

BPG columns

Operating Instructions

Original instructions





Table of Contents

1	Intr				
	1.1	Important	t user information	5	
	1.2	About this	s manual	6	
	1.3	Abbreviat	ions	7	
2	Safe	etv instruc	ctions	8	
	2.1	-	ecautions		
	2.2		d symbols		
	2.3		cy procedures		
3	Colu	ımn descr	riptions	17	
	3.1				
	3.2		140 and 200 columns		
	3.3		column		
	3.4		column		
	3.5		nd adapter		
	3.6		ies		
4	Inst	allation		30	
	4.1		rements		
	4.2	•			
	4.3	•	g		
	4.4	•	yel the column		
	4.5		g kit		
	4.6	,	9 11		
	4.7		acking extension tube		
5	Ope	ration		44	
	5.1		on		
	5.2	•	paration		
	5.3		acking		
	0.0	5.3.1	Preparing the column		
		5.3.2	Preparing the adapter		
		5.3.3	Column flow packing method		
		5.3.4	Packing the column - settling phase	65	
		5.3.5	Packing the column - compression phase	71	
		5.3.6	Storage and unpacking of packed columns		
	5.4	Performa	nce evaluation of the column	. 77	
6	Mai	ntenance		80	
	6.1	Service ar	nd preventive maintenance	82	
	6.2	Disassem	bling column	84	
	6.3	Replacements of nets and O-rings			
	6.4	Assembling column 1			
	6.5	Disassemble and assemble the adapter 1			
	6.6	Leakage t	est	119	
	6.7	Cleaning		122	

	6.8	Storage		124
7	Troub	oleshootii	ng	126
8	Refer			129
	8.1	Specification	ons	130
		8.1.1	BPG 100	132
		8.1.2	BPG 140	133
		8.1.3	BPG 200	134
		8.1.4	BPG 300	135
		8.1.5	BPG 450	
	8.2		esistance	
	8.3	Recycling in	nformation	140
	8.4	Regulatory	information	141
		8.4.1	Contact information	142
		8.4.2	European Union and European Economic Area	143
		8.4.3	Great Britain	144
		8.4.4	Other regulations and standards	145
Ind	ех			146

1 Introduction

About this chapter

This chapter contains important user information, descriptions of safety notices, and the intended use of BPG columns.

In this chapter

Section	on	See page
1.1	Important user information	5
1.2	About this manual	6
1.3	Abbreviations	7

1.1 Important user information

Introduction

This section contains important user information about the product and this manual.

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 can be exposed or expose others to hazards that can lead to personal injury and you can cause damage to the equipment.

Intended use of the product

BPG columns are intended for industrial applications that demand high standards of hygiene. BPG columns can be used with BioProcess™ Chromatography resin. The pressure specifications and low flow resistance make these columns suitable for use with BioProcess resin in ion exchange, hydrophobic interaction, affinity, and gel filtration chromatography.

BPG columns are intended for production use only and should not be used for diagnostic purposes in any clinical or in vitro procedures.

Prerequisites

In order to operate the equipment in the way it is intended, the following prerequisites must be fulfilled:

- You must have read and understood the safety instructions in the user documentation.
- You must have experience of working with pressure vessels.
- You must have knowledge of the entire system and process that the column is part
 of.
- All operations should be performed by qualified personnel who are adequately trained.

1.2 About this manual

Purpose of this manual

The *Operating Instructions* provide you with the information needed to install, operate, and maintain the product in a safe way.

Scope of this document

This document describes the basic components and principles of operation of BPG columns. Specific configuration information can be found in the product documentation provided with each column and on the column label.

Detailed information regarding process systems, resins and buffer tanks is not covered in this document.

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 proce-

dures.

Typographical conventions

The text on a graphical user interface is identified in this manual by **bold italic** text.

The text on the label of a hardware item is identified in this manual by **bold** text.

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

1.3 Abbreviations

Introduction

This section explains abbreviations that appear in the user documentation for BPG columns.

Abbreviations

The abbreviations used in this manual are defined as follows:

Abbreviation	Description	Translation
A _s	Peak asymmetry factor	Peak asymmetry factor
ВВ	Big Beads	Big Beads
CIP	Cleaning-in-place	Cleaning-in-place
CV	Column volume	Column volume
EPDM	Ethylenepropylenedienemo- nomer rubber	Ethylenepropylenedienemo- nomer rubber
FEP	Fluoroethenepropene	Fluoroethenepropene
FF	Fast Flow	Fast Flow
HETP	Height equivalent to a theoretical plate	Height equivalent to a theoretical plate
L	Bed height	Bed height
N	Number of theoretical plates	Number of theoretical plates
PA	Polyamide	Polyamide
PF	Pack factor	Pack factor
PEEK	Polyetheretherketone	Polyetheretherketone
PFR	Perfluor rubber	Perfluor rubber
PTFE	Polytetrafluoroethane	Polytetrafluoroethane
THF	Tetrahydrofurane	Tetrahydrofurane
UV	Ultra violet	Ultra violet
V _e	Elution volume	Elution volume
WFI	Water for injection	Water for injection
W _h	Peak width at half peak height	Peak width at half peak height

2 Safety instructions

About this chapter

This chapter describes safety precautions and the label attached to the equipment. In addition, the chapter describes emergency and recovery procedures.

In this chapter

Section	on	See page
2.1	Safety precautions	9
2.2	Labels and symbols	14
2.3	Emergency procedures	16

2.1 Safety precautions

Introduction

Before installing, operating, or maintaining the system, you must be aware of the hazards described in this manual.

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.

General precautions



WARNING

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



WARNING

All installation, maintenance, operation, and inspection must be carried out according to local regulations by adequately trained personnel.



WARNING

Accessories. Use only accessories supplied or recommended by Cytiva.



WARNING

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

Flammable liquids and explosive environment



WARNING

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



WARNING

Flammable liquids. This product is not approved for handling liquids under conditions when they can be flammable.



WARNING

Explosion hazard. When the product is operated or maintained in a hazardous location, it **must be correctly 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.

Personal protection



WARNING

For personal safety when handling columns, use 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



WARNING

Hazardous substances and biological agents When using hazardous chemical or biological agents, take all suitable protective measures, such as working in a well ventilated area, wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and national regulations for safe operation and maintenance of the column.

Installing and moving



WARNING

Unpacking and moving the delivery crates must be performed by trained personnel, in accordance with local regulations.



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

Heavy object. A minimum of two people and the appropriate lifting equipment for the task are required when moving or lifting the column.



WARNING

Do not unscrew the wheels too far. If the wheels are unscrewed completely, the column may fall and cause injury or damage.



WARNING

Make sure that the wheel support is fully engaged into the stand before starting to move the column.



CAUTION

Place the column on an even floor surface. After moving the column to its production location, lock the wheel brakes and level the column.



CAUTION

Make sure that the pressure relief valve is correctly adjusted and that connecting tubing has an unrestricted flow path. Connect the pressure relief valve outlet to waste.

Operation



WARNING

Max. column pressure. The working pressure of the column must never exceed the maximum pressure stated on the nameplate and in the documentation package, 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

There must **never** be air or gas under pressure in the column.



WARNING

Crush risk. Be careful when fitting the adapter to the column as there is a risk of crushing your hands.



CAUTION

Do not use chemicals at temperatures above the specified limits.

Maintenance



WARNING

Thoroughly check the glass column tube to make sure that it is not cracked or severely scratched. Pay extra attention to the end parts of the column tube. The pressure specifications are only valid if the glass tube is undamaged.



WARNING

Decontaminate before maintenance or service. Before performing any maintenance or service work on the column, make sure that the column has been emptied and decontaminated.



WARNING

For continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere, the user must test the piping system for leakage at maximum operating pressure.

- Always perform a leakage test after assembly or maintenance.
- Always perform a leakage test before operation or CIP.



CAUTION

After assembly or maintenance, make sure that the column is tight and not leaking.



CAUTION

Removing the top plate and adapter often causes spillages and splashing. Remove any spillage on the floor immediately to minimize the risk of accidents.

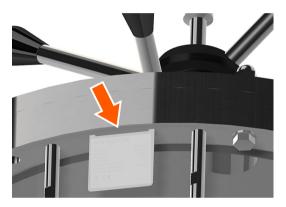
2.2 Labels and symbols

Introduction

This section describes the nameplate, labels, and other safety and regulatory labels and symbols.

Nameplate

The nameplate is located on the back of the equipment. The nameplate identifies the equipment and shows regulatory compliance, and warning symbols.



Description of symbols on the nameplate

The following symbols and text may be present on the nameplate:

Label text	Description
Code number	Code number identifying the column as a BPG column product.
Serial number	Serial number.
Mfg date	Year and month the instrument was produced (YYYY/MM).
Tare mass	Weight of the instrument when empty. (Tare mass is not always presented on the label.)
Operating temp. TS	Permitted operating temperature range.
Design pressure PS	Pressure the instrument has been designed to tolerate.
PED fluid group / cat.	Pressure Equipment Directive fluid group and category classification.

Label text	Description
\triangle	Warning! Read the user documentation before using the system.
CE	The equipment complies with applicable European directives. Applicable to BPG 200/950, 300/500, 300/750, 300/950, 450/500, 450/750 and 450/1000.
CA	The equipment complies with applicable European directives. Applicable to BPG 200/950, 300/500, 300/750, 300/950, 450/500, 450/750 and 450/1000.

2.3 Emergency procedures

Precautions



WARNING

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

Emergency shutdown

The operation of BPG columns is controlled by the equipment that is connected to the column. All operations are therefore stopped by stopping the operation of the connected equipment.

If the column is connected to a system, the procedure for the emergency shutdown of the system should be followed. This may involve pressing the **EMERGENCY STOP** button to stop the operation of the equipment controlling the pumping system connected to the column. Alternatively, the process system may require to be paused or shutdown. Follow the manufacturer's instructions for the system.

Restart

To restart after an emergency shutdown:

Step	Action
1	Make sure that the condition that was the root cause of the emergency stop has been corrected.
2	If an EMERGENCY STOP button was used to shutdown the equipment connected to the column, reset this before restart.
3	Reduce pump settings prior to reactivation to avoid immediate high pressure increases.
4	Restart the equipment connected to the column.

