOTICE**

To retain sanitary surfaces, it is recommended that clean gloves be worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Biohazardous substances

The cleaning procedures described in this section do not apply to columns that have been exposed to biohazardous substances. If your column or associated equipment has been contaminated with a biohazardous substance, contact your local Cytiva service representative for further information about decontamination procedures.

Cleaning agents

All column parts can be cleaned with the agents most commonly used in column chromatography, such as detergents, 20% ethanol, weak acids, sodium hydroxide and salt solutions. For details refer to the chemical resistance guidelines in [Section 8.2 Chemical resistance, on page 176](#).

**WARNING**

Risk assessment! Perform a risk assessment for any risks due to the process or process environment. Evaluate the effects the use of the equipment and operational processes have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.

**NOTICE**

Do not use chemicals harmful to the column.



NOTICE

For acrylic-tubed columns, do not use solutions containing more than 20% ethanol.

Rinse the column after cleaning

Water is the preferred rinse solution due to the solubility properties of sodium chloride in water compared to other possible rinsing solutions such as ethanol. It is recommended that a water rinse of at least five column volumes is used.

6.3 Cleaning stainless steel bed supports

Introduction

Stainless steel bed supports may clog if not cleaned regularly. The supports must be cleaned at least after 5 to 10 packings, but preferably after each packing. If the supports cannot be cleaned properly using the method below, contact your local Cytiva representative for advice.

Note: *The compressed air that is used for cleaning bed supports must be completely free from lubricating oils and particles.*

Procedure

Step	Action
1	Remove the bed support from the column.
2	Flush the bed support free from entrapped resin using water.
3	Place a shut-off valve on a compressed air supply hose. Fit a 0.5 to 1 inch plastic TC-clamp connector or similar to the valve.
4	Use a shallow container to soak the bed support in 0.5 to 1 M NaCl solution for a maximum of 5 minutes.
5	Place the bed support with the coarse side up on a clean non-scratching (plastic) support, that allows air and resin to flow through the bed support.
6	Open the valve and start blowing air through the bed support from a close distance. Make sure the whole surface of the bed support is properly blown and that special care is used at the outer rim and at the center.
7	Rinse the bed support thoroughly in water immediately after the above procedure is finished.
8	Repeat the procedure, this time using at least 20% ethanol solution. If 20% ethanol cannot be used, use water. Finish by carefully rinsing with water.

6.4 Maintenance schedule

The table below provides recommendations for the frequency of regular maintenance procedures for columns in continuous use.

Note: *Make sure that the product file is kept updated and together with the product documentation. The file should contain information about inspections, maintenance procedures that have been performed, spare parts that have been exchanged and any other relevant information for safe operation.*

Procedure	Frequency
Change all O-rings	At least once every two years
Clean O-ring grooves	Every time O-rings are changed
Change liquid in flushing channels	Every two months and preferably before each packing
Check all seals for leakage	Regularly
Change O-rings and seals on moving parts (e.g. adapter and nozzles)	Every 5 to 10 packings, more frequently if required
Clean stainless steel bed supports	Every 5 to 10 packings, more frequently if required
Replace plastic bed supports	Every 5 to 10 packings, more frequently if required



NOTICE

Make sure that cleaning of O-ring grooves is performed each time O-rings are removed and replaced. Clean O-ring grooves manually and gently with 20% ethanol, taking great care not to damage the sealing surface.

6.5 Maintenance procedures

In this section

Section	See page
6.5.1 Remove the adapter	116
6.5.2 Remove automatic nozzles and exchange O-rings	122
6.5.3 Remove manual nozzles and exchange O-rings	129
6.5.4 Exchange adapter O-rings	134
6.5.5 Exchange plastic bed supports	135
6.5.6 Exchange the adapter stainless steel bed support	142
6.5.7 Exchange the bottom unit O-rings and plastic bed support	149
6.5.8 Exchange the bottom unit O-rings and stainless steel bed support	152
6.5.9 Refit the adapter	156

Introduction

This section describes the specific maintenance procedures when cleaning or replacing parts and provides information about service.

Maintenance work shall be performed according to Cytiva recommendations. Contact your Cytiva representative for advice and before performing any maintenance procedure you are not sure about.

Precautions



WARNING

Decontaminate before maintenance. To avoid personnel being exposed to potentially hazardous substances, make sure that the column is properly decontaminated and sanitized before maintenance or service.



WARNING

Do not perform any type of maintenance work on the column while the column is pressurized. Note that the column can be pressurized even when the system is closed down.



NOTICE

Do not leave O-rings or seals in hot water for longer than the time period recommended in Cytiva product documentation.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.



NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.



NOTICE

Make sure that tools are properly calibrated before use. Use of uncalibrated tools such as torque wrenches may result in over-tightening or under-tightening of bolts and result in damage to column components.



NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

6.5.1 Remove the adapter

Introduction



WARNING

Pay extra attention when moving the adapter as there is a risk of crushing your hands.



WARNING

To remove the adapter or rebuild the equipment, a hoist with a minimum lifting capacity to safely lift the weight of the column components is required.



NOTICE

When working on columns fitted with automatic nozzles, a packing station must be used as the nozzles are controlled from this unit. Columns with manual nozzles can use a pressurized water supply with a shut off valve.



NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

Tools required

The following tools are required for removing the adapter:

- A crane or hoist, with suitable lifting capacity
- Three slings
- Three adapter legs
- Stainless steel wrenches

Adapter removal procedure



WARNING

During removal of the adapter, or any other movement performed through applying hydrostatic pressure, a pressure gauge must be connected to the system so that the internal column pressure can be monitored.



NOTICE

The adapter is moved up or down by pressurizing the column with liquid, usually water. DO NOT raise/lower the column by hand as this may damage the threads on the adapter locking rods.

The procedure contains three steps:

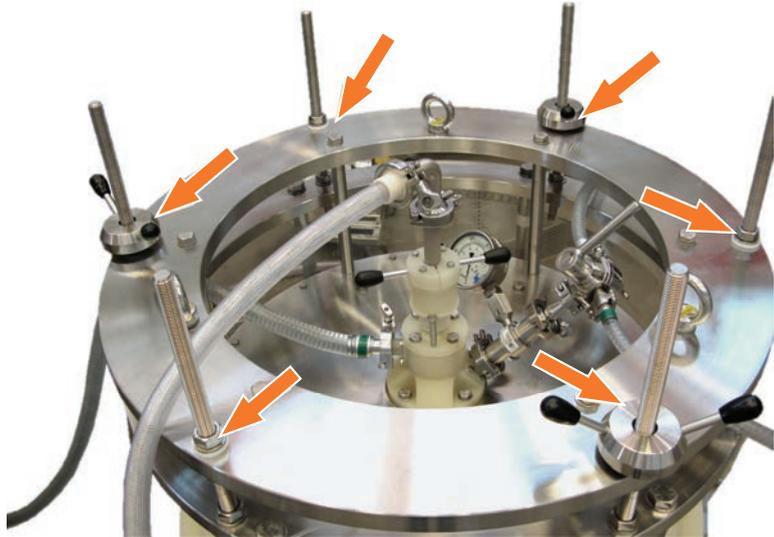
1. Prepare the adapter, raising it to its highest position within the column.
2. Re-level the adapter (if it is tipped out of alignment during raising).
3. Lift the adapter out of the column.

Prepare the adapter

Step	Action
1	<p>Make sure the column is full of liquid (usually distilled water) before starting the process to remove the adapter. Make sure the column tube wall above the top piston seal is perfectly clean and sprayed with 20% ethanol.</p> <p>Lubricate the seals (see Lubricate the adapter, on page 76) and prime the flushing ports (see Prime the flushing ports, on page 70).</p>
2	<p>Prime to remove all the air in the column, packing station, pipes and vessels. See instructions in Section 5.2.5 Prime the column and bed supports, on page 72.</p>

Step	Action
------	--------

- | | |
|---|---|
| 3 | Remove all fasteners on the adapter flange. |
|---|---|



- | | |
|---|---|
| 4 | Set the bottom nozzle to PACK and the top nozzle to RUN . |
| 5 | Open the pathway to SIB . |
| 6 | Start the packing pump and fill the column to move the adapter upwards.
The pressure needed to move the adapter should be less than 0.5 bar. |

Note:

If the pressure rises above 0.5 bar, or if the adapter is tilting, then stop the pump and repeat the procedure to lubricate the seals (see [Lubricate the adapter, on page 76](#)) and prime the flushing ports (see [Prime the flushing ports, on page 70](#)).

If the adapter is tilting, then re-level it according to instructions in [Re-level the adapter, on page 119](#) before proceeding to the next step.

- | | |
|---|---|
| 7 | Check to see if anything other than the seals are preventing movement, or if the adapter has tipped out of alignment. |
|---|---|

Note:

Adapter misalignment of a few mm is acceptable and will normally even itself out.

- | | |
|---|--|
| 8 | Stop the pump as the top O-ring of the adapter approaches the upper edge of the column tube. |
|---|--|

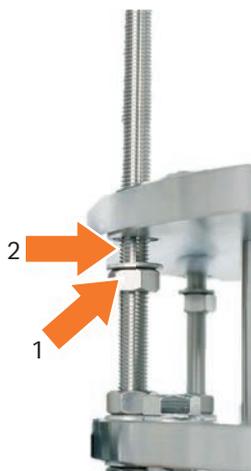
Re-level the adapter

If leveling is required follow these instructions.

Step	Action
1	Stop the packing pump or inflow of water.
2	Lubricate the O-rings.
3	Fit a nut and washer to the clamp rod at the highest point on the adapter flange.
4	Start the pump and watch the adapter as it straightens itself out. Once the adapter is level, stop the pump and remove the nut and washer.

Lift the adapter

Step	Action
1	Position the lower nuts and washers equally spaced just below the lower face of the adapter flange on all adapter locking rods. The nuts (1) must remain in contact with the flange (2) to provide additional safety during the following steps.



Step	Action
------	--------

- | | |
|---|---|
| 2 | Pass three slings from an overhead crane or hoist through the three eyebolts on the adapter flange. |
|---|---|



- | | |
|---|---|
| 3 | Carefully lift the the column just off the floor and then lower it again. This will position the center the column aligned with the hoist. |
| 4 | Raise the adapter further by packing the column with water very slowly until the lower O-ring is just clear of the upper edge of the tube (i.e. the column begins to leak). Then stop the pump. |



Note:

Refer to the assembly drawing in the documentation package provided with the column for additional information on lifting.

Step	Action
5	Keep the hoist steady in a position where it holds the adapter and prevents it from tipping.
6	Set the top nozzle to RUN , then turn off the packing station.
7	Disconnect the multi-connector lines from the top nozzle (these are attached at the quick connection points). On automatic nozzles, disconnect all air supply lines and piping from the nozzle assembly.
8	Tension the slings using the hoist to begin to move the adapter slowly upwards. Continue the gradual upward movement of the adapter and carefully lift the adapter free from the column.
9	Mount the legs and bolts of the adapter legs provided with the column to the underside of the adapter. The adapter legs are mounted in the positions otherwise occupied by the locking rods. Make sure to use washers between the top of the adapter legs and the adapter flange.



10	Gently lower the adapter, now fitted with adapter legs, to the floor to rest on the adapter legs.
----	---

6.5.2 Remove automatic nozzles and exchange O-rings

Introduction

Nozzles in a Chromaflow™ column are either automatic or manual. This section describes how to remove an automatic nozzle from a Chromaflow™ column, how to replace O-rings and how to refit the nozzle in the column again. There is an option of fitting a pre-assembled new nozzle tip unit to the Chromaflow™ nozzle. These can be ordered separately. Refer to the Spare Part List, or cytiva.com for further information.

Note: *When performing maintenance on nozzles it is recommended to use a table with an approximately 40 mm diameter hole or to use supports with a central gap of 40 mm (for example two support blocks that the nozzle is balanced on). This will allow you to place the nozzle assembly upright.*

Nozzle O-ring tool (option)

The replacement of nozzle tip O-rings can be performed by either:

1. fitting a pre-assembled new nozzle tip unit to the Chromaflow™ nozzle, or
2. removing and replacing the existing nozzle tip O-rings.

If the second method is chosen, an optional tool is available for expanding and fitting the new nozzle tip O-rings into place.



Remove an automatic nozzle



NOTICE

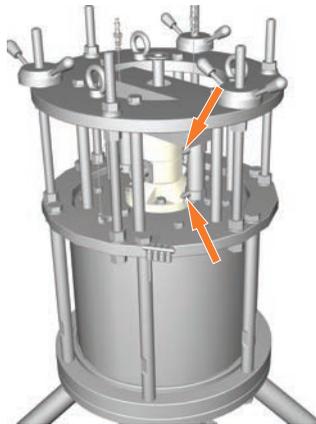
Before disassembling the nozzle unit, check that all associated air connections and piping are marked correctly - this will ensure proper re-connection.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Set the nozzle you are servicing to RUN .
2	Make sure the unit controlling the automatic nozzle is set to OFF .
3	Disconnect the multi-connector lines from the nozzle attached at the quick connection points.
4	Disconnect all air supply lines and piping from the nozzle assembly.



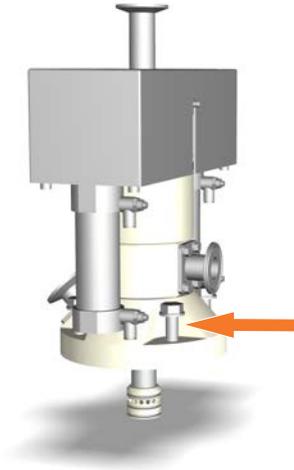
6 Maintenance

6.5 Maintenance procedures

6.5.2 Remove automatic nozzles and exchange O-rings

Step	Action
------	--------

- | | |
|---|--|
| 5 | Remove the four bolts and washers that secure the nozzle assembly to the column backing plate. |
|---|--|



- | | |
|---|---|
| 6 | Carefully pull the entire nozzle assembly straight out. |
|---|---|



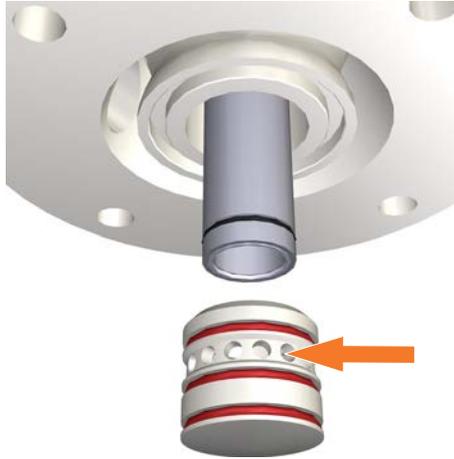
NOTICE

Take extreme care not to damage the nozzle tip and seals.

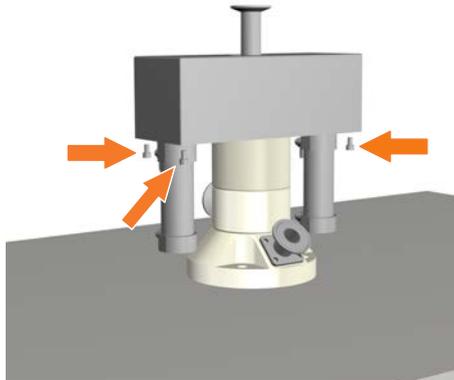
Exchange automatic nozzle O-rings

Step	Action
------	--------

- | | |
|---|--|
| 1 | Unscrew the nozzle tip from the nozzle tube. |
|---|--|



- | | |
|---|---|
| 2 | Place the nozzle assembly in the upright position on a table with a hole or on supporting blocks. |
| 3 | Remove the four screws securing the cover to the nozzle assembly. |



- | | |
|---|-------------------|
| 4 | Remove the cover. |
|---|-------------------|

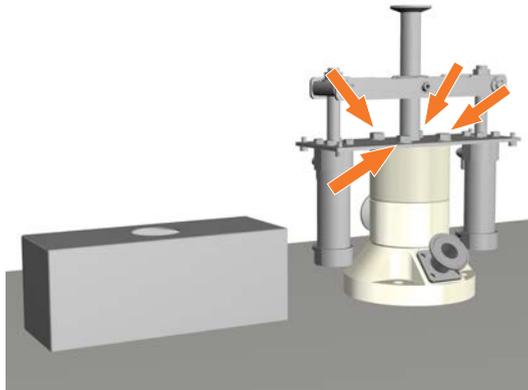
6 Maintenance

6.5 Maintenance procedures

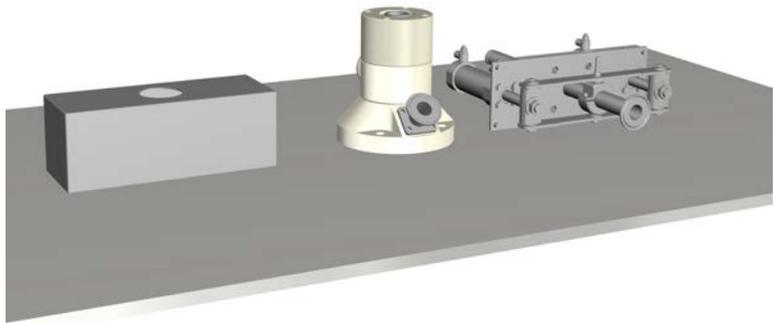
6.5.2 Remove automatic nozzles and exchange O-rings

Step	Action
------	--------

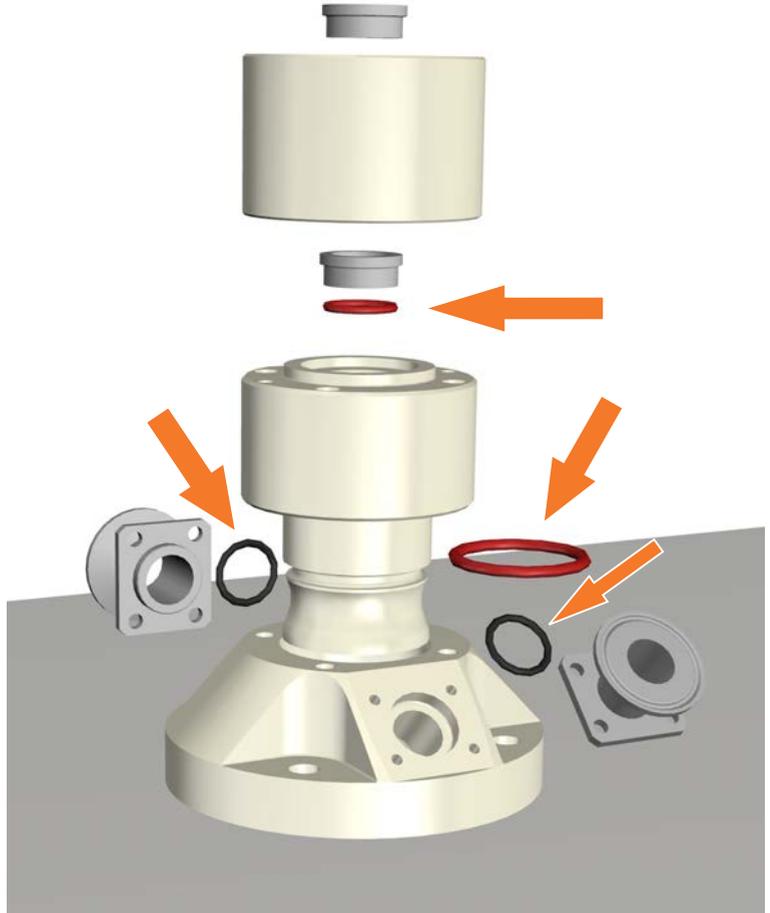
- | | |
|---|---|
| 5 | Remove the four bolts holding the mounting plate. |
|---|---|



- | | |
|---|--|
| 6 | Lift and remove the mounting plate assembly. |
|---|--|



Step	Action
7	Remove the screws, disassemble the remaining nozzle parts, and remove the O-rings, as illustrated below, from the tube.



- 8 Exchange the nozzle O-rings using the assembly drawing (found in the documentation package) as a guide.



NOTICE

Visually inspect all O-rings for signs of handling damage before mounting.

6 Maintenance

6.5 Maintenance procedures

6.5.2 Remove automatic nozzles and exchange O-rings

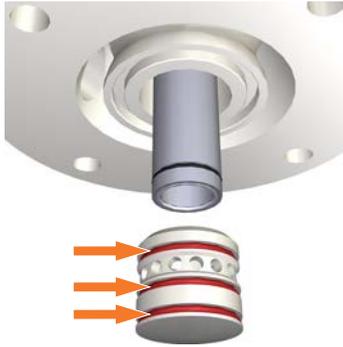
Step	Action
------	--------



NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

- 9 Exchange the three seals on the nozzle tip.



- 10 Reassemble the nozzle in the reverse order of steps.

6.5.3 Remove manual nozzles and exchange O-rings

Introduction

Nozzles in a Chromaflow™ column are either automatic or manual. This section describes how to remove a manual nozzle from a Chromaflow™ column, how to exchange O-rings and how to refit the nozzle in the column again. There is an option of fitting a pre-assembled new nozzle tip unit to the Chromaflow™ nozzle. These can be ordered separately. Refer to the Spare Part List, or cytiva.com for further information.

Note: *When performing maintenance on nozzles it is recommended to use a table with an approximately 40 mm diameter hole or to use supports with a central gap of 40 mm (for example two support blocks that the nozzle is balanced on). This will allow you to place the nozzle assembly upright.*

Nozzle O-ring tool (option)

The replacement of nozzle tip O-rings can be performed by either:

- fitting a pre-assembled new nozzle tip unit to the Chromaflow™ nozzle, or
- removing and replacing the existing nozzle tip O-rings.

If the second method is chosen, an optional tool is available for expanding and fitting the new nozzle tip O-rings into place.