3 Column descriptions

About this chapter

This chapter contains a general description of BPG columns and illustrations of the BPG models.

In this chapter

Section		See page
3.1	Overview	18
3.2	BPG 100, 140 and 200 columns	21
3.3	BPG 300 column	23
3.4	BPG 450 column	25
3.5	Column and adapter	27
3.6	Accessories	28

3.1 Overview

Introduction

The BPG column can be used with a BioProcess system or as a stand-alone unit connected to a pump. There are five sizes of the BPG columns.



The BPG columns are listed in the table below with the internal diameters, specific design pressure and the various heights.

Characteristics	BPG 100	BPG 140	BPG 200	BPG 300	BPG 450
Internal diameter (mm)	100	140	200	296	446
Design pressure (bar)	8	6	6	4	2.5
Column height (mm)	500, 750 , 950	500,750, 950	500, 750 , 950	500, 750 , 950	500, 750, 1000

For detailed specifications of the BPG columns, refer to Section 8.1 Specifications, on page 130.

All BPG columns are equipped with wheels with brakes, except the BPG 100 column, for which wheels with brakes are an option.

Column material

The materials used in the BPG columns are compatible with the solvents most commonly used in chromatography separations of biomolecules, column maintenance and cleaning processes. All polymeric materials in contact with the process stream meet the requirements for USP class VI, described in USP <88> Biological Reactivity Tests, In Vivo.

Adapter and rods

The adapter top plate and the adapter height adjuster are bolted to the column flange. The adapter height adjuster nut is allowed to rotate on a bushing ring in the adapter top plate, altering the height of the adapter plate in the column tube.

The adapter O-ring forms a seal between the adapter and the glass column when it is compressed by the sealing unit. This is controlled by the seal adjuster knob.

The rods hold the flange and end-piece to the glass tube which is sealed by O-rings. The O-rings effectively isolate the glass tube from direct contact with the stainless steel components.

Nets

The column is delivered with 23 μ m (PolyPropylene) nets. For resin with an average particle diameter <70 μ m, change to 10 μ m (Polyamide), or 12 μ m (PEEK) in both adapter and end-piece. For SepharoseTM Big Beads, use 54 μ m nets, see *Recommended net porosities, on page 47*.

Connections

BPG columns can be connected to different types of tubing and systems. To protect the packed bed it is advisable to have isolation valves on both the inlets and outlets of the column.

The hygienic, sanitary clamp fittings on BPG columns permit cleaning-in-place. The outer diameter on all BPG clamp fittings is 25 mm. Refer to *General Specification* for more information.

Explosive atmosphere

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

If potentially explosive liquids are used with BPG columns, consult local authorities regarding local rules and regulations before installing or operating.

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

Definition of the zone according to the directive: Zone 2

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.

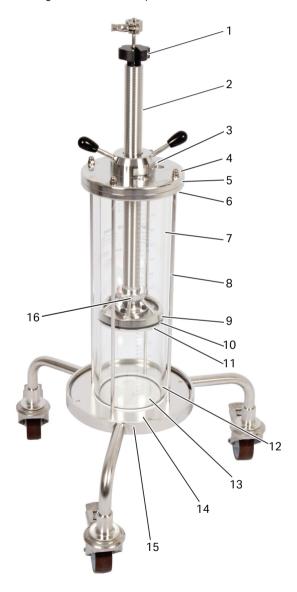
The column does not fall under the ATEX equipment directive, and as a result it is not marked with EX labels, but it fulfills the explosion safety requirements in the directive's harmonized standards, for example EN 13463-1 or EN ISO 80079-36.

This is valid for the BPG column in its standard execution and with a grounding cable, see *Grounding kit*, on page 29. Any additional accessory or equipment must carry EX-marking or be assessed to be in conformity with the EU ATEX workplace directive.

3.2 BPG 100, 140 and 200 columns

BPG 100-200 parts

The image below shows the parts of BPG columns 100, 140 or 200.



Part	Description
1	Seal adjuster
2	Adapter tube, outer
3	Adapter height adjuster
4	Domed nut
5	Adapter top plate
6	Flange
7	Column glass tube
8	Support rod
9	Adapter plate
10	O-ring adapter
11	Net (adapter)
12	O-ring bottom
13	Net (column)
14	End-piece
15	Stand
16	Adapter height adjuster

3.3 BPG 300 column

BPG 300 parts

The image below shows the parts of BPG 300 column.

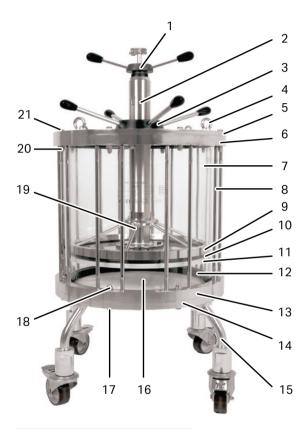


Part	Description
1	Seal adjuster
2	Adapter tube, outer
3	Adapter height adjuster
4	Lifting grip
5	Nut
6	Washer
7	Adapter top plate
8	Flange
9	Screw and washer
10	Column glass tube
11	Support rod
12	Adapter plate
13	O-ring adapter
14	Net (adapter)
15	O-ring bottom
16	Net (column)
17	End-piece
18	Stand
19	Adapter height adjuster

3.4 BPG 450 column

BPG 450 parts

The image below shows the parts of BPG 450 column.



Part	Description
1	Seal adjuster
2	Adapter tube, outer
3	Adapter height adjuster
4	Eyebolt
5	Adapter top plate
6	Upper flange
7	Column glass tube
8	Support rod

Part	Description	
9	Adapter plate	
10	O-ring adapter	
11	Net (adapter)	
12	O-ring bottom	
13	Bottom flange	
14	End-piece	
15	Stand	
16	Net (column)	
17	Screw and washer	
18	Nut and washer	
19	Adapter height adjuster	
20	Nut and washer	
21	Screw and washer	

3.5 Column and adapter

The image below shows an example of the column and adapter parts.



Part	Description
1	Column
2	Adapter

3.6 Accessories

Packing extension tube

Packing extension tubes are available for all BPG columns allowing larger amount of slurry and higher final bed heights. The packing tubes are made of glass for BPG 100 to 300, and of stainless steel for BPG 450. The illustration below shows two examples of extension tubes. For a complete list of spare parts and ordering information, refer to the spare parts list and the accessory list.



Stand

BPG 300 and 450 columns are delivered with a stand. For BPG 100 to 200 columns, the stand is available as an accessory.

Pressure relief valve

The pressure relief valve is connected between the pump and column inlet. It releases pressure if the calibrated value is exceeded. It is to be used if the column may exceed its design pressure and no other safety equipment is included in the chromatographic system to prevent this.

Top and bottom valves

Top and bottom valves are available as separate accessories. Refer to the accessory list for more information.

Air trap

The air trap kit includes the air trap, bracket, steel valves, clamps and gaskets.

For air traps for BPG 100, 140 and 200, tubing is included. A manually operated valve is recommended at the top of the air trap as an air outlet control.

Pressure gauge kit

The pressure gauge kit contains a pressure gauge, T-junction, and necessary clamps and gaskets for sanitary connections.

Grounding kit

To avoid discharge from static electricity, the column can be properly grounded (earthed). For this purpose, a suitable grounding kit is available as an accessory and a grounding connection terminal is provided. The equipment must be grounded when it is to be used in a potentially explosive atmosphere.

4 Installation

About this chapter

This chapter contains information about BPG installation, site requirements, unpacking, transport and how to set up the BPG columns for use.

In this chapter

Section		See page
4.1	Site requirements	31
4.2	Transport	32
4.3	Unpacking	33
4.4	How to level the column	35
4.5	Grounding kit	37
4.6	Setup	39
4.7	Fitting a packing extension tube	42

4.1 Site requirements

Space and floor load

For space and floor load requirements, refer to the dimensions and weight information in the product documentation provided with each column. Make sure the floor can handle the weight of the BPG columns at fully loaded conditions.

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

Ambient environment

The following should be avoided:

- · Direct sunlight
- Vibrations
- Corrosive gas
- Dust

4.2 Transport

Check the delivery

On receipt of the crate check to see if there is any apparent damage to the crate and the equipment. When unpacking BPG columns check that all equipment is enclosed in the transport crate according to the packing list.

If any damage is observed do not continue with the installation of the column, record this on the receiving documents. Contact your Cytiva representative for advice and further instruction

Transport in crate



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.

Use suitable lifting equipment with a minimum capacity to match the empty weight of the column plus the crate. If maneuvering is performed with a crane or hoist, keep lifting heights to the minimum necessary to make sure that there is sufficient clearance space to allow passage of the column when lifted from the floor. Refer to the product documentation for information about column weight and dimensions for each column.

Moving the column



WARNING

Make sure that the wheel support is fully engaged into the stand before starting to move the column.



WARNING

Do not unscrew the wheels too far. If the wheels are unscrewed completely, the column may fall and cause injury or damage.

The wheels should be fully engaged into stand before moving the column.

4.3 Unpacking

Tools required for crate unpacking

The following tools are recommended when removing larger BPG columns from the transport crate:

- Suitable lifting equipment, such as a crane or hoist
- Round slings or lifting chains with the capacity to safely lift the weight of the column
- Lifting eyebolts (included and used in BPG 450 column)
- Screwdriver
- Suitable wrench or ratchet wrench

Crate unpacking



WARNING

Unpacking and moving the delivery crates must be performed by trained personnel, in accordance with local regulations.

Unpack the BPG 300 and 450 columns by following the unpacking instructions attached to the outside of the transport crate. In the absence of unpacking instructions, unpack BPG column from the transport crate as follows:

Step	Action
1	Move the crate to a protected indoor location. Make sure that the transport crate is located on a level and even floor surface.
2	Remove the screws marked with black paint from the side panel of the transport crate, and carefully remove that side panel from the crate.
3	Remove the top panel from the crate.
4	Remove the enclosed accessories that are packed inside the crate.
5	Place the ramp that is provided inside the crate on the floor surface.
6	Secure the ramp tightly against the open side of the crate. Make sure there is a smooth transition between the bottom of the crate and the floor.
7	Release the column wheel brakes and carefully roll the column out of the crate and onto the floor.