Remove a manual nozzle



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

6 Maintenance

6.5 Maintenance procedures

6.5.3 Remove manual nozzles and exchange O-rings

Step	Action
------	--------

- | | |
|---|---|
| 1 | Set the nozzle you are servicing to RUN . |
| 2 | Loosen the 4 bolts attaching the nozzle to the adapter. |



- | | |
|---|--|
| 3 | Carefully pull the nozzle straight up and out. |
|---|--|

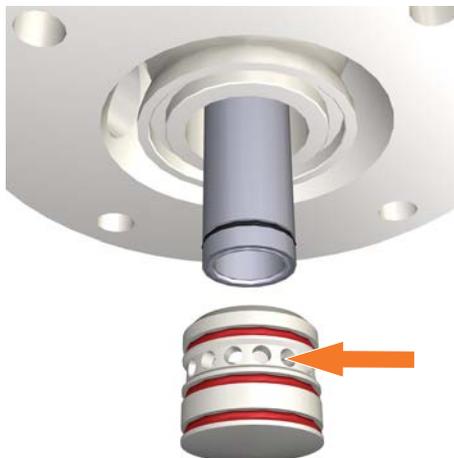


Exchange manual nozzle O-rings

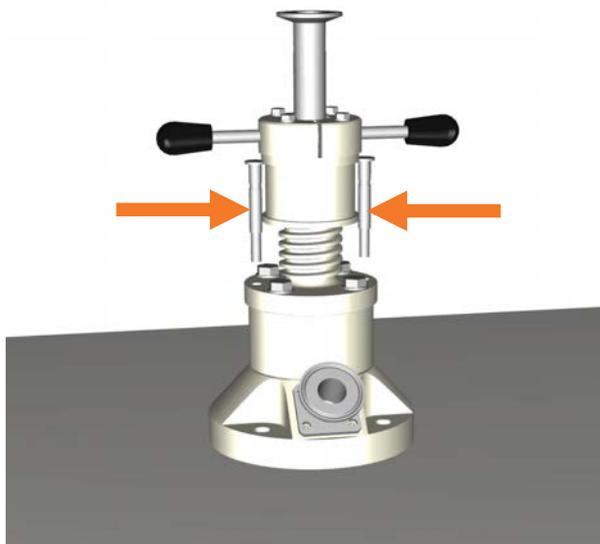
Follow the procedure below to exchange nozzle O-rings on manual nozzles.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Unscrew the nozzle tip from the nozzle tube. |
|---|--|



- | | |
|---|---|
| 2 | Place the nozzle assembly in the upright position on a table with a hole or on supporting blocks. |
| 3 | Remove the nozzle posts (see below) and the 4 nozzle bolts and washers that secure the nozzle assembly and lift the assembly straight up. |



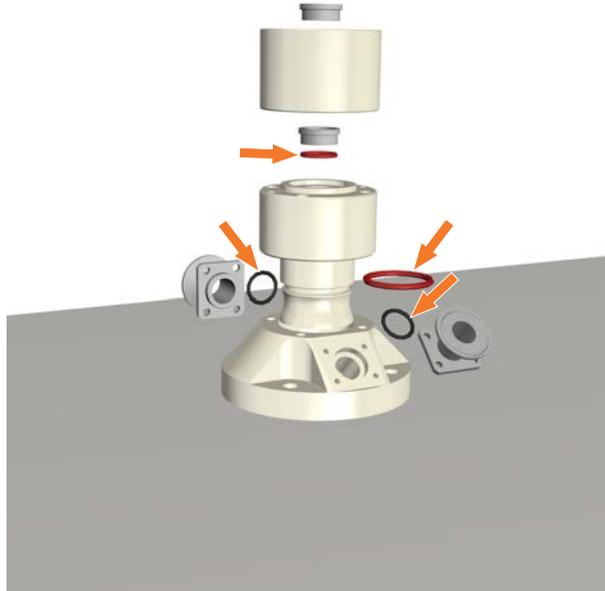
6 Maintenance

6.5 Maintenance procedures

6.5.3 Remove manual nozzles and exchange O-rings

Step	Action
------	--------

- | | |
|---|---|
| 4 | Disassemble the remaining nozzle parts and remove the O-rings, as illustrated below, from the tube. |
|---|---|



- | | |
|---|---|
| 5 | Exchange the nozzle O-rings using the assembly drawing (found in the documentation package) as a guide. |
|---|---|

**NOTICE**

Visually inspect all O-rings for signs of handling damage before mounting.

**NOTICE**

Lubricate all O-rings and seals thoroughly with 20% ethanol.

Step	Action
------	--------

6	Exchange the three seals on the nozzle tip.
---	---



7	Reassemble the nozzle in the reverse order of steps.
---	--

6.5.4 Exchange adapter O-rings

Introduction

This section describes the general procedure for exchanging the adapter O-rings on Chromaflow™ columns.

Procedure

Follow the instructions below to exchange the adapter O-rings.

Step	Action
1	Remove the adapter as described in Section 6.5.1 Remove the adapter, on page 116 .
2	Use a plastic spatula and pry both the O-rings from their grooves and remove them.
	<div style="border: 1px solid black; padding: 10px;">NOTICE Do not to use a knife or other sharp-edged object as these may damage column components.</div>
3	Inspect the O-ring grooves for damage and dirt. Exchange damaged or dirty O-rings. Clean and undamaged O-rings can be refitted.
4	Lubricate the O-rings with 20% ethanol and place them in their grooves. Make sure that the O-rings are not twisted.
	<div style="border: 1px solid black; padding: 10px;">NOTICE Twisted or deformed O-rings will cause leakage.</div>

6.5.5 Exchange plastic bed supports

Introduction

The bed support of Chromaflow™ columns may be made of plastic or stainless steel. This section describes how to exchange plastic bed supports.



CAUTION

Be careful when handling the distribution assembly as it is heavy.



CAUTION

When working with 20% v/v 1-propanol solutions follow these instructions:

- Work in a well-ventilated area.
- Wear appropriate personal protective equipment.
- Keep storage containers tightly closed.



NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

Wet the new bed support

Note: *It is recommended to start to wet new plastic bed supports the day ahead of the exchange.*

Step	Action
1	Carefully open a 20 to 30 cm hole in the plastic bag containing the new bed support.
2	Pour 1-propanol on the new bed support inside the plastic bag until it covers the bed support.
3	Make sure the liquid is evenly spread over both sides of the bed support, then seal the the plastic bag.
4	Leave the bag for at least 2 hours, but preferably overnight.

Remove adapter distributor



NOTICE

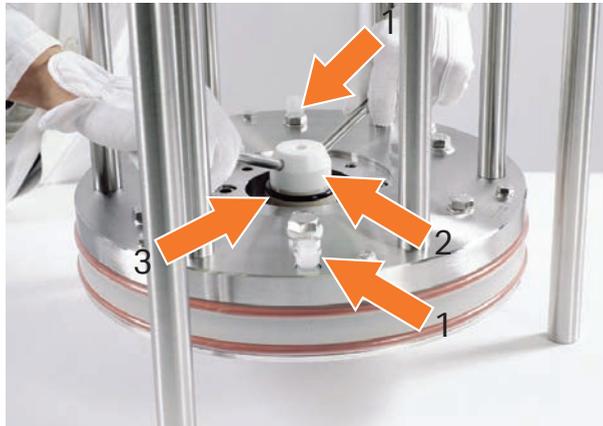
At least two people are required to perform this procedure.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Remove the adapter as described, see Section 6.5.1 Remove the adapter, on page 116 .
2	Remove the nozzle as described, see Section 6.5.2 Remove automatic nozzles and exchange O-rings, on page 122 or Section 6.5.3 Remove manual nozzles and exchange O-rings, on page 129 .
3	Remove the flush extension tubes (1), then remove the bed retainer nut (2) and the distance ring (3).



Tip:

Use the nut fixation tool provided in the bed support spare and accessory kit to remove the bed retainer nut. This will prevent the nut from rotating.

Step	Action
4	Remove the bolts and washers holding the bed support to the distributor plate. When removing the last bolts, make sure that the bed support is not dropped.
5	Carefully remove the adapter distributor and place it upside down on a clean surface.

Note:

On Chromaflow™ columns 800 and 1000, position the adapter over a suitable flat surface that will support the bed support when it is lowered.



NOTICE

Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.

Remove and exchange bed support

For this procedure it is recommended to be at least two people.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Make sure the bed supports are properly wetted according to the instructions in Wet the new bed support, on page 135 .

6 Maintenance

6.5 Maintenance procedures

6.5.5 Exchange plastic bed supports

Step	Action
------	--------

- | | |
|---|---|
| 2 | On Chromaflow™ column 800 and 1000 sizes, the plastic bed supports are held in with additional bed support nuts. Remove the bed support nuts. |
|---|---|



- | | |
|---|--|
| 3 | With one person holding down the distributor, the other person can firmly remove the current bed support by placing a hand in the center hole and pulling upwards. |
| 4 | To insert the new bed support, bend the bed support on one edge and fit it into the distributor, working slowly around until you meet stiff resistance. |



- | | |
|---|---|
| 5 | One person presses down on the portion of the bed support that is not seated, while the other person pulls up on the center of the bed support. The unseated portion of the bed support should then slide into place. |
|---|---|

Step	Action
6	On Chromaflow™ column 800 and 1000 sizes, make sure to align the holes for the bed support nuts. Then refit the bed support nuts using three or four turns at this stage. Finally, tighten the bed support nuts to a torque of 1.8 Nm.



Refit the adapter distributor

For this procedure it is recommended to be at least two people.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Refit the adapter O-rings.
2	Lift the adapter up and fit the bolts by hand to seat the adapter into place.

Step	Action
------	--------

- | | |
|---|---|
| 3 | Center the distributor plate to the backing plate by placing the distance ring and the two flushing extension tubes in place. |
|---|---|



- | | |
|---|--|
| 4 | Use a torque wrench to tighten the inner bolts, working around the backing plate tightening diametrically opposite pairs of bolts sequentially. Gradually tighten the bolts in increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached. |
| 5 | Remove the distance ring. |
| 6 | Use a torque wrench to tighten the outer bolts, using the same technique as for inner bolts. Refer to the AD for recommended torque settings. |



NOTICE

Always recheck bolt tensions after the column has been pressurized and retighten to recommended bolt torque settings when necessary.

- | | |
|---|--|
| 7 | If applicable, refit the bed retainer screw. |
| 8 | Fit new O-rings to the bed retainer nut. |



NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

Step	Action
------	--------



- | | |
|----|---|
| 9 | Use the bed retainer nut tool to screw in the bed retainer nut until it bottoms-out in the middle of the bed support. |
| 10 | Fit new O-rings to the distance ring and push the ring down into the adapter backing plate. |
| 11 | Refit the nozzle assembly. |
-

The adapter is now ready to put back into the column.

6.5.6 Exchange the adapter stainless steel bed support

Introduction

This section describes how to exchange stainless steel bed supports on Chromaflow™ columns. Note that stainless steel bed supports can be cleaned and reused. This instruction is suitable for both new or cleaned stainless steel bed supports.



CAUTION

Be careful when handling the distribution assembly as it is heavy.



NOTICE

Handle stainless steel parts with care and use only stainless steel tools. Using inappropriate tools will cause damage to surfaces and may lead to leakage and corrosion.



NOTICE

Do not use sharp metallic tools that may scratch the stainless steel surface. If scratched, the stainless steel should be re-passivated prior to use. Contact Cytiva for additional information



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Tools required

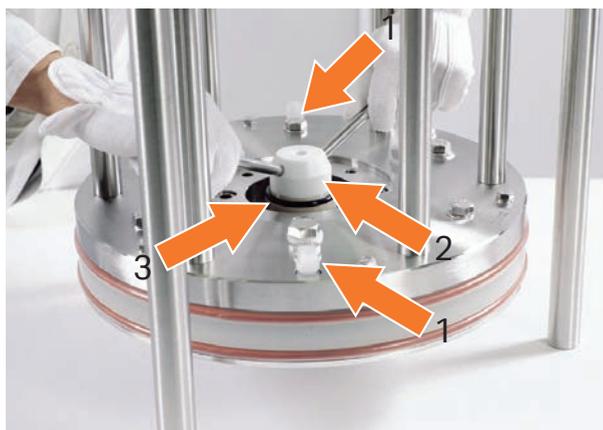
The following tools are required to replace stainless steel bed supports on Chromaflow™ columns:

- 13 mm open torque wrench (calibrated)
- Bed support retainer nut fixation tool
- Table or other suitable clean surface

Procedure

Follow the instructions below to exchange the adapter stainless steel bed support.

Step	Action
1	Remove the adapter as described in Section 6.5.1 Remove the adapter, on page 116 .
2	Remove the nozzle as described in Section 6.5.2 Remove automatic nozzles and exchange O-rings, on page 122 or Section 6.5.3 Remove manual nozzles and exchange O-rings, on page 129 .
3	Remove the flushing port extension tubes (1), then remove the bed retainer nut (2) and the distance ring (3).



Tip:

Use the bed support retainer nut fixation tool provided in the bed support spare and accessory kit to remove the bed retainer nut. This will prevent the nut from rotating.

- 4 Remove the bolts and washers holding the bed support to the distributor plate. When removing the last bolts, make sure that the bed support cannot drop.
- 5 Carefully remove the adapter distributor and place it upside down on a clean, protective surface.

Note:

On Chromaflow™ column 800 and 1000, position the adapter over a suitable flat surface that will support the bed support when it is lowered.



NOTICE

Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.

6 Maintenance

6.5 Maintenance procedures

6.5.6 Exchange the adapter stainless steel bed support

Step	Action
6	Exchange the O-rings listed in the Spare Parts List in the documentation package provided with the column.
7	Attach an O-ring to the welded bed retaining screw, then attach the new bed support unit.
8	Lift the bed support assembly into place and screw in the bolts and washers by hand. <i>See Fit bed support retaining screws, on page 145 for the detailed procedure of mounting the bed support retaining screws.</i>



9	Using a stainless steel torque wrench, tighten diametrically opposite bolts in ¼-turn increments to the recommended torque setting specified on the assembly drawing (AD) for the column.
10	Use the nut fixation tool to screw in the bed retainer nut.
11	Fit new O-rings to the distance ring and push the ring down into the adapter backing plate.
12	Refit the nozzle assembly.

The bed support assembly is now ready to put back into the column.

Fit bed support retaining screws

The following tools are required to mount the bed support retaining screws to Chromaflow™ columns with stainless steel bed supports.

- PTFE tape
- Stainless steel 13 mm open torque wrench (calibrated)
- Stainless steel ring spanner or socket spanner for M8 bolts

Follow the instructions below to mount and tighten the adapter unit bed support retaining screws when fitting steel bed supports to Chromaflow™ columns.

Step	Action
1	Wrap the threads of each of the bed support retaining screws (M8 bolts) with PTFE tape, as shown in the illustration.

Note:

Make sure that the tape extends far enough up the bolt so that there is PTFE tape between all metal-to-metal contact surfaces of the bolt when it is in place.



2	Fit the new bed support assembly to the adapter backing plate, being careful to align the screw holes.
---	--

6 Maintenance

6.5 Maintenance procedures

6.5.6 Exchange the adapter stainless steel bed support

Step	Action
------	--------

- | | |
|---|--|
| 3 | Mount the bed support retaining screws, washers and nuts, into the bed support retaining screw holes, as shown in the illustration, and finger tighten the screws. |
|---|--|



Step	Action
------	--------

- | | |
|---|---|
| 4 | Screw down the nut on each of the bed support retaining screws. |
|---|---|



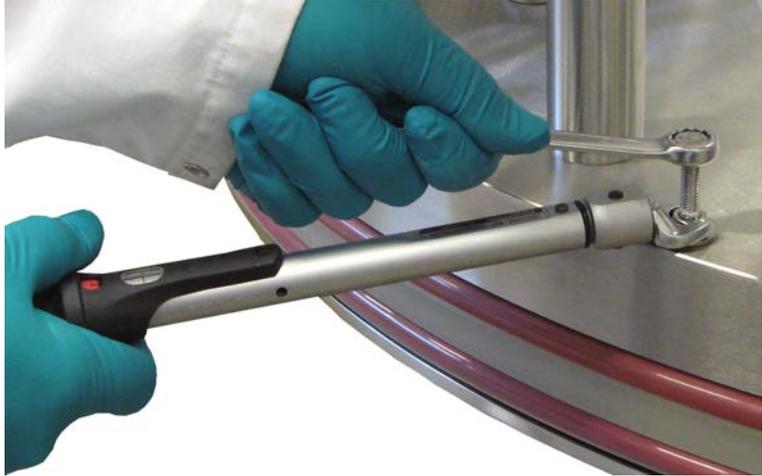
6 Maintenance

6.5 Maintenance procedures

6.5.6 Exchange the adapter stainless steel bed support

Step	Action
-------------	---------------

- | | |
|---|--|
| 5 | Hold the top of the bed support retaining screw in place with a ring spanner or socket spanner, and then slowly tighten the nut on each of the screws using a calibrated open torque wrench, as shown in the illustration. |
|---|--|



- | | |
|---|---|
| 6 | Work around the adapter crosswise and gradually tighten diametrically opposite pairs of screws in turn to the recommended torque setting specified on the assembly drawing (AD) for the column. |
| 7 | Remove the excess PTFE tape from the thread of the bed support retaining screw, until no tape can be seen on the screw thread above the nut. |

6.5.7 Exchange the bottom unit O-rings and plastic bed support

Introduction

This section describes how to replace the bottom unit O-rings on Chromaflow™ columns with plastic bed supports.



CAUTION

Be careful when handling the distribution assembly as it is heavy.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.



NOTICE

Extreme care must be taken to protect the bottom surface of the column tube, as this is a sealing area and must be kept clean and scratch free.

Procedure

Note: *Make sure there is PTFE tape on the threads of all bolts before tightening. This will stop the threads from binding.*

Step	Action
1	Remove the adapter as described Section 6.5.1 Remove the adapter, on page 116 .
2	Empty the column of water or buffer.
3	Remove the bolts and washers on the top column flange. Then remove the top column flange.
4	Remove the tie bars. Then carefully remove the column tube and place it on a soft, clean surface.

6 Maintenance

6.5 Maintenance procedures

6.5.7 Exchange the bottom unit O-rings and plastic bed support

Step	Action
------	--------

5	Remove the nozzle and, if applicable, the bed support nuts.
---	---

Note:

Bed support nuts are only fitted on Chromaflow™ columns 800 and 1000.

6	Using the special tool, remove the bed retainer nut and then remove the distance ring.
---	--

7	Remove the bed support, then remove the O-ring in the distributor and replace with a new O-ring.
---	--



NOTICE

Lubricate O-rings thoroughly with 20% ethanol.

8	Place a new bed support assembly on the bottom distributor.
---	---

Note:

On Chromaflow™ columns, also refit the bed support nuts.

9	Screw in the bed support retaining nut and then the distance ring.
---	--

Note:

On Chromaflow™ columns 800 and 1000, also tighten the bed support nuts with a torque wrench to the recommended torque setting specified on the assembly drawing (AD) for the column.

10	Refit the nozzle assembly.
----	----------------------------



NOTICE

Make sure the bottom of the tube is clean and flawless.

11	Carefully lower the column tube onto the bed support assembly, centering it as best as possible.
----	--

12	Refit the tie bars and their associated bolts and washers.
----	--

13	Very carefully position the column flange onto the tie bars and refit the bolts and washers. Do not tighten at this point. Make sure that the column tube and the column flange are properly aligned.
----	---

Step	Action
14	Begin by tightening the bolts on the bottom of the tie bars by hand. Then use a stainless steel torque wrench to slowly tighten all the bolts in turn, turning diametrically opposite bolts in ¼-turn increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached.
15	Once the bolts at the bottom of the tie bars are secure continue with the top bolts of the tie bars. Use the same procedure as for the bottom bolts.

6.5.8 Exchange the bottom unit O-rings and stainless steel bed support

Introduction

This section explains what to do when performing these maintenance procedures on Chromaflow™ columns with stainless steel bed supports.



CAUTION

Be careful when handling the distribution assembly as it is heavy.



NOTICE

Extreme care must be taken to protect the bottom surface of the column tube, as this is a sealing area and must be kept clean and scratch free.



NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Remove the stainless steel bed support

Step	Action
1	Remove the adapter according to the appropriate instructions for your Chromaflow™ column.
2	Empty the column of water or buffer.
3	Remove the bolts and washers on the top column flange, then remove the flange.
4	Remove the tie bars and then carefully remove the column tube and place it on a soft, clean surface.
5	Remove the bottom nozzle.

Step	Action
6	Use the bed support retainer nut fixation tool to remove the bed retainer nut and then remove the distance ring.
7	Unscrew and remove the bed support retaining screws holding the bed support in place, then carefully lift the bed support assembly and set it aside on a clean surface.

**Note:**

Chromaflow™ columns 800 and 1000 are fitted with threaded holes on the top outer surface of the bed support ring to allow eye bolts to be screwed in, to simplify the lifting procedure.

**NOTICE**

Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.

Remove O-rings and fit new or cleaned bed support

Note: *Make sure there is PTFE tape on the threads of all bolts before tightening. This will stop the threads from binding.*

6 Maintenance

6.5 Maintenance procedures

6.5.8 Exchange the bottom unit O-rings and stainless steel bed support

Step	Action
------	--------

- | | |
|---|---|
| 1 | Remove the O-ring from the bottom distributor plate and exchange it for a new, lubricated O-ring. |
|---|---|



NOTICE

Lubricate O-rings thoroughly with 20% ethanol.

- | | |
|---|--|
| 2 | Place a new O-ring on the bed retainer screw. |
| 3 | Place a new (or a cleaned) bed support assembly on the adapter backing plate, being careful to align the screw holes with the ring. Screw in the bed support retaining screws. |
| 4 | Exchange all O-rings on the bed support retainer nut and the distance ring, then refit the bed retainer nut (using the nut fixation tool) and the distance ring. |
| 5 | Refit the nozzle assembly.
On Chromaflow™ columns 800 and 1000, and columns equipped with automatic nozzles, reconnect the air supply lines and any necessary pipe work. |
| 6 | Refit the column tube O-ring, ensuring that it is not twisted. |



NOTICE

Make sure the bottom of the tube is clean and flawless.