Positioning the column



WARNING

Heavy object. A minimum of two people and the appropriate lifting equipment for the task are required when moving or lifting the column.

The BPG column can be rolled to the intended operational location on its own wheels, or the larger columns can be lifted into position using a crane or hoist.

Tip:

It is recommended to wrap PTFE tape around the threads of the eyebolts used with BPG column.

Cleaning column surfaces

Before beginning to prepare the column for operation it is recommended that any surface residues acquired during transport or removal of the column from the packing crate are removed using warm water and/or 20% ethanol.

4.4 How to level the column

Precautions



WARNING

Do not unscrew the wheels too far. If the wheels are unscrewed completely, the column may fall and cause injury or damage.



CAUTION

Place the column on an even floor surface. After moving the column to its production location, lock the wheel brakes and level the column.

Lock the wheels of BPG columns

After positioning the column in its operational location, lock the wheels as illustrated below.



Use the level adjusters

Before operation the column must be level. Use a spirit level to check if the column is level. Measure in two positions perpendicular to one another and repeat the measurements at several points around the exterior of the column.

If column is not level screw all wheels fully into the stand and then level on the correct wheel to avoid adjusting any wheel to far out.

- If the column must be raised, turn the column leg level adjustor clockwise.
- If the column must be lowered, turn the column leg level adjustor counter-clockwise.

Check if the column is level after adjusting each leg. Adjust the column legs in turn until the column is level.



4.5 Grounding kit

Precautions



WARNING

Explosion hazard. When the product is operated or maintained in a hazardous location, it **must be correctly grounded** to avoid static discharge.



WARNING

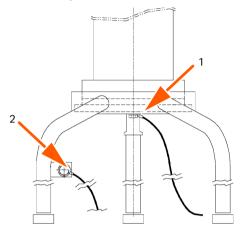
Explosion hazard. When connecting the grounding cable, make sure that there is no explosive atmosphere present. Static electricity discharge could occur when connecting the grounding cable.

Ground the column

To ground the column, connect one end of the grounding cable to an earth terminal in the plant grounding network and the other end to the ground terminal located on the column stand. The column ground terminal has an M6 thread.

Illustration

A grounding kit is available as an accessory for BPG columns and a grounding connection terminal is provided on the column stand as shown below.



Part	Function
1 Grounding terminal/cable on BPG 100, 140, 200 and 450 col	
2	Grounding terminal on BPG 300 column.

4.6 Setup

Precautions



WARNING

Max. column pressure. The working pressure of the column must never exceed the maximum pressure stated on the nameplate and in the documentation package, 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.



CAUTION

Make sure that the pressure relief valve is correctly adjusted and that connecting tubing has an unrestricted flow path. Connect the pressure relief valve outlet to waste.



NOTICE

The bottom outlet should be above the top of the column to eliminate any chance of a siphon.



NOTICE

A pressure relief valve or rupture disc must be connected between the pump and column inlet.

Column setup

The following setup description is an example setup that works for column packing and processing. The setup includes valves, tubing, pump, and sensors to monitor the packing or processing through a chromatography system.

The setup illustrated below uses 3-port 2-way diaphragm valves on the column top inlet and bottom outlet. The ports on each valve are designated S1, S2, and S3.

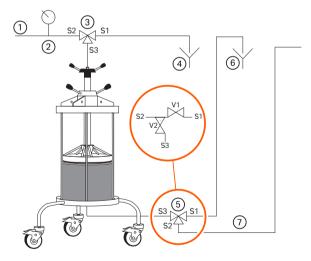
Each valve is fitted with two manual actuators, V1 and V2, to control the flow direction.

The valves are available as accessories.

In the illustration below, the bottom valve is illustrated in more detail. The top valve has a similar design. See *Column top and bottom valves Instructions for Use* for more details and illustrations of the valves.

- The top valve is fitted to the top adapter inlet using port S3. Port S2 is connected to a
 pressure gauge followed by a tubing connected to a system. Port S1 is directed to
 waste for purging the system and column.
- The bottom valve is fitted to the bottom adapter outlet using port S2. Port S1 is
 connected to the tubing directs the flow to waste. Port S3 is connected to the tubing
 to recycle the packing solution back to the column bottom inlet port on the process
 system. The bottom valve can be connected vertically to the column, as illustrated,
 or horizontally. In both cases, port S2 should be connected to the column.

Note: The use of a safety valve at position 2 or just before position 2 in the illustration below is recommended.



Part	Description	
1	From system	
2	Pressure gauge and safety valve (not visible in the illustration)	
3	3-port, 2-way valve	

Part	Description	
4	Drain	
5	3-port, 2-way valve	
6	Drain	
7	To the process system, if connected	

Three-port valve positions, top valve

Flow	Valve position	Description
S2 to S1	S2 S3	Mobile phase from pump to waste V1: Open V2: Closed
\$2 to \$3	S2 S1 S3	Mobile phase to column V1: Closed V2: Open
S3 to S1	S2 S1 S3	Column to waste V1: Open V2: Open

Three-port valve positions, bottom valve

Flow	Valve position	Description	
\$3 to \$2	\$3 \$1 \$2	Return to the process system V1: Closed V2: Open	
S3 to S1	S3 S1 S2	Column to waste V1: Open V2: Open	

4.7 Fitting a packing extension tube

Precautions



NOTICE

When using an extension tube, do not pack the column to bed heights higher than the length of the column. The adapter must seal against the column wall, <u>not</u> against the extension tube wall. A minimum of 10 cm is required between the top of the bed and the top of the column tube to install the adapter if the packing extension is removed.

BPG 100, 140 and 200 columns

The extension tube is assembled when delivered.

Follow the instructions below to fit an extension tube onto the BPG column.

For the extension tubes, the same torque is applicable as for the column itself. See *Torque specifications, on page 101* for torque specifications.

Step	Action
1	Remove the adapter from the column and place the extension tube onto the upper flange. Note that the column rods fit into the open holes in the flange on the extension tube.
2	Make sure that the thin O-ring is positioned properly in the flange of the extension tube.
3	Use the washers and domed nuts from the adapter to secure the extension tube to the column. $ \\$
4	Use the screws delivered with the extension tube to secure the adapter to the extension tube.
5	The extended column is now ready for the first packing phase in the two-phase packing procedures.

BPG 300 columns

The extension tube is assembled when delivered.

Follow the instructions below to fit an extension tube on the BPG 300 column.

For the extension tubes, the same torque is applicable as for the column itself. See *Torque specifications, on page 101* for torque specifications.

Step	Action
1 Remove the adapter from the column and place the extension tube upper flange.	
2	Make sure that the thin O-ring is properly positioned in the flange of the extension tube.
3	Use the screws and washers delivered with the extension tube to secure the extension tube to the column.
4	Use the screws, nuts and washers from the adapter to secure the adapter to the extension tube.
5	The extended column is now ready for the first packing phase in the two-phase packing procedures.

BPG 450 columns

Follow the instructions below to fit an extension tube on the BPG 450 column.

For the extension tubes, the same torque is applicable as for the column itself. See *Torque specifications, on page 101* for torque specifications.

Step	Action
1	Assemble the small O-ring around the protrusion on the extension tube bottom flange and the large O-rings on each flange on the extension tube.
2	Remove the adapter from the column, and place the extension tube onto the column with the protrusion facing towards the column.
3	Use the screws, nuts and washers from the adapter to secure the extension tube to the column.
4	Use the screws and washers delivered with the extension tube to secure the adapter to the extension tube.
5	The extended column is now ready for the first packing phase in the two-phase packing procedures.

5 Operation

About this chapter

BPG columns can be used to perform a number of different chromatographic purifications, depending on the chromatography resin and specific methods selected for use. This chapter provides general instructions on how to prepare and to operate the BPG columns.

In this chapter

Section		See page
5.1	Preparation	46
5.2	Slurry preparation	48
5.3	Column packing	51
5.4	Performance evaluation of the column	77

Precautions



WARNING

All installation, maintenance, operation, and inspection must be carried out according to local regulations by adequately trained personnel.



WARNING

Thoroughly check the glass column tube to make sure that it is not cracked or severely scratched. Pay extra attention to the end parts of the column tube. The pressure specifications are only valid if the glass tube is undamaged.



WARNING

There must **never** be air or gas under pressure in the column.



WARNING

Use a harmless fluid in the beginning of the process. This makes it possible to detect leakage with minimized consequences and the risk for potential leakage of hazardous fluids is avoided.



CAUTION

Do not use chemicals at temperatures above the specified limits.



NOTICE

Make sure that any water used with the column is particle-free (WFI), as particles can block and damage the bed support.

5.1 Preparation

About this section

This section contains information how to prepare the BPG columns before column packing. Information and support related to optimal column packing can be obtained from your Cytiva representative.

Location

Take care in selecting the position of the column before proceeding. System, tanks, drains and handling devices for the larger columns all require space and easy, safe access during all operations of the column. Take care to route the hoses carefully to avoid tripping risks and always try to keep hose lengths to a minimum.

Arrange connections



WARNING

Only use tubing and clamps approved for the maximum pressure of the column and resistant to the chemicals intended to be used.

Make sure the tubing inner diameter is constant throughout your system setup to minimize unwanted volume and keep a consistent flow and pressure in the process stream. The valves, tubing, and gaskets used should have the same inner diameter as the column outlets or slightly larger. See *Connections, on page 19* for more information.

Materials

The following materials are suitable for the column packing:

- BPG column with suitable net for the resin
- Resin in about 50% slurry concentration in water or other suitable packing solution
- WFI for packing or other suitable packing buffer
- Tank/containers for resin slurry, packing buffer and test solutions
- Liquid delivery system, a pump or a chromatography system
- Sensors measuring flow rate, conductivity, UV, or other parameters that are required to monitor and evaluate the packing
- A spirit level
- A resin stirrer
- A column setup as described in

Recommended net porosities

It is important to select the appropriate adapter and end-piece nets. Nets are available in 10, 12, 23 and 54 μ m porosities. Improperly sized nets can lead to back pressure problems or resin leakage. The table below shows recommended net porosities.

Net porosity (µm)	Material	Bead size (µm)
10	PA ¹	30 to 70
12	PEEK ²	30 to 70
23	PP ³	90 (FF-resin)
54	PP ³	120 (BB-resin)

¹ Polyamide

Packing extension tube

The bed height and volume are achievable when retaining the extension tube during the final packing step. The adapter must seal at least 5 cm into the column tube to avoid high tensions on the glass tube.

Note:

Before packing the column for the first time we recommend that the column and tubing are flushed with CIP solution e.g. NaOH to ensure that all surfaces are clean.

For optimum performance from BPG columns, we recommend the column packing protocols described in *Section 5.3 Column packing, on page 51*. Before using the columns, it is recommended that you read the complete chapter.

² Polyetheretherketone

³ Polypropylene

5.2 Slurry preparation

Slurry concentration

Mix the packing buffer with the resin to form a 50% slurry, i.e., sediment volume/total slurry volume = 0.50. This slurry volume is appropriate for BioProcess resin.

Make sure that the correct amount of slurry is used to end up with the target bed height and optimal bed compression.

More information regarding how to determine slurry concentration using the Slurry Concentration Kit can be found in *Slurry Concentration Kit Instructions for Use* 29112530.

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)

Compression factor should read for most Cytiva resins, between 1.10 and 1.15.

Amount of resin and slurry volume

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

 $A = (\pi \times r2 \times H \times Cf/1000)$

Where:

A = volume of resin needed, liter

 $\pi = 3.14$

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

H = bed height in column, cm

Cf = Compression factor of resin

The 50% slurry volume is calculated as: A/0.5

Resin quantities required per cm bed height

The table shows the amount of resin per centimeter of packed bed. The compression factor is 1.15.

BPG column	Column diameter (mm)	(π×r2×H×Cf/1000)	Amount of resin ¹ per cm of packed bed
BPG 100	100	78.5 × 1.15	90.3 mL
BPG 140	140	154 × 1.15	177 mL

BPG column	Column diameter (mm)	(π×r2×H×Cf/1000)	Amount of resin ¹ per cm of packed bed
BPG 200	200	314 × 1.15	361 mL
BPG 300	296	707 x 1.15	812 mL
BPG 450	446	1590 x 1.15	1.83 L

DEAE Sepharose FF, Q Sepharose FF, CM Sepharose FF, S Sepharose FF, Phenyl Sepharose FF, Sepharose 4 FF and 6 FF, Sephacryl™ High Resolution, Superdex™ 75 and 200 prep grade, Q and SP Sepharose HP, Phenyl Sepharose HP

Sephadex G-25

The Sephadex $^{\text{TM}}$ G25 resin swell 4 to 5 times (1 g = 4-5 mL of resin). The table shows the amount of resin per centimeter of packed bed.

Note: The figures in the table below are based on four-fold swelling and that there can be batch-to-batch variation.

Resin	Amount of resin per liter of packed column (g)	Pack factor (PF) (I)	Amount of resin per cm of packed bed (g)				
			BPG 100	BPG 140	BPG 200	BPG 300	BPG 450
Sephadex G-25 C and M grades	250	1.151	22.6	43.9	90.3	202.4	453
Sephadex G-25 grade SF	280	1.151	22.6	43.9	90.3	202.4	453

Prepare the slurry

Follow the instructions below to mix the slurry for column packing:

Step	Action
1	Exchange the storage buffer of the resin slurry in a glass filter funnel or in the column.
2	If the resin is cleaned outside of the column, prepare the resin slurry in a container suitable for pouring it into the column tube after homogenization of the resin slurry and measurement of concentration. Make sure to keep the homogenization of the resin slurry.
	Tip: To save time, and not needing to prepare resin slurry again, it is recommended to prepare the column up to the step when it is time to fill it with resin slurry.
3	Measure the slurry concentration to make sure the correct amount of resin is added to the column. Also, make sure that the concentration is enough to enable the mounting of the adapter into the column tube at its highest position at least.

5.3 Column packing

About this section

This sections describes the steps that must be taken in order to pack the BPG columns.

In this section

Section		See page
5.3.1	Preparing the column	52
5.3.2	Preparing the adapter	55
5.3.3	Column flow packing method	62
5.3.4	Packing the column - settling phase	65
5.3.5	Packing the column - compression phase	71
5.3.6	Storage and unpacking of packed columns	76

5.3.1 Preparing the column

Column preparation

Operation of the adapter, integrity of the adapter O-ring and porosity of the support nets should be checked before starting. The column tube and all internal surfaces must be clean. Perform a leakage test, see <u>Section 6.6 Leakage test</u>, on page 119. Also, prime the column top valve and the column bottom valve.

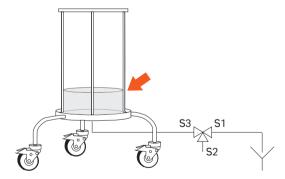
Remove air from bottom net

Note: Make sure to run an upflow for the top valve and a downflow for the bottom valve to remove any trapped air from the valve ports before operation.

Every effort must be made to remove air from bottom net and tubing. Air is removed from under the bottom net using suction and about 1 cm buffer left in the column.

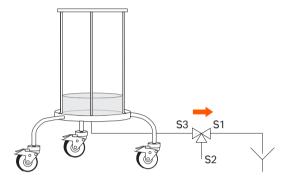
Step	Action
1	Make sure that the column is level, see Section 4.4 How to level the column, on page 35.
2	Make sure that the column is grounded, see Section 4.5 Grounding kit, on page 37.

3 Connect port S3 of a 3-port 2-way valve to the column and port S1 to the drain. Make sure that actuators V1 and V2 are closed. (For illustrations of the port and actuator positions, refer to *Column top and bottom valves Instructions for Use*.

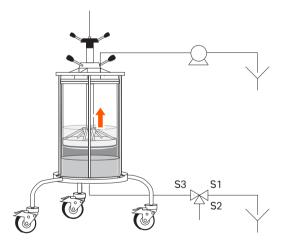


4 Wet the column net with 20% ethanol and pour 2 to 3 cm of packing buffer into the column.

Open actuators V1 and V2 to allow liquid and air to drain from the bottom distributor plate (port S3 to port S1).

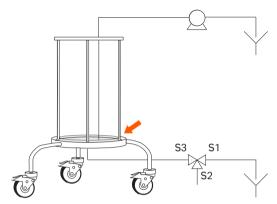


- 6 Close actuators V1 and V2.
- 7 Connect a pipe to a pump, or a vacuum line, and lower the pipe onto the surface of the bottom net. Make sure that the pipe end is soft and cannot damage the bottom net.
- 8 Start the suction to remove any remaining air from under or on the surface of the bottom net.



9 Continue until there is only 1 cm of buffer in the column and no air bubbles are left under the net or under the flow stopper.

On the bottom valve, connect port S2 to tubing, directing the packing solution back to the column bottom inlet port on the process system.



Pocult

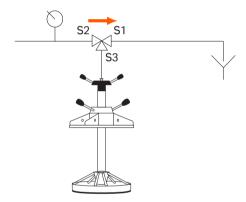
The column tube is now ready for packing.

5.3.2 Preparing the adapter

Position and seal the adapter

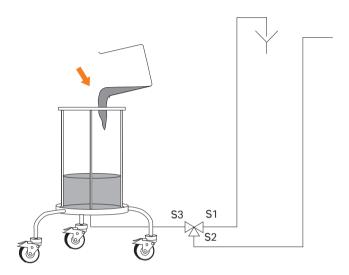
Step Action

- Connect port S3 of a 3-port, 2-way valve to the top of the adapter of the column, port S2 to the pump and port S1 to waste. (For illustrations of the port and actuator positions, refer to *Column top and bottom valves Instructions for Use*.
- 2 Open actuator V1 and close actuator V2 to direct the flow from the pump to waste (port S2 to port S1). Start the pump to purge air.



- 3 Stop the pump once all air is removed.
- 4 Close actuator V1.

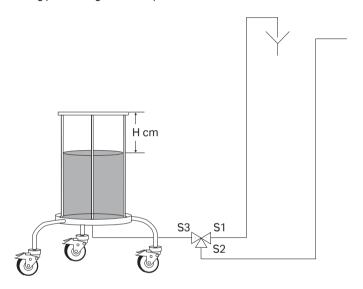
Carefully pour the homogenous slurry into the column. Pour the slurry along a rod or by the column wall to avoid that air bubbles are trapped within the slurry. Rinse off the column tube wall or rod with packing solution.



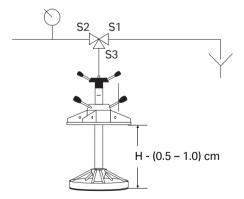
6 Measure the height (H) from the top of the column to the surface of the slurry.

Note:

The height (H)is measured to enable adjustment of the adapter position on the threaded rod. The adapter must not go too deep into the resin slurry during positioning of the adapter into the column tube.



7 Set the adapter height to [H - (0.5 - 1.0)] cm.



8 Wait until a small clear zone is visible above the slurry.

9



WARNING

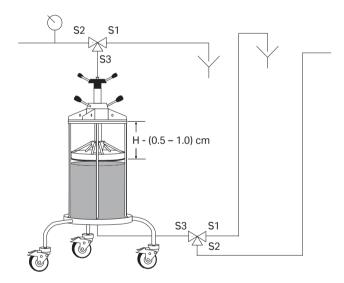
Heavy object. A minimum of two people and the appropriate lifting equipment for the task are required when moving or lifting the column.



WARNING

Crush risk. Be careful when fitting the adapter to the column as there is a risk of crushing your hands.

Insert the adapter in the column just above the slurry level in the column with the seal fully relaxed.



10 Secure the column adapter in place with the dome nuts to the top flange.





Seal the adapter O-ring by turning the seal adjuster knob or handle on the top of the adapter, clockwise.