- | | |
|---|--|
| 7 | Carefully lower the column tube onto the bed support assembly, centering it as much as possible. |
|---|--|

Step	Action
8	Refit the tie bars and their associated nuts and washers. Do not tighten.
9	Very carefully position the column flange onto the tie bars and replace the associated bolts and washers. Do not tighten.
10	Beginning with the bolts on the bottom of the tie bars, use a torque wrench and slowly tighten all of the bolts in turn. Work sequentially around the column, tightening diametrically opposite pairs of bolts in 1/4-turn increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached.
11	Once the bottom bolts are secure, tighten the bolts at the top of the tie bars.

6.5.9 Refit the adapter

Introduction

This section describes how to refit the adapter after completing service and maintenance procedures.



NOTICE

Refitting the adapter assembly into the column requires the column to be filled with liquid.



NOTICE

Make sure that the adapter is horizontal and perpendicular to the tube. Take great care that the steel bed support edge does not contact the acrylic tube.

Procedure

Step	Action
1	If the column is not filled with water, fill it to approximately 5 cm of the top of the column tube.
2	Lubricate the adapter O-rings and/or column walls with 20% ethanol.
3	Lift the adapter using the slings and hoist as described in Section 6.5.1 Remove the adapter, on page 116 .
4	Remove the three legs on which the adapter stood and, using the hoist, carefully move the adapter to the column tube.
5	Lower the adapter very carefully over the tie bars and into the column tube.
6	On Chromaflow™ column 800 and 1000, the top nozzle may have been removed when removing the adapter. To replace the top nozzle, first lower the adapter into the column until the first seal is engaged with the column wall and then support the weight of the adapter on nuts and washers from the underside of the adapter flange. Remove the hoist and fit the nozzle. At this point, do not fit any air lines or extra piping to the top nozzle. Open MPT .
7	Make sure the nozzles are set to RUN during assembly.

Step	Action
8	Refit all the clamp nuts, and washers onto the locking rods. <div data-bbox="435 323 1204 496" style="border: 1px solid black; padding: 10px;"><p>NOTICE Do not fully tighten the extender rod nuts until the extender rods have been fitted to the adapter.</p></div>
9	Tighten down each in turn, diametrically opposite, until the first adapter O-ring is engaged. To ensure alignment, measure the gap between the column and adapter flanges at diametrically opposite points. Adjust the nuts and handles accordingly until the gap is uniform. Use a steel rule or vernier caliper.
10	On Chromaflow™ column 800 and 1000, and columns with automatic nozzles, reconnect any air supply lines and external piping.
11	Follow the instructions for moving the adapter down that are appropriate for your type of Chromaflow™ column.

When maintenance procedures have been completed and the adapter refitted a leakage test should be performed to validate the integrity of the column. See [Section 5.2.7 Perform a leakage test, on page 85](#) for instructions.

Following successful completion of the hydrostatic pressure test the column is fully assembled and ready to be prepared as described in [Section 5.2 Prepare the column, on page 55](#).

6.6 Storage

Introduction

This section describes how to store Chromaflow™ columns and the preparation necessary for storage of columns that have previously been used in production and for continued storage of new Chromaflow™ columns.

The instructions vary depending on the type of bed support. Plastic bed supports need to be adequately wetted throughout the procedures.



NOTICE

The column must be unpacked from the crate and washed as soon as possible after delivery, preferably within 2 months. Storage temperature must be 4°C to 24°C.

Storage conditions

The following conditions shall be maintained while the column is in storage:

- Store the column indoors in a dust-free environment with a well-controlled climate.
- Maintain the storage area at a constant temperature, between 4°C to 24°C.
- Make sure the relative humidity is between 0% to 95%, non-condensing (preferably low humidity).
- Keep air humidity and air temperature differences as low as possible to prevent condensation and corrosion.
- Acrylic tube columns must be protected from sunlight, and other sources of UV light.
- Do not expose the column to chlorides.
- Change the storage solution regularly, at least once every three months.



WARNING

If the column is equipped with caster wheels, the wheel brakes must be activated at all times during operation or storage.

Before storage

The following procedure describes how to prepare the column for long-term storage. The procedure provides that the column is set up with associated equipment as instructed in [Section 4.4 Set up associated equipment, on page 44](#).

Step	Action
1	Clean the column according to your established cleaning procedure (for example using 1.0 M NaOH).
	<div style="border: 1px solid black; padding: 10px;">  <p>NOTICE Make sure that the column is free of Cl residue before storage. Otherwise there is a risk of corrosion of applicable steel components (column tube and bed supports).</p> </div>
2	Prime the column with a suitable storage solution, for example 18% EtOH samt 2% IPA or 0.01 M NaOH. Refer to Section 8.2 Chemical resistance, on page 176 for more information.
3	Move both nozzles to RUN .
4	Rinse and disconnect tubing.
5	Fit blind caps to nozzle port inlets and outlets.

Prepare a column without resin for storage

Follow this procedure to prepare a column without packed resin for storage.

	<div style="border: 1px solid black; padding: 10px;">  <p>NOTICE Chlorides and low pH can cause corrosion of stainless steel. Rinse thoroughly with clean water after use.</p> </div>
--	---

Step	Action
1	Clean the column according to your established cleaning procedure (for example using 1.0 M NaOH). Refer to Section 8.2 Chemical resistance, on page 176 for more information.
2	Prime the column with a suitable storage solution (for example 18% EtOH and 2% Isopropanol or 0.01 M NaOH).
3	Open the pathway to SIT .
4	Make sure that top and bottom nozzles are set to RUN .
5	Start the packing pump to clean the tubing pathways to SIT via SOT and to slurry.

Step	Action
6	Disconnect the tubing and fit blind caps to nozzle port inlets and outlets.
7	Exchange the liquid in the chamber between the adapter seals by priming the flushing ports. Refer to Prime the flushing ports, on page 70 for details.

Prepare a new column for further storage

A new column can be stored as delivered for up to three months in recommended storage conditions. After this period, or earlier if the recommended storage conditions cannot be met, the storage solution needs replacing if the column shall be stored longer.

When replacing storage solution, it is important to use a procedure that will prevent air from being introduced into the bed supports, especially in columns with plastic bed supports.



WARNING

Hazardous substances and biological agents. When using hazardous chemical and biological agents, 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 this product.



WARNING

For personal safety during the transportation, installation, operation, maintenance and service of columns, use protective glasses and other personal protective equipment appropriate for the current application at all times. The following personal protective equipment should always be available:

- Protective glasses
- Working gloves to protect against sharp edges
- Protective footwear, preferably with steel toe-cap
- Disposable gloves

Always use clean disposable gloves when manually handling parts.



NOTICE

Do not strain nozzle connections and be careful when connecting piping. Failure to do so may result in damage.

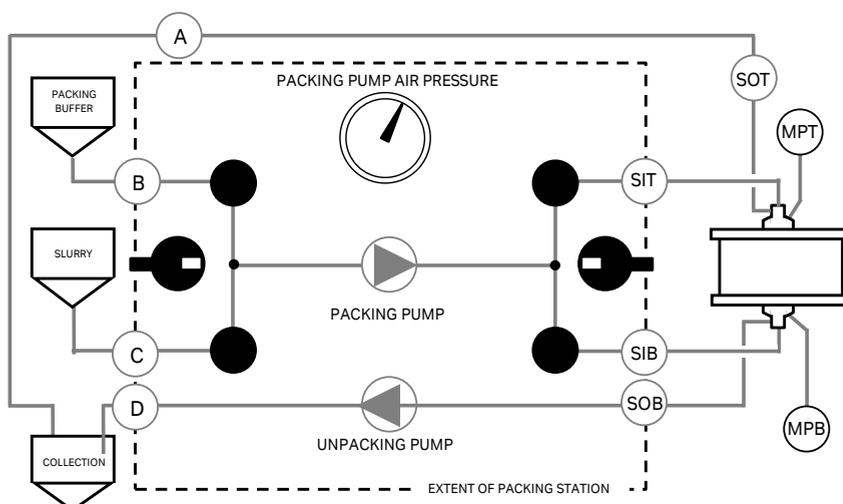


NOTICE

It is recommended to exchange the storage solution regularly, at least every 3 months.

Prerequisites

- The column is equipped with isolating valves on **MPT** and **MPB** and a pressure gauge on the **MPT** before the isolating valve.
- The column is connected to a packing station or similar equipment. Refer to [Section 5.2.2 Connect a Chromaflow™ Packing Station to the column, on page 60](#) for details.
- The column is connected to a vessel via pathway B containing suitable storage solution (see the illustration below).
- The column is collected to a collection vessel via pathway D (see the illustration below).
- The tubing A is not submerged in the collection vessel.



Empty slurry lines of air

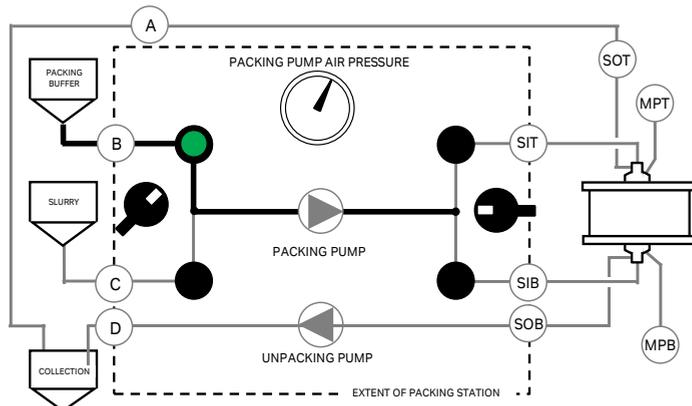


NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

Follow this procedure to first empty the slurry lines of air.

Step	Action
1	Close the MPT and MPB ports.
2	Set both top and bottom nozzles to RUN .
3	Open the pathway to SIT .
4	Start the packing pump. Pump until liquid is flowing out through SOT . <i>Result:</i> Liquid is pumped through the top nozzle and back to the collection vessel and removes any air in the lines.
5	Stop the pump and open the pathway to SIB .
6	Start the packing pump. Pump until liquid is flowing out through SOB . <i>Result:</i> Liquid is pumped through the bottom nozzle and back to the collection vessel and removes any air in the lines.
7	Close the pathway to the column. On the packing station this is done by switching the SIT/SIB control to neutral (the mid position). See the illustration below.



Step	Action
8	Stop the pump.

Prepare a column with packed resin for storage

A chromatography system should be used to fill a packed column with storage solution to provide low enough flow rate not to disturb the packed bed. Refer to the equipment's user documentation for instructions.



NOTICE

Chlorides and low pH can cause corrosion of stainless steel. Rinse thoroughly with clean water after use.

Replace storage solution

Follow this procedure to replace the storage solution. Make sure the packing buffer vessel contains the relevant replacement solution.



WARNING

There should **NEVER** be air or gas under pressure in the column.



CAUTION

The storage solution may evaporate over time when the column is stored. This may create an increase in pressure in the column. Ensure that all valves are closed before removing the TC-blind caps from the bottom slurry inlet and carefully open the valve to release the pressure.



NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

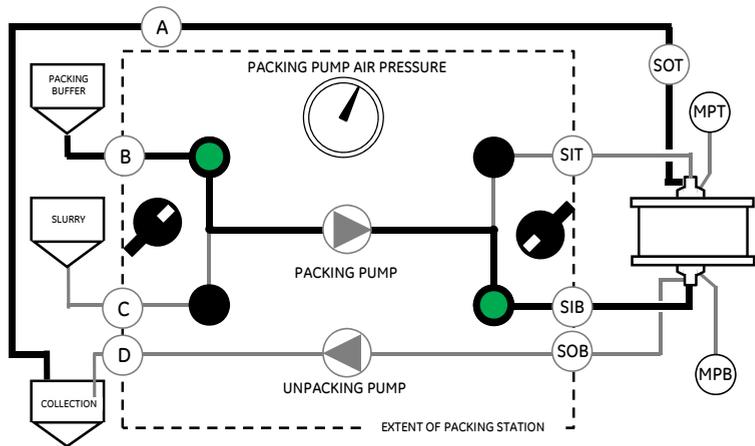


NOTICE

Make sure that the end of the tube connection **A** remains above the level of the liquid in the collection tank at all times.

Step Action

- 1 Make sure the **MPT** and **MPB** ports are closed.
- 2 Set the top and bottom nozzle to **UNPACK**.
- 3 Start the unpacking pump carefully and start to empty the column of liquid via **SOB** into the collection tank.
When the column is empty, stop the unpacking pump.
- 4 Set the bottom nozzle to **PACK**.
- 5 Open the pathway **B** from the packing buffer and open the pathway to **SIB**.



- 6 Start the packing pump. Pump until the column is filled and fluid is flowing back into the vessel, then stop the pump.
Result:
Liquid is pumped through the bottom nozzle (upflow) until all air is evacuated from the column via **SOT**.
- 7 Set the top nozzle to **RUN**.
- 8 Start the packing pump.

Step	Action
9	Allow the packing pump to build up 1 bar g pressure.
	<div style="display: flex; align-items: center;">  <div> <p>NOTICE</p> <p>Make sure to carefully monitor the pressure on the pressure gauge.</p> </div> </div>
10	Quickly open the MPT to drain, then close it again. <i>Result:</i> This quick release of pressure and fast flow through the bed support will purge the the air via the column top nozzle.
11	Repeat the previous two steps (steps 9 to 10) at least three times or until no more air can be noticed in the outlet when the MPT port is opened.
12	Allow the packing pump to build up 1 bar g pressure again.
13	Quickly open the MPB port to drain, then close it again. <i>Result:</i> This quick release of pressure and fast flow through the bed support will purge the air via the column bottom nozzle.
14	Repeat the previous two steps 12 to 13 at least three times or until no more air can be noticed in the outlet when the MPT port is opened.
15	Stop the packing pump and set both top and bottom nozzles to RUN . <i>Result:</i> The column is sealed.
16	Proceed to rinse the slurry lines.

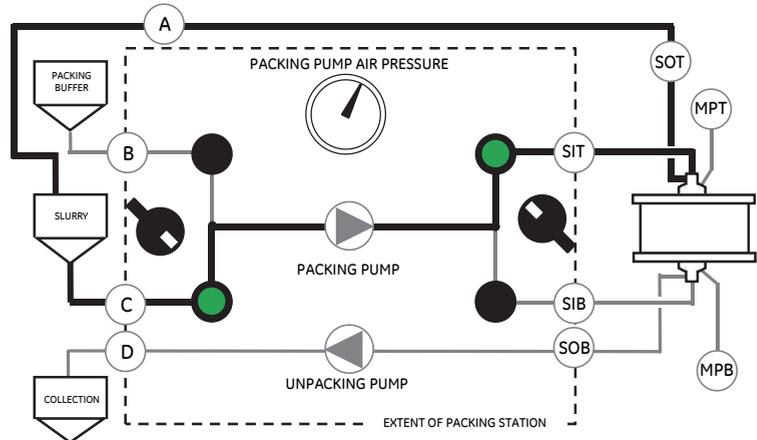
Rinse slurry lines

Follow the instructions to rinse the slurry lines and exchange the fluid in the flushing ports and adapter seal.

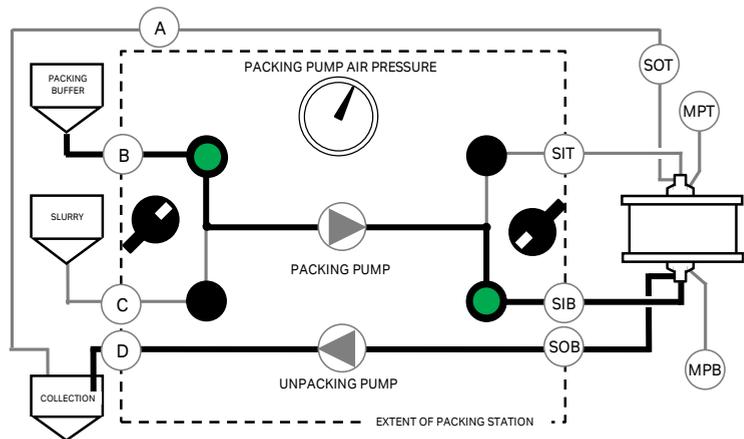
Step	Action
1	Open the pathway to a vessel containing water (in the illustration below this is the vessel labeled PACKING BUFFER), which will allow the tubing and piping to be cleaned from resin.

Step Action

- 2 Open the pathway to **SIT** to clean the tubing to and from the top nozzle.



- 3 Start the packing pump and pump until the tubing is clean.
4 Open the pathway to **SIB**, to clean the tubing to and from the bottom nozzle.



- 5 When the tubing is thoroughly rinsed, stop the packing pump.
6 Remove the tubing to the column and fit blind caps on the nozzle ports.
7 Exchange the liquid in the flushing ports and adapter seals. For instructions see [Prime the flushing ports, on page 70](#).

The column is prepared for further storage.

7 Troubleshooting

About this chapter

This chapter provides information to assist users and service personnel to identify and correct problems that may occur when operating Chromaflow™ columns.

If a problem persists or cannot be resolved using the suggested actions in this guide, contact your Cytiva representative for advice.

High back pressure

Symptom	Possible cause	Suggested action
High back pressure	Pressure gauge is faulty	Check that the pressure gauge is functioning properly.
	Closed valves between the pump and collection vessel	Make sure that the valves between the pump and collection vessel are open.
	Dirty valves or internal blockage	Inspect the valves and clear any blockages.
	Incorrect flow rate selected	Adjust the flow rate.
	Blocked or clogged bed supports, or the presence of residual resin	<ul style="list-style-type: none"> • Clean or replace the bed support. • Check the resin quality.
	Trapped air in the bed support	Always wet/prime the bed supports before use.
	Equipment in use, either before or after the column, generating back pressure.	Inspect the equipment and make the necessary adjustments.
	Internal diameter of tubing is too small.	Change to larger tubing.
	The collection vessels are at a higher level than the pump.	Adjust the relative heights.
	The resin has been packed to a greater compression factor than recommended.	Evaluate whether a repack is necessary.
There is tightly bound sample material in the chromatography resin.	Perform CIP according to the procedure established for the resin in use.	

Leakage

Symptom	Possible cause	Suggested action
Leakage at the pump	The connections to and from the pump are not correct or properly tightened.	Inspect the equipment and make the necessary adjustments.
Leakage at the column tube	The adapter is not properly positioned with respect to the column tube.	Check that the adapter is properly positioned and adjust if required.
Resin leakage	There is media in the buffer return line. Possible damage to the bed support, nozzle tip, bed support fastening apparatus, or seals.	<ul style="list-style-type: none"> Inspect nozzle, column seals, and O-ring for damage. Replace if wear is detected. Make sure there is no gap between the O-ring and the bed support or between the O-ring and the column tube. Make sure that pressure tests have been performed. Typically, a pressure test should be performed in an empty column, after maintenance, and before packing the column. (For more information see Section 6.4 Maintenance schedule, on page 113 and Service frequency, on page 108.)
Leakage at distributor plate	Damage to the seal or O-ring The natural characteristic of polypropylene is to revert back into the original shape that it had prior to molding into sheet form. This can result in shrinkage over time. The rapidity of shrinkage increases with chemical exposure and temperature cycling.	<p>Inspect distributor seals for damage.</p> <p>Inspect the nozzle seals and bed support for damage.</p> <p>Make sure a tight seal is formed around the distributor plate. Possible solution is to change the O-ring or distributor plate.</p>

Symptom	Possible cause	Suggested action
Continuous leakage	An O-ring or seal does not form a tight sealing surface.	<ul style="list-style-type: none"> Regularly inspect O-rings and seals. Replace if wear is detected. Check that O-rings are not twisted or damaged. Refit or replace as necessary. Make sure that O-rings are thoroughly wetted when installed. Replace damaged parts with new parts. If no damaged parts are found, reinstallation may resolve the issue. Perform regular preventive maintenance, dependent on process needs. Contact your Cytiva service representatives for advice.

Chromatograms not as expected

Symptom	Possible cause	Suggested action
Chromatogram is not as expected	Detectors	Inspect the UV detectors.
	The linear flow rate is not as intended.	Change the linear flow rate.
	The adapter is not positioned correctly in close contact with the bed.	The column needs to be repacked.
	Back-mixing has occurred in the column set-up (e.g. the sample has passed through an air trap).	By-pass the air trap during sample load.
	There is a gap between the packed bed and the bed support.	This may be due to the flow rate during the run being higher than the packing flow rate, the column may be too loosely packed, or the run pressure may be too high.

Symptom	Possible cause	Suggested action
	The column has been incorrectly equilibrated.	Inspect the equipment and make the necessary adjustments.
	There has been a change in how the sample has been handled prior to its application to the column (e.g. there are sample batch variations).	Any such changes should be analyzed for their effect on the chromatogram.
	In the process, changes have been made to one of the following: nets, separation resin, time intervals between different operations, buffer constituents, precipitation procedures, chromatographic system hardware.	Any such changes should be analyzed for their effect on the chromatogram.
	All buffers are not compliant with the defined specifications below. <ul style="list-style-type: none"> • Dilution • Filtration • Age • Temperature • Preparation of the sample buffers 	Evaluate and make the necessary adjustments.
	The bed support is clogged.	Clean or replace the bed supports.
	The resin is damaged	Test the resin and take the necessary action.
	The packed bed is cracked or is contaminated with foreign materials. Note: <i>Smaller cracks, less than 5% of the bed height, may be present without affecting the packing performance.</i>	Clean and repack the column.

Air trapped in the column

Symptom	Possible cause	Suggested action
Air is trapped in the column	The buffer contains air in it.	Make sure that the buffers are prepared correctly to eliminate air in the buffers.
	The buffers and the columns are at different temperatures.	Equilibrate the temperature before use.
	The connections are not fitted together tightly.	Adjust the connections and perform new priming if needed.
	The valves are not tightly shut.	Adjust the valves and perform new priming if needed.
	If using an air trap, the air trap volume is not adequate.	Evaluate and make the necessary adjustments.