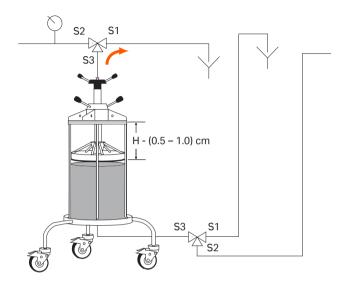




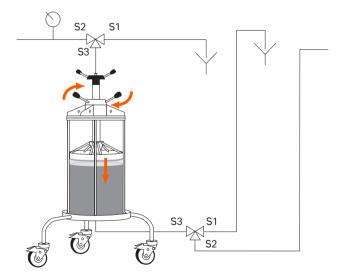
Note:

The seal should be tight, but it should still be possible to move the adapter down into the column tube in the following steps.

On the top valve, open actuators V1 and V2 to direct the flow to waste (port S3 to port S1).

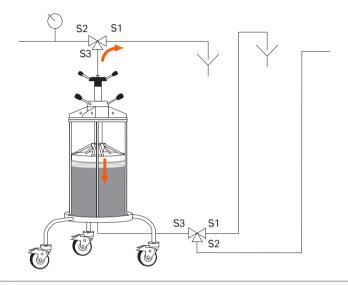


13 Move the height adjuster handle clockwise to position the adapter 0.1 to 0.5 cm below the top surface of the liquid.



Result:

Air, and then liquid, is pushed up through the adapter and out to waste as the adapter is pushed downwards.



5.3.3 Column flow packing method

Introduction

There are different packing methods, each dependent on the column and type of resinused.

For BPG columns, we recommend the packing method described in *Method description*, on page 62 or in the application notes.

The optimum packing flow rate is dependent on temperature, type of resin, batch and quantity. Consequently, for each individual system, the optimum flow rate must be determined empirically by producing a pressure/flow rate curve. The recommended methods are described comprehensively in *Determine optimum packing flow rate*, on page 63, Method description, on page 62 and in the application notes.

Packing flow rate

The packing flow rate is dependent on:

- temperature
- · type of resin
- · resin slurry concentration
- · packing buffer
- · bed height
- · packing method

Consequently, the optimal flow rate or rates must be determined empirically by producing a pressure/flow rate curve on an open bed.

The pressure flow curve of the resin must be performed with the resin volume required for the packed bed including its estimated compression.

Refer also to the application notes on packing methods for each respective resin or resin type.

Method description

Constant flow packing is a two-phase method that is applicable to CL4-6B to Fast Flow and Big Beads resins types of resin. Contact your Cytiva representative if you have questions on what parameters to use or start with. Assuming a suitable liquid delivery system is available, the main requirements are that the column must have a moveable adapter and a pressure rating which allows the optimal packing flow rate to be obtained.

 In the settling phase, a low flow rate is used to form a loosely packed bed from the slurry. This allows the adapter to be lowered the majority of the distance towards its final packed position with little or no expansion of the bed during travel time. 2. In the compression phase, the flow rate is increased to a predetermined optimal value to obtain the applicable packing density. When the flow is stopped, the proximity of the adapter to the bed minimizes the time necessary to successfully reposition the adapter to fixate the packed bed.

Determine optimum packing flow rate

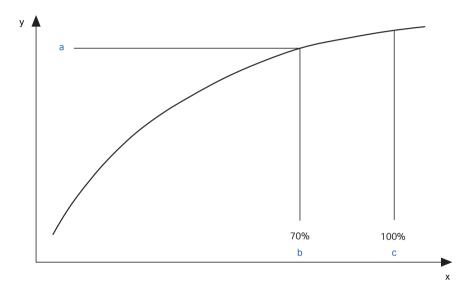
Follow the steps below to determine the optimum packing flow rate.

Step	Action
1	Start the pump and remove air in the tubing from the pump by pumping with a low flow rate.
2	While pumping at the low flow rate, connect a manometer between the pump and the column. Then connect the tubing to the top valve port S2, with V1 open and V2 closed (system to waste).
3	Open V2 on the bottom valve, directing the flow from the column back to the system (port S3 to port S2). Close V1 and open V2 on the top valve to direct the flow into the column (port S2 to port S3).
4	Establish a pressure/flow rate curve by beginning with a low flow rate. The resin will begin to settle, leaving a clear space between the bed and the adaptor.
	Note: Do not adjust the adapter.
5	Slowly increase the pressure in increments and record the flow rate when the pressure is stabilized. The time taken for the pressure to stabilize is dependent on the type of media, it can take from a few minutes to a few hours.
6	Continue to increase the pressure until a flow rate plateau is reached (increased pressure does not give higher flow rates). For some rigid gel resins, there will be no plateau. In those cases, use the flow rate at maximum allowable pressure for the column.
7	When the maximum pressure is reached, the determination should be concluded.

Pressure flow curve

Plot the pressure in the flow rate, as indicated in the below example. The optimal packing flow rate is about 70% of the maximum flow rate. For some very rigid resin there is no plateau. In those cases, use the flow rate at maximum allowable pressure for the column. The figure below shows the pressure flow curve.

Refer to the application notes on how to perform the open bed pressure test and how to set the packing parameters.



The description of the axis are:

x = Pressure

y = Flow rate

The characters in the table are described below:

Value	Meaning
а	Packing flow rate
b	Packing pressure
С	Max pressure

5.3.4 Packing the column - settling phase

Description of the settling phase

The settling phase of constant flow packing produces a consolidated bed at a low flow rate (20 to 60 cm/h), and positions the adapter near the final bed height. The pump is started and the tubing to the column is purged of air.

The flow should be quickly adjusted to approximately the preferred flow for the settling phase. The top valve is then changed to set the column inline. The bottom valve is opened to direct the flow back to the process system. The flow rate is adjusted as needed.

After the bed height of the resin stabilizes in one to two column volumes (1 to 2 CV), the bottom valve is closed. The top valve is configured from column to waste. The adapter O-ring is loosened so that buffer passes above it. The adapter is lowered to 0.5 to 1.0 cm above the top of the bed.

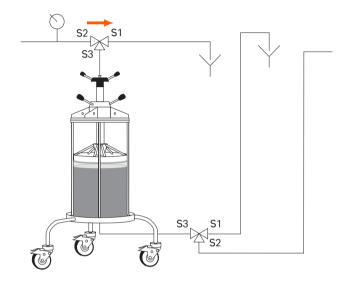
The O-ring is re-tightened, and the top valve is configured from system to column. The settling flow rate is re-started. After stabilization of the consolidated bed, the adapter is lowered to 0.1 to 0.2 cm above the bed with buffer passing out the top valve to waste.

Settling phase

Note:

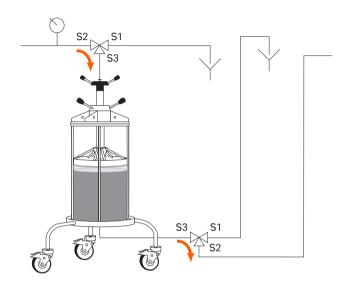
The use of a safety valve at or just before the pressure gauge position in the illustration below is recommended.

On the top valve, open actuator V1 open and close actuator V2 to direct the flow from the pump to waste (port S2 to port S1). Start the pump to remove air.

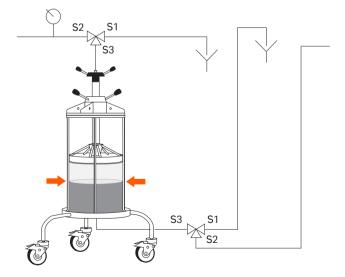


- 2 Pause the flow when all air is removed.
- On the top valve, close actuator V1 and open actuator V2 to direct the flow from the pump to the column (port S2 to port S3).

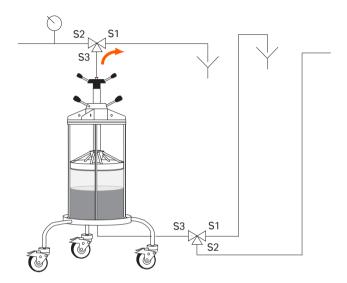
4 On the bottom valve, open actuator V2 to direct the flow back to the column bottom inlet port on the process system.



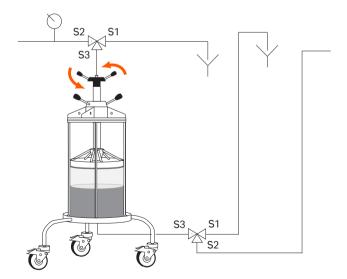
- 5 Restart the flow.
- 6 Monitor the packing flow rate and adjust as necessary.
- 7 Pause the flow when the bed has settled, normally 1 to 2 CV.



When the column is depressurized, close actuator V2 on the bottom valve and open actuator V1 on the top valve to direct the flow from the column to waste (port S3 to port S1). Both actuators V1 and V2 on the top valve should now be open.

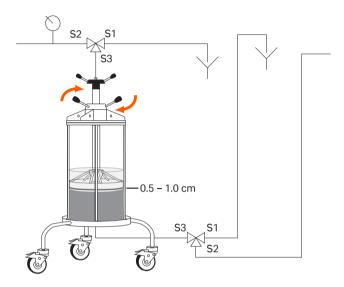


9 Loosen the adapter O-ring seal by turning the adjuster knob, or the seal adjuster handle, on the top of the column counter-clockwise.

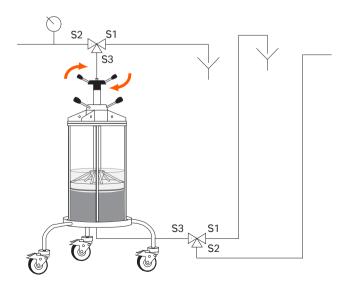


Lower the adapter quickly by turning the height adjuster handle clockwise.

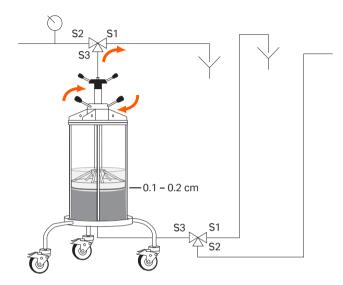
Maintain some compression on the adapter O-ring and lower the adapter smoothly downwards in a steady motion. Stop lowering the adapter when it is 0.5 to 1.0 cm above the bed.