Removing small amounts of air from the column

Note: *In general, if air has entered the column then the column should be re-packed and primed. If only a small amount of air has been trapped in the column, however, the air can be removed.*

Follow the instructions below to remove small amounts of air from the column.

Step	Action
1	Reverse the flow direction.
2	Recycle the liquid and monitor the UV signal. If air is coming out of the column, the UV signal will be erratic. It will stabilize when the air is removed.
3	Test the column again before use.

Realigning the adapter

The most common factor contributing to the adapter moving out of alignment is insufficient lubrication of the O-rings. Follow the instructions below if the adapter is not level.

Step	Action
1	Stop the packing pump or inflow of water.
2	Lubricate the O-rings.

Step	Action
3	Check to see if anything other than the seals are preventing movement, or if the adapter has tilted out of alignment.
4	Measure adapter dis-alignment. Adjust upper adaptor nuts, compensating for any dis-alignment. (For more information see Level the adapter, on page 77.)
5	Pump water into the column, allowing for the adaptor to move upwards, back into alignment.
6	Start the pump and watch the adapter as it straightens itself out. Open sufficient flow to create back-flow with pump.

Note: *Adapter misalignment of a few mm is acceptable and will normally even itself out.*

Bed supports

This section outlines troubleshooting for issues related of the bed supports.

Symptom	Possible cause	Corrective action
The plastic bed support released from the distributor.	The bed support is blocked or partially blocked. This can result in an increased pressure behind the bed support and increase the load on the bed support.	Exchange the bed support.
	The bed support is subjected to high unsupported flow. A higher flow means a higher pressure drop across the bed support and therefore an increased load.	
	The column is badly or loosely packed. This will manifest itself as a gap between the packed bed and the bed support results in unsupported flow.	

Symptom	Possible cause	Corrective action
	Bed support not wetted with corrective action to follow instructions for the wetting	
	Air in column with corrective action to prime the column	
Serious deformation or breakage occurred at the center of the stainless bed support.	The bed support is subjected to rapid pressure or flow changes. This pulsation causes flexing of the stainless steel bed supports and the structure will eventually fail.	Replace the stainless bed support
	Bed support clogged or in some way blocked.	

8 Reference information

About this chapter

This chapter lists the technical specifications of Chromaflow™ 400-1000 columns. More detailed information and specifications for individual columns can be found in the documentation package provided with your column. The chapter also includes a chemical resistance guide, recycling information, regulatory information and ordering information, and Health and Safety Declaration form for service.

In this chapter

Section	See page
8.1 Specifications	175
8.2 Chemical resistance	176
8.3 Recycling information	181
8.4 Regulatory information	182
8.5 Health and Safety Declaration Form	186

8.1 Specifications

Introduction

This section contains a short summary of general technical specifications, requirements and storage conditions for Chromaflow™ columns. Note that the specifications for individual columns may differ. Specific information for each column can be found in the documentation package provided with your column.

General technical specifications

Parameter	Specification
Dimensions	See documentation package
Net weight	See documentation package
Compressed air requirements	N/A
Air consumption	N/A
Operating temperature range	4°C to 30°C
Safety standards	See Section 8.4 Regulatory information, on page 182
Design pressure	See documentation package
Test pressure (ASME)	1.3 × design pressure
Test pressure (PED)	1.43 × design pressure
Maximum pressure	See documentation package
Maximum altitude	2000 m
Storage temperature range	4°C to 24°C

8.2 Chemical resistance

Introduction

This section gives some general guidelines concerning chemical resistance for Chromaflow™ components. Regarding exposure to solutions not covered by these guidelines, contact your Cytiva representative for recommendations.

**NOTICE**

Make sure that any water used with the column is particulate-free (down to 1 µm). Particles can block and damage the bed supports.

**NOTICE**

Only use chemicals listed in the Chemical Resistance information. The wetted parts of the product may be damaged by chemicals not listed in the Chemical Resistance information. Contact your Cytiva representative before using chemicals that are not listed.

Resistance to buffers

Chromaflow™ columns are resistant to chemical agents used in protein recovery, including buffer solutions for adsorption, elution and washing, and to solutions effective in cleaning, sanitization and storage.

Resistance to sodium chloride

Depending on configuration, Chromaflow™ columns can be equipped with wetted components of stainless steel and must therefore be appropriately maintained when exposed to sodium chloride (NaCl) during chromatographic processes.

Stainless steel bed supports have a high degree of resistance to corrosion but may corrode under certain conditions, such as extended exposure to NaCl. The degree of susceptibility varies according to conditions of use. Low pH in combination with NaCl, high temperatures and damage to the steel surface all increase the risk of corrosion.

If a stainless steel surface is damaged (scratches, impact marks etc.) it must be cleaned and passivated before being taken into operation. Refer to *Application Note 28943377 Handling of stainless steel column parts in sensitive environments*. Your Cytiva representative can provide guidance on suitable cleaning and passivation procedures if required.

It is recommended that a water rinse of at least five column volumes is used to remove NaCl from stainless steel surfaces. Water is the preferred rinse solution due to the solubility properties of sodium chloride in water compared to other possible rinsing solutions (such as ethanol solutions).

Columns should be stored with solutions free from chloride ions.

Handling of stainless steel columns

For further information refer to Application note *Handling of stainless steel column parts in sensitive environments (28943377)*.

Chemicals to avoid

When using Chromaflow™ columns it is recommended that the following chemicals are avoided:

- Extreme oxidizers
- Fluorine and halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters
- Aromatic hydrocarbons (such as toluene)
- Alcohols at concentrations higher than those specified in the table below
- Chloride solutions in combination with pH below 4. Always wash the column with at least five column volumes of a pH-neutral solution (water) between and after use of chloride solutions and low pH buffers.



NOTICE

Ethanol >20% v/v and other chemicals not recommended in the chemical resistance table may cause cracks in the acrylic materials of Chromaflow™ columns and must not be used.

Chemical resistance guidelines



WARNING

Risk assessment! Perform a risk assessment for any risks due to the process or process environment. Evaluate the effects the use of the equipment and operational processes have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.



WARNING

Some of the chemicals used with Chromaflow™ columns may be flammable under certain conditions. Make sure to use chemicals only under conditions where they are not flammable. Refer to local and/or national classifications of flammable liquids.

**CAUTION**

Do not use chemicals with temperatures above the specified limits.

**NOTICE**

Do not use chemicals harmful to the column.

**NOTICE**

Chlorides and low pH can cause corrosion on stainless steel. Rinse thoroughly with clean water after use.

Inspect the column regularly for signs of corrosive attacks, which may cause column damage if untreated. Note that the stainless steel bed supports are especially vulnerable to corrosion.

Note: *The following table presents a summary of information obtained from several published sources, not from individual tests on column components. It should be used only as a guide. The effect of chemicals will generally be more severe at higher temperatures. Note also that the combined effects of agents have not been taken into account in this table. The applicable chemical resistance depends on the configuration of the column and the associated materials of construction.*

The recommended temperature range for the use of each of the chemicals listed below is 4°C to 30°C, unless otherwise specified.

Chemical	Concentration ¹	Time/cycle restrictions	Comments	Temperature limits ²	CAS no. ³
1-propanol		16 h	For wetting plastic bed supports		71-23-8
Acetic acid	25%	3 h	Cleaning-In-Place (CIP)	2°C to 30°C	64-19-7
Acetone	2%	1 h	Efficiency test	2°C to 30°C	67-64-1
Ammonium sulfate	2 M ⁴	5 h	Adsorption	2°C to 30°C	7783-20-2
Benzyl alcohol	2.0% (w/w) ⁵	12 months	Storage	2°C to 24°C	100-51-6

Chemical	Concentration ¹	Time/cycle restrictions	Comments	Temperature limits ²	CAS no. ³
Ethanol	20%	12 months and max. 0.5 bar	Storage	2°C to 24°C	64-17-5
Ethanol/ acetic acid	20%/10%	3 h	CIP	2°C to 30°C	64-17-5/6 4-19-7
Guanidine hydrochloride	6 M ⁶	5 h	CIP	2°C to 30°C	50-01-1
Hydrochloric acid ⁷	0.1 M (pH = 1)	1 h	CIP	2°C to 30°C	7647-01-0
Isopropanol	30%	1 h	CIP	2°C to 30°C	67-63-0
Phosphoric acid	5%	8 h	For passivation of stainless steel bed supports	2°C to 30°C	7664-38-2
1-Propanol	20%	16 h	Wetting plastic bed supports	2°C to 30°C	71-23-8
Sodium chloride	0 to 3 M ⁸	3 h	Purification, CIP	2°C to 30°C	7647-14-5
Sodium hydroxide	1 M (pH = 14)	24 h, room temp. to 30°C	CIP	2°C to 30°C	1310-73-2
Sodium hydroxide	0.01 to 0.1 M (pH = 12 to 13)	12 months	Storage	2°C to 30°C	1310-73-2
Sodium hydroxide/ ethanol	1 M/ 20%	3 h	CIP	2°C to 30°C	1310-73-2/ 64-17-5
Sodium sulphate	1 M ⁴	3 h	Adsorption	2°C to 30°C	7757-82-6
Urea	8 M ⁴	5 h	Purification, CIP	2°C to 30°C	57-13-6
Aqueous buffers commonly used in chromatography	10 to 250 mM, pH 2 to 10	24 h	Equilibration, adsorption, elution	2°C to 30°C	

¹ When a concentration is given as a percentage, this is v/v, except in the case of benzyl alcohol, see table note 5.

² Do not exceed the operating temperature specifications for the individual column size.

³ CAS no.: Registration number assigned by the Chemical Abstract Services (CAS), American Chemical Society.

⁴ pH in these solutions depends on the pH of the buffer, which can vary between 3 and 13.

⁵ Take care when preparing solutions for both accuracy and allowing sufficient time for the benzyl alcohol to fully dissolve.

⁶ Not for use with columns containing wetted components of stainless steel.

8 Reference information

8.2 Chemical resistance

⁷ pH below 4 for stainless steel is not recommended.

⁸ For columns containing wetted stainless steel components max 1.0 M NaCl is recommended. For additional important information, see [Resistance to sodium chloride, on page 176](#).

8.3 Recycling information

Introduction

This section contains information about the decommissioning of the product.



CAUTION

Always use appropriate personal protective equipment when decommissioning the equipment.

Decontamination

The product must be decontaminated before decommissioning. All local regulations must be followed with regard to scrapping of the equipment.

Disposal of the product

When taking the product out of service, the different materials must be separated and recycled according to national and local environmental regulations.

8.4 Regulatory information

Introduction

This section lists the regulations and standards that apply to the product.

In this section

Section	See page
8.4.1 Contact information	183
8.4.2 European Union and European Economic Area	184
8.4.3 Other regulations and standards	185

8.4.1 Contact information

Contact information for support

To find local contact information for support and sending troubleshooting reports, visit [cytiva.com/contact](https://www.cytiva.com/contact).

Manufacturing information

The table below summarizes the required manufacturing information.

Requirement	Information
Name and address of manufacturer	Cytiva Sweden AB Björkgatan 30 SE 751 84 Uppsala Sweden
Telephone number of manufacturer	+ 46 771 400 600

8 Reference information

8.4 Regulatory information

8.4.2 European Union and European Economic Area

8.4.2 European Union and European Economic Area

Introduction

This section describes regulatory information for the European Union and European Economic Area that applies to the equipment.

Conformity with EU Directives

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards.

A copy of the EU Declaration of Conformity is available on request.

A copy of the EU Declaration of Conformity is included in the documentation package.

Directive	Title
2006/42/EC	Machinery Directive (MD)

Chromaflow™ columns also fulfill the requirements of, but do not fall under and are therefore not marked according to, the European Pressure Equipment Directive (PED) 2014/68/EU.

CE marking



The CE marking and the corresponding EU Declaration of Conformity is valid for the instrument when it is:

- used according to the *Operating Instructions* or user manuals, and
- used in the same state as it was delivered, except for alterations described in the *Operating Instructions* or user manuals.

8.4.3 Other regulations and standards

Introduction

This section describes the standards that apply to the product.

Biological and chemical compatibility

The wetted parts of the product meet the material requirements of the following standards and regulations:

Requirement	Description
USP <88> Class VI	Material requirements from United States Pharmacopeia. Biological Reactivity Tests, "In Vivo".
EMA/410/01	Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products. Animal origin-free material.
21 CFR 177	Indirect food additives: Polymers

Machinery standards

Standard requirements fulfilled by this product are summarized in the table below.

Standard	Description
EN ISO 12100	Safety of machinery. General principles for design. Risk assessment and risk reduction.

Additional design considerations

For additional design considerations, such as ASME-BPE, GMP and cGMPs information refer to the product-specific specification information provided in the documentation package delivered with each equipment.

8 Reference information

8.5 Health and Safety Declaration Form

8.5 Health and Safety Declaration Form

On site service



On Site Service Health & Safety Declaration Form

Service Ticket #:	
--------------------------	--

To make the mutual protection and safety of Cytiva service personnel and our customers, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts a repair. To avoid delays in the servicing of your equipment, complete this checklist and present it to the Service Engineer upon arrival. Equipment and/or work areas not sufficiently cleaned, accessible and safe for an engineer may lead to delays in servicing the equipment and could be subject to additional charges.

Yes	No	Review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below.
<input type="radio"/>	<input type="radio"/>	Instrument has been cleaned of hazardous substances. Rinse tubing or piping, wipe down scanner surfaces, or otherwise make sure removal of any dangerous residue. Make sure the area around the instrument is clean. If radioactivity has been used, perform a wipe test or other suitable survey.
<input type="radio"/>	<input type="radio"/>	Adequate space and clearance is provided to allow safe access for instrument service, repair or installation. In some cases this may require customer to move equipment from normal operating location prior to Cytiva arrival.
<input type="radio"/>	<input type="radio"/>	Consumables, such as columns or gels, have been removed or isolated from the instrument and from any area that may impede access to the instrument.
<input type="radio"/>	<input type="radio"/>	All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.
Provide explanation for any "No" answers here:		
Equipment type / Product No:		Serial No:
I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.		
Name:		Company or institution:
Position or job title:		Date (YYYY/MM/DD):
Signed:		

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

© 2020 Cytiva.

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact.
28980026 AD 04/2020

Product return or servicing



Health & Safety Declaration Form for Product Return or Servicing

Return authorization number:		<i>and/or</i> Service Ticket/Request:	
-------------------------------------	--	---	--

To make sure the mutual protection and safety of Cytiva personnel, our customers, transportation personnel and our environment, all equipment must be clean and free of any hazardous contaminants before shipping to Cytiva. To avoid delays in the processing of your equipment, complete this checklist and include it with your return.

1. Note that items will NOT be accepted for servicing or return without this form
2. Equipment which is not sufficiently cleaned prior to return to Cytiva may lead to delays in servicing the equipment and could be subject to additional charges
3. Visible contamination will be assumed hazardous and additional cleaning and decontamination charges will be applied

Yes	No	Specify if the equipment has been in contact with any of the following:	
<input type="radio"/>	<input type="radio"/>	Radioactivity (specify)	
<input type="radio"/>	<input type="radio"/>	Infectious or hazardous biological substances (specify)	
<input type="radio"/>	<input type="radio"/>	Other Hazardous Chemicals (specify)	
Equipment must be decontaminated prior to service / return. Provide a telephone number where Cytiva can contact you for additional information concerning the system / equipment.			
Telephone No:			
Liquid and/or gas in equipment is:		<input type="checkbox"/>	Water
		<input type="checkbox"/>	Ethanol
		<input type="checkbox"/>	None, empty
		<input type="checkbox"/>	Argon, Helium, Nitrogen
		<input type="checkbox"/>	Liquid Nitrogen
		<input type="checkbox"/>	Other, specify
Equipment type / Product No:			Serial No:
I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.			
Name:		Company or institution:	
Position or job title:		Date (YYYY/MM/DD)	
Signed:			

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

© 2020 Cytiva.

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact.
28980027 AD 04/2020

To receive a return authorization number or service number, call local technical support or customer service.

Index

A

Adapter, *64, 70, 76–78, 81, 116, 136, 139, 156, 171*
 leveling, *77*
 leveling downwards, *78*
 leveling upwards, *81*
 lubrication, *76*
 out of alignment, *171*
 priming flushing ports, *70*
 refit distributor, *139*
 refitting, *156*
 removal, *116*
 remove distributor, *136*
 safety valves, *64*

Air trapped in column, *171*

Ambient environment, *38*

Associated documentation, *7*

ATEX directive, *20*

Automatic nozzle, *122*
 removal, *122*

B

Bed support, *135, 136, 142, 149, 152, 172*
 plastic, *135, 149*
 replacing plastic bed supports, *136*
 stainless steel, *142, 152*
 troubleshooting, *172*

Bed support retaining screws, *145*
 fitting procedure, *145*

Biohazardous substances, *110*

C

CE, *184*
 marking, *184*

CE conformity, *184*

Chromaflo™ Packing Station, *60, 63*
 connecting to tanks, *63*
 pneumatic connections, *60*

Chromatograms not as expected, *169*

Clean empty column, *105*

Cleaning, *109–112*
 agents, *110*
 CIP (refer to system documentation), *109*
 rinsing after cleaning, *111*
 stainless steel bed supports, *112*
 when to clean, *109*

Cleaning-in-place, CIP, *67*

Column, *27*
 options, *27*

Component documentation, *7*

Connecting, *62, 63*
 column to Chromaflo™ Packing Station, *62*
 column to vessels, *63*
 vessels to Chromaflo™ Packing Station, *63*

Connection diagrams, *85*
 column leakage test, *85*

D

Documentation, *7*
 system specific documentation, *7*

E

Emergency shutdown, *24*

Empty column before use, *65*

Empty column of slurry, *103*

Explosive atmosphere, *20*

H

Hazardous location, *20*

HETP, *98*

High back pressure, *167*

I

Important user information, *5*

Insert bed support, *138*

Intended use, *5*

L

- Labels, 21–23
 - column label, 21
 - safety labels, 22, 23
- Leakage, 168, 169
- Leakage test, 85
- Level the column, 57
- Loosen packed bed, 100

M

- Maintenance, 113
 - schedule, 113
- Manual nozzle, 129
 - removal, 129
- Manufacturing information, 183
- Moving the adapter, 78, 81
 - downwards, 78
 - upward, 81
- MPB, 31
- MPT, 31

N

- Notes and tips, 6
- Nozzle, 29–32
 - assembled on column, 31
 - inlet, 29
 - mobile phase port, 30
 - outlet, 29
 - parts, 29
 - positions, 32
 - principles of operation, 32
- Nozzle (automatic), 123, 125
 - removal, 123
 - replace O-rings, 125
- Nozzle (manual), 130, 131
 - Removal, 130
 - Replace O-rings, 131
- Nozzle function, 33, 34
- Nozzle illustration, 32
 - in pack position, 32
 - in run position, 32
 - unpack position, 32
- Nozzle O-ring tool, 122, 129

O

- O-rings, 134
 - replacement, 134
- On site service, 186

- Operation, 52
 - work flow example, 52

P

- Packing, 34, 94
 - procedure, 94
- Plastic bed support, 149
 - exchanging bottom unit O-rings, 149
- Plastic bed supports, 135–137, 139
 - exchanging, 135
 - refit adapter distributor, 139
 - removal and exchange, 137
 - remove adapter distributor, 136
 - wetting, 135
- Position the adapter, 78
- Preparation, 55
 - before packing, 55
- Preparing the adapter, 76
- Pressure sensor, 64
- Priming and purging, 73
- Product return or servicing, 187
- Purpose of this manual, 6

R

- Recommended buffer, 72
- Recycling information, 181
 - decontamination, 181
- Regulatory information, 182
- Remove automatic nozzle, 122
- Removing the adapter, 117
- Resin supply, 44
- Restart after emergency shut-down, 24
- Rinse slurry lines, 97, 165
- Rinse the column, 69
- Running, 35
 - the chromatographic phase, 35

S

- Safety assessment, 10
- Safety labels, 22, 23
- Safety notices, 9
- Safety valves, 64
 - isolation valves, 64
 - pressure sensor, 64

Service agreements, 108
 Service and preventive maintenance, 106, 108
 Service frequency, 108
 Setup, 27, 45, 46, 49
 example, 27
 grounding, 49
 packing, 45
 pressure relief, 49
 running, 46
 SIB, 31
 SIT, 31
 Slurry, 91, 92
 calculation, 91
 Priming top inlet, 92
 Slurry preparation, 89
 bed volume table, 89
 formula, 89
 Slurry preparations, 90, 91
 compression factor, 90
 concentrations, 90
 final check, 91
 flow rates, 90
 procedure, 91
 SOB, 31
 SOT, 31
 Space and floor load, 38
 Stainless steel, 176
 resistance to sodium chloride, 176
 Stainless steel bed support, 152
 exchanging bottom unit O-rings, 152
 Stainless steel bed supports, 112, 142
 cleaning, 112
 exchanging, 142
 Stainless steel columns, 177
 special handling, 177
 Standards, 185
 Storage, 158–160, 163
 columns packed with resin, 163
 columns without packed resin, 159
 conditions, 158
 new column preparations, 160
 procedures before storage, 158
 replace storage solution, 163

T

Transport in crate, 39
 Troubleshooting, 167–169, 171, 172
 adapter out of alignment, 171
 air trapped in column, 171
 bed supports, 172
 chromatograms not as expected, 169
 high back pressure, 167
 leakage, 168
 Typographical conventions, 6

U

Uncrating, 42
 Uncrating procedure, 42
 Unpacking, 35, 43
 User documentation, 7

W

Wetting plastic bed support, 73

Page intentionally left blank



cytiva.com

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

Chromaflow is a trademark of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

All other third-party trademarks are the property of their respective owners.

© 2020–2021 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit [cytiva.com/contact](https://www.cytiva.com/contact)

28962232 AE V:14 05/2021