Seal the adapter O-ring by turning the seal adjuster knob, or seal adjuster handle, clockwise.



Lower the adapter further down, causing buffer solution to pass the top valve to waste (port S3 to port S1). Stop lowering the adapter when it is 0.1 to 0.2 cm above the bed.



13 Close the top valve.

Result:

The column is now ready for the compression phase.

Proceed immediately to the compression phase.

5.3.5 Packing the column - compression phase

Description of the compression phase

The compression phase of flow packing compresses the bed at a flow rate that is predetermined from the pressure flow curve data, and rapidly sets the adapter position to maintain the packed bed density. See *Determine optimum packing flow rate*, on page 63 for more information.

The top valve is configured to direct the flow from the pump to waste. When the pump starts, the tubing to the column is purged of air. Thereafter, the flow is directed from the pump to the column. The pump is adjusted quickly to the preferred packing flow rate to obtain the final bed height. The integrity of the adapter O-ring seal is monitored and further tightened if necessary.

When the bed stabilizes in about 1 to 2 CV, the bed height is marked on the column tube. The top valve is then configured from column to waste.

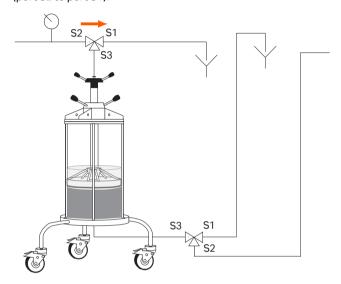
The adapter is rapidly lowered into place with minimal disturbance of the packed bed. To minimize the disturbance of the packed bed, the adapter O-ring is just loose enough for the adapter to move inside the tube. The adapter O-ring is also just loose enough for the buffer to exit out of the top valve, not passing the O-ring.

Once the adapter is in place, the O-ring is re-tightened immediately, and the top and bottom valve closed. If the packed bed decompresses significantly above the marked bed height before the adapter is lowered into place, the compression phase can be repeated once.

Compression phase

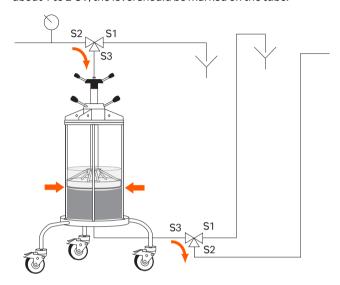
Step Action

As a result of the settling phase the O-ring should be in a tightened state. On the top valve, open actuator V1, directing the flow from the pump to waste (port S2 to port S1).



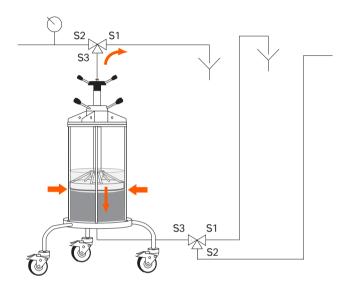
- 2 Pause the flow.
- On the top valve, close actuator V1 and open actuator V2 to direct the flow from the pump to the column (port S2 to port S3).
- 4 On the bottom valve, open actuator V2 to direct the flow from the column to the system.

Resume the flow. This moves the bed down and once stabilized, usually in about 1 to 2 CV, the level should be marked on the tube.



- 6 Pause the flow.
- When the column has depressurized, open actuator V1 on the top valve to direct the flow from the column to waste (port S3 to port S1).

Move the adapter down by pushing it into the resin until it reaches the mark on the column tube. The adapter O-ring must be just loose enough for the buffer to exit out of the top valve and not around the O-ring. The O-ring must also be loose enough to be able to move the adapter. Once the adapter is in place, re-tighten the O-ring immediately.



9 Close actuators V1 and V2 on the top valve and actuator V2 on the bottom valve.

Result:

The column is now packed.

Wait at least 5 minutes for the bed to cure before moving the column. Test the packed column, for instructions see Section 5.4 Performance evaluation of the column, on page 77.

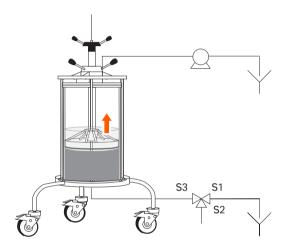
Remove buffer that remains on top of adapter

The design of BPG columns allows buffer to be on top of the adapter while packing. When packing is complete, and the O-ring is sealed it is essential that all buffer (especially salt) is removed from that space to avoid corrosion over time. For more information about NaCl, see Section 8.2 Chemical resistance, on page 137.

Follow the instructions below to remove buffer from adapter.

Step Action

1 Siphon or pump out the buffer.



- 2 Wash, if necessary.
- 3 Repeat.

5.3.6 Storage and unpacking of packed columns

Storage

After equilibrating the column in a storage solution, both column outlets should be sealed. The packed column should be stored in a clean and temperature-controlled environment. Before storage, the column area above the adaptor should be rinsed with 20% ethanol and kept dry and clean. Make sure that there are no chloride ions left on top of the adapter.

Unpacking



NOTICE

If the adapter O-ring seal is dry, it may be damaged by forced movement of the adapter. Wet the O-ring with water or 20% ethanol solution. Do not grease the O-ring.

Wet the O-ring with water or 20% ethanol solution. Fully loosen the wing nut that seals the adapter O-ring. Make sure the adapter outlet is open to waste. If the adapter is not easily raised, try slightly lowering it to break the O-ring seal contact on the glass tube. If the O-ring rolls, tighten the wing nut a bit to prevent rolling of the O-ring during adapter ascent. A firm twist on the threaded adapter shaft is also useful when attempting to unseal the O-ring.

Raise and remove the adapter. If the packed bed is difficult to reslurry, pumping buffer up through the bottom valve helps to lift and to disperse the resin more rapidly and clear the bottom net.

5.4 Performance evaluation of the column

About this section

This section describes how to measure HETP and A_s . To perform a test of the packed column, read the information supplied with the resin.

Column efficiency test

The efficiency of a column depends on how well it is packed. A poorly packed column gives rise to uneven flow, resulting in zone broadening and reduced resolution. A column efficiency test should be performed directly after packing and at regular intervals during the working life of the packed bed. The test is also useful when the separation performance is seen to deteriorate. Poor test results indicate that the resin should be repacked or replaced.

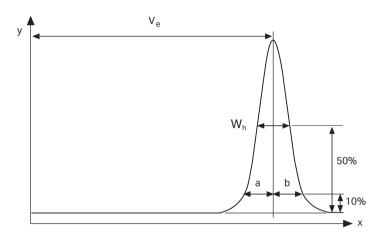
A widely used method for expressing the efficiency of a packed bed is in terms of the height equivalent to a theoretical plate (HETP) and the peak asymmetry factor (A_s). These values can easily be determined by applying a sample of for example acetone or NaCl solution to the column.

Measuring HETP and As

Measuring the HETP and A_s values is the best way to judge the condition of the packed column. A packed column can look good, but still need repacking for optimal performance. Always check the column after packing and regularly between runs to make sure the best column performance. Follow the specific instructions for the packed resin to optimize the testing protocol.

Test chromatogram

The following test chromatogram shows the parameters used to calculate peak broadening and peak symmetry.



The description of the axis are:

x = Volume or time

y = UV absorption or conductivity

HETP calculation

Calculate the height equivalent of a theoretical plate (HETP) from the UV curve if acetone is used, or conductivity curve if sodium chloride is used as sample, as follows:

HETP = L/N

 $N=5.54 (V_e/W_h)^2$

Variable	Meaning
L	Bed height
N	Number of theoretical plates
V _e	Elution volume
W _h	Peak width at half peak height

 V_e and W_h are in the same units

Asymmetry calculation

The asymmetry factor A_s describes the deviation from an ideal Gaussian peak shape and is calculated from the peak width at 10% of peak height.

 $A_s = b/a$

Calculate the asymmetry factor (A_S) from the UV curve or conductivity curve if sodium chloride is used as sample, as follows:

The peak should be symmetrical and the asymmetry factor as close to 1 as possible. Values between 0.8 and 1.8 are usually acceptable. A change in the shape of the peak is usually the first indication of bed deterioration due to use.

6 Maintenance

About this chapter

This chapter provides a general overview of cleaning, sanitization, replacements, and maintenance procedures.

In this chapter

Section		See page
6.1	Service and preventive maintenance	82
6.2	Disassembling column	84
6.3	Replacements of nets and O-rings	91
6.4	Assembling column	103
6.5	Disassemble and assemble the adapter	107
6.6	Leakage test	119
6.7	Cleaning	122
6.8	Storage	124

Precautions



WARNING

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



WARNING

Decontaminate before maintenance or service. Before performing any maintenance or service work on the column, make sure that the column has been emptied and decontaminated.

Note:

During maintenance or service work, Cytiva service engineers cannot use fork lifts, cranes, or other lifting equipment at customer's site. The customer must provide personnel for performing lifting.

6.1 Service and preventive maintenance

Precautions



WARNING

All installation, maintenance, operation, and inspection must be carried out according to local regulations by adequately trained personnel.



WARNING

Accessories. Use only accessories supplied or recommended by Cytiva.

Note:

During maintenance or service work, Cytiva service engineers cannot use fork lifts, cranes, or other lifting equipment at customer's site. The customer must provide personnel for performing lifting.

Service frequency

Regular service and maintenance of the column is necessary to maintain the column in optimal condition and extend the operational lifetime of column components. Nets and and worn O-ring seals should be checked regularly for wear.

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 visit is recommended.

Contact your local Cytiva representative for information about frequency of service requirements to suit individual application needs.

Note:

Note that the recommendations may not apply to your specific use of the system. The system owner is solely responsible for establishing applicable routines for periodic maintenance.

Cleaning before planned maintenance/service

To protect the safety of service personnel, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts maintenance work.

Health and safety declaration forms

Health and safety declaration forms are available for copying or printing on the digital media supplied with the user documentation.

Passivation procedure

It is recommended to include a passivation procedure into a service and preventive maintenance program. Contact your Cytiva representative for additional information.

6.2 Disassembling column

Introduction

This section describes how to disassemble the column before performing maintenance, service or cleaning procedures.

Note:

As a precaution for easy removal of the O-rings in the end piece, pour 50°C warm water into the column and let stand for some minutes.

Equipment required for disassembly/ assembly

- Crane or hoist, depending on column size
- Forklift or other suitable mechanical lifting equipment for lowering and lifting the bottom end plate
- Wrenches
- Hex/Allen keys
- 20% ethanol for cleaning and lubricating
- Clean rubber gloves to prevent grease from fingers becoming transferred to metal surfaces

Remove the adapter

The instructions below show an example how to remove the adapter from the column tube.

Note:

The instructions show a BPG 300 column but is applicable even to other BPG columns.

Step Action

Turn the handles on the seal adjuster several turns to loosen the adapter Oring seal, counter-clockwise.

Tip:

Move the adapter down a few millimeters to loosen the O-ring from the glass wall. Then move it up as far as it will go.





NOTICE

If the adapter O-ring seal is dry, it may be damaged by forced movement of the adapter. Wet the O-ring with water or 20% ethanol solution. Do not grease the O-ring.

2 Remove the domed nuts and washers from the adapter top plate with help of a spanner. Loosen the screws cross-wise.



NOTICE

Nuts must be loosened incrementally.

3



WARNING

Heavy object. A minimum of two people and the appropriate lifting equipment for the task are required when moving or lifting the column.



CAUTION

Removing the top plate and adapter often causes spillages and splashing. Remove any spillage on the floor immediately to minimize the risk of accidents.

Loosen the adapter top plate and lift out the adapter. Remove the adapter without letting the stainless steel touch the glass.

Note:

Lift the BPG 300 column and the BPG 450 column with a hoist or a crane.



Disassemble the 100, 140, 200 and 300 columns

The instructions below show an example of how to disassemble the column.

Note:

The instructions show a BPG 140 column but is applicable to other BPG columns except for BPG 450. For disassembly of BPG 450 columns, see Disassemble the 450 column, on page 90.

Step Action

1 Loosen the nuts on the flange cross-wise in two steps to avoid damage to the column tube.



2 Remove the flange carefully.

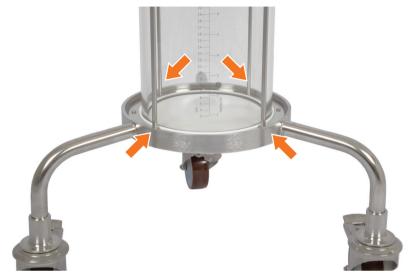




NOTICE

When the support rods are removed, the column tube is unsupported. Make sure that the column tube does not tip over.

3 Use a spanner to unscrew the support rods from the end-piece.



Note:

Make sure not to get the spanner in contact with the column tube to avoid damage to the tube.

4 Lift off the column tube. Carefully place the tube on a secure surface.

If required, clean the end piece separately by using a spanner to remove the bolts and washers that secure the end-piece to the stand.



6 Remove the nets and O-ring.

Tip:

To be able to remove the O-rings without damaging other parts, spray 20% ethanol over the flange or seal.



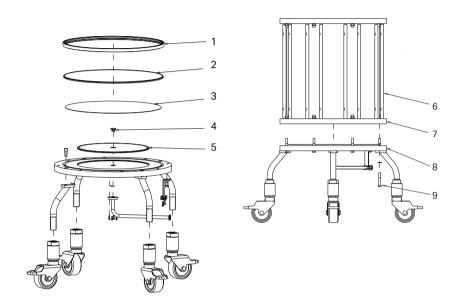
NOTICE

Make sure not to scratch any steel surfaces of the column when removing seals and O-rings. If scratched, the sealing of the column may be compromised and leakage may result. If a steel surface becomes scratched it must be re-passivated prior to use.

Disassemble the 450 column

The instructions below show an example of how to disassemble the column.

Note: For disassembly of BPG 100, 140, 200 and 300 columns, see Disassemble the 100, 140, 200 and 300 columns, on page 87.



Step	Action	
1	Untighten the bolts (9) with a torque wrench and remove them.	
2	Carefully remove the column tube from the end plate (8).	
3	Remove the following items in the bottom end plate:	
	U-shaped seal (1; mounted on the filter net)Filter net (2)	
	Support net (3)	
	Snap plug (4)	
	Distribution plate (5)	

6.3 Replacements of nets and O-rings

Nets

Blockage of the nets causes malfunction of the columns and an over-used net can affect distribution. To prevent blockage follow the instructions listed below.

- When columns are to be re-packed, always check the nets and wash them in a detergent solution or replace them.
- It is important to select the appropriate adapter and end-piece nets, see Recommended net porosities, on page 47.
- Check the HETP and A_s regularly to prevent poor performance due to old nets.

O-rings and seals

The sealing O-rings may lose their flexibility with time and need to be replaced regularly. Worn O-rings may not seal properly.

Tip:

Cytiva recommends that column O-rings and seals are changed at least every second year. It can be necessary to change O-rings and seals exposed to some form of movement in the column more frequently, for example the O-rings on the adapter.

Adapter items

Order Top- Down	Part	Description	BPG 100- 140-200	BPG 300	BPG 450
1		Adapter plate	Yes	Yes	Yes
2		O-ring	Yes	Yes	Yes
3		Distribution plate	N/A	Yes	Yes
4	п	Snap plug	N/A	N/A	Yes
5		Support net	Yes	Yes	Yes

Order Top- Down	Part	Description	BPG 100- 140-200	BPG 300	BPG 450
6		Net	Yes	Yes	Yes

For detailed views, refer to the product documentation.

End-piece items

Order Top- Down	Part	Description	BPG 100 - 140 - 200	BPG 300	BPG 450
1		O-ring	Yes	Yes	N/A
2		Guide ring	Yes	N/A	N/A
3		U-shaped seal on net	N/A	N/A	Yes
4		Net	Yes	Yes	Yes
5		Support net	Yes	Yes	Yes
6	п	Snap plug	N/A	N/A	Yes
7		Distribution plate	N/A	Yes	Yes
8		End-piece	Yes	Yes	Yes

Tools required for changing nets and O-rings

The following tools are recommended when replacing nets and O-rings on the BPG columns.

Illustration	Part
	Hex keys
2 11400 - 6975/9E-1600/31090 - 16	Spanners
•	Torque wrenches for hexagonal socket fittings and torque settings of 4, 5, 6, 6.5, 8 Nm
	12-point opening sockets
	Screwdrivers

Liquids required for changing nets and O-rings

- 100% ethanol for cleaning
- 20% ethanol for lubricating seals
- Warm water (approximately 50°C)

Replace net in the adapter

The instructions below show an example how to replace the adapter net.

Note: The instructions show a BPG 300 column but is applicable to other BPG

columns.

Step Action

- 1 Locate the small slot at the base of the net ring.
- 2 Remove the net by bending it carefully.



Note:

BPG 450: Remove the net by twisting a spatula or similar stainless blunt blade in the slot.

Remove the support net. Be careful not to scratch the adapter as this may cause leakage.



4 Fit new net items according the order in the table *Adapter items*, on page 91.



Wet the net with 20% ethanol to remove all air bubbles trapped in the net. If the net is tight when refitting, warm it in 50°C water to soften it and to get it in place easier.

Note:

Inspect visually and examine with fingers that the net is fitted evenly and tightly.

Replace the O-ring in the adapter



NOTICE

Make sure not to scratch any steel surfaces of the column when removing seals and O-rings. If scratched, the sealing of the column may be compromised and leakage may result. If a steel surface becomes scratched it must be re-passivated prior to use.

Follow the instructions below to replace the O-ring in the adapter.

Step	Action
1	Pull the O-ring off.
2	Attach a new O-ring lubricated with 20% ethanol.

Replace nets in the end-piece

The instructions describe how to replace nets in the end-piece.

Note:	The instructions show a BPG 140 column but is applicable also to other BPG
	columns.

Step Action

1 Fit new end-piece items according the order in table *End-piece items*, on page 93.

Note:

Make sure to mount the distributor plate in the correct direction.

Note:

Wet the net with 20% ethanol to eliminate trapped air.

- For the 100, 140, 200 and 300 columns: Secure the end-piece to the stand using the bolts and washers (left image below).
 - For the 450 column, if required: Secure the end-piece to the wheels (right image below).



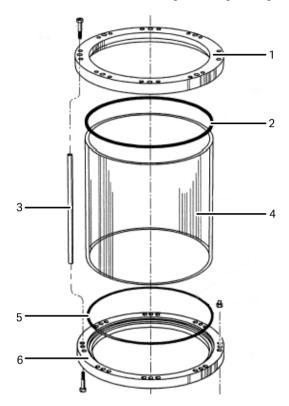
Change top and bottom flange Orings in 100, 140, 200 and 300 columns

Follow the instructions below to change the top and bottom flange O-rings in 100, 140, 200 and 300 columns.

Step	Action
1	Disassemble the column according to <i>Disassemble the 100, 140, 200 and 300 columns, on page 87</i> .
2	Position and fit the bottom flange O-ring. For more detailed positioning of the flange, see <i>BPG 100–200 parts, on page 21</i> and <i>BPG 300 parts, on page 23</i> .
3	Put back the glass tube.
4	Position and fit the new top flange O-ring.
5	Assemble the column according to <i>Assemble the 100, 140, 200 and 300 columns, on page 103</i> .

Change top and bottom flange Orings in the 450 column

Follow the instructions below to change the flange O-rings in the BPG 450 column.



Step	Action
1	Remove the upper flange (1).
2	Remove the tie rods (3).
3	Remove the column tube (4).
4	Replace the bottom O-ring (5) in the bottom flange (6).
5	Put back the column tube.
6	Put back the tie rods.
7	Replace the upper O-ring in the upper flange.
8	Fit the upper flange (2) on top of the column tube. Make sure the tube is correctly aligned to the flange.

Step	Action
9	Put back and fasten the bolts and nuts according to the torque specifications in <i>Torque specifications</i> , on page 101.

Torque specifications

Use the torque setting listed in the following table when assembling the column and extension tubes. Torques for other fastening sequences are also shown.

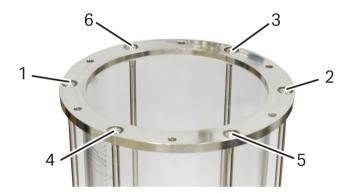
Note: Extension tube support rods do not exist for BPG 450 column.

Tighten the bolts in a cross- and stepwise sequence according to the figure below.



NOTICE

Failure to use crosswise and incremental tightening can damage the column tube.





NOTICE

Always use a calibrated torque wrench. Do not exceed the stated torque because that could damage the column.

BPG column	Description	100	140	200	300	450
	Support rod into top flange ¹	4 Nm	5 Nm	5 Nm	6 Nm	4 Nm

BPG column	Description	100	140	200	300	450
5	Support rod into end piece ²	4 Nm	5 Nm	5 Nm	6 Nm	4 Nm
	Adapter top plate ³	4 Nm	5 Nm	5 Nm	6.5 Nm	8 Nm
5 8	End-piece into bottom flange ⁴	N/A	N/A	N/A	N/A	8 Nm

¹ For BPG 100–300 a nut is used to fasten the support rods into the flange. For BPG 450 a screw is used. The same torque applies for extension tube support rods.

For extension tube to column for BPG 100-200, a nut and support rod are used. For BPG 300, a screw is used and for BPG 450, a screw and nut are used. For BPG 100-200 and 450, the adapter top plate is fastened to the extension tube with a screw. For BPG 300, a screw and nut are used.

² For BPG 100-300, the support rod is fastened directly into the end piece. For BPG 450 a screw is used instead. The same torque applies for extension tube support rods, except for BPG 450 because the extension tube does not have support rods.

³ For BPG 100-200, the adapter top plate is fastened to the column with a nut and support rod. For BPG 300 and 450 a screw and nut are used. The same torques apply for fitting extensions to the column.

 $^{^4\,}$ For BPG 450, the end-piece is fastened to the bottom flange with a screw and nut.

6.4 Assembling column

Assemble the 100, 140, 200 and 300 columns

The instruction below is an example how to assemble the 100, 140, 200 and 300 columns, assuming that the end-piece is already assembled including all end-piece items, see *End-piece items*, on page 93.

Note:

The instruction shows a BPG 140 column but is applicable to other BPG columns except for BPG 450. For information about the 450 column, see Assemble 450 column, on page 105.

Step Action

1 Carefully place the glass tube on the O-ring in the end-piece. Make sure that the glass tube does not touch the stainless steel. Align the glass tube with the bottom net.



NOTICE

The glass tube can crack if it is in contact with stainless steel.

Screw the support rods into the end-piece, see Torque specifications, on page 101.



- 3 Fit the column tube to the end-piece with the bolts.
- 4 Inspect the O-ring in the flange and exchange it if needed.

5 Position the flange so that the support rods fit into the appropriate holes.



6 Align the inner surface of the glass tube with the inner surface of the flange.



NOTICE

Twisted or deformed O-rings can cause leakage.

- 7 Fit the washers and the nuts on the support rods and fingertighten the nuts.
- 8 Set the torque wrench according to specifications, see *Torque specifications*, on page 101.



NOTICE

Do not exceed stated torque settings. Overtightening may cause damage.

9 Tighten the nuts evenly crosswise and in steps as shown in *Torque specifications*, *on page 101*.



CAUTION

After assembly or maintenance, make sure that the column is tight and not leaking.

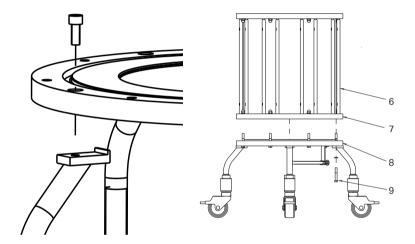


NOTICE

Failure to use crosswise and incremental tightening can damage the column tube.

Assemble 450 column

Follow the instructions below to assemble the 450 column.



Step Action

- 1 Make sure that the following items are positioned properly in the bottom end-piece:
 - Distribution plate (5)
 - Snap plug (4)
 - Support net (3)
 - Filter net (2)
 - U-shaped seal (1; mounted on the filter net)

Note:

Wet the filter net with 20% ethanol in order to eliminate trapped air.

Step	Action				
2	Carefully place the column tube (6) on the U-shaped seal.				
	Note:				
	Ensure that the flange (7) is centered on the U-shaped seal.				
3	Secure the tube to the end plate (8) with the bolts (9).				
4	Tighten the bolts in crosswise and in steps with the torque wrench set on 8 Nm. Each bolt has to be tightened 3 times.				

6.5 Disassemble and assemble the adapter

Autoclaving

If the adapter is going to be autoclaved it must be disassembled in its parts. This section shows how to disassemble and assemble the adapter.

Disassemble the adapter

The instructions below show an example how to disassemble the adapter.

Note: The instructions show a BPG 300 column but is applicable to other BPG

columns.

Step Action

- 1 Remove the adapter from the column tube according to instructions in *Remove the adapter, on page 84*.
- 2 For 200 columns and smaller sizes: Unscrew and remove the cap screws that secure the stopper on the adapter top plate with the hex key.



3 For 200 columns and smaller sizes: Remove the stopper.



For 300 and 450 columns only: Remove the stop screw and locking nut from the adapter top plate.



5 Carefully unscrew and remove the seal adjuster. This releases the tension on the spring situated under the inner adapter tube.



6 Remove the top-plate assembly by turning it in a clockwise direction.



7 Unscrew the bolts on the upper ring of the sealing unit. This will release the sealing unit and the inner adapter tube.



8 Disassemble the top plate assembly if the parts are worn.



9 Remove the sealing unit and the adapter O-ring.



- 10 Unscrew and remove the bolts in the adapter plate.
- 11 Remove the inner adapter tube from inside the threaded outer adapter tube.
- 12 If not already done, remove the O-ring, the nets, the distributor plate and the distributor plug.

Assemble the adapter

The instructions below is an example how to assemble the adapter.

Note: The instructions show how to assemble a BPG 300 column but is applicable

to other BPG columns.

Step Action

1 Position the bushing ring(s) in the recess on the adapter top plate.

Note:

This image shows BPG 140 column but is applicable to other BPG columns.



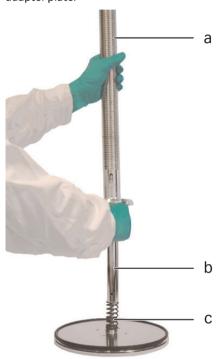
2 Using the screws secure the adjuster nut insert to adjuster nut body through the adapter top plate so that the adjuster nut body turns on the bushing ring.

Note

This image shows BPG 140 column but is applicable to other BPG columns.



3 Slide the spring (c), inner adapter tube (b) and outer adapter tube (a) onto the adapter plate.



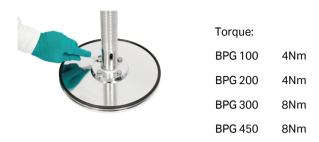
4 Fasten the bolts by hand to assemble the outer adapter tube to the adapter plate.





Step	Action
OLOP	AUCIUII

5 Tighten the bolts with a ring spanner to complete the assembly.



6 Slide the sealing unit onto the adapter tube.



7 In a counter clockwise direction, screw down the top plate assembly 5 cm along the outer adapter tube.



8 Assemble the seal adjuster on top of the adapter.

Turn the seal adjuster clockwise onto the top of the adapter until the holes in the inner adjusting tube are level with the holes in the outer adapter tube.

(See Chapter 3 Column descriptions, on page 17 for respective size of the seal adjuster.)



Use a flashlight and a screwdriver through one hole to adjust the inner tube in position. Add two adapter head screws but do not tighten them.



11 Remove the screwdriver and fit the third screw.



12 Tighten the screws with a spanner.



13 Locate the stop screw and locking nut in the top plate.



Put the spring inside the plastic end socket and assemble them together with the stop screw and locking nut on the top plate.

Note:

Do not overtighten the stop screw through the plastic end socket. The inner spring should remain flexible and push the plastic end socket towards the outer adapter tube, preventing sideways movement without preventing vertical movement. A suitable amount of travel for the plastic end socket is approximately 2 mm.



- Lock the stop screw in position with the locking nut.
- 16 Attach the distributor plate and the nets on the adapter plate.

6.6 Leakage test

Precautions



WARNING

For continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere, the user must test the piping system for leakage at maximum operating pressure.

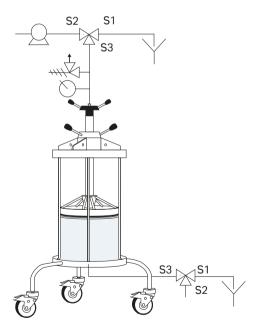
- Always perform a leakage test after assembly or maintenance.
- Always perform a leakage test before operation or CIP.

Perform leakage test

Follow the instructions below to test for leakage across the adapter.

Step Action

1 Close actuators V1 and V2 on the bottom valve. Fill the column with water.



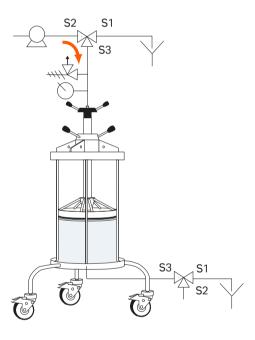
2 Position the adapter in the column to the height intended to be used when running the column. Make sure there is no air trapped in the column.

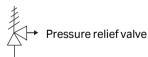


NOTICE

If the adapter O-ring seal is dry, it may be damaged by forced movement of the adapter. Wet the O-ring with water or 20% ethanol solution. Do not grease the O-ring.

3 Seal the adapter with the seal adjuster.







WARNING

Max. column pressure. The working pressure of the column must never exceed the maximum pressure stated on the nameplate and in the documentation package, 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.

4 Close actuator V1 and open actuator V2 on the top valve, directing the flow from the pump to the column (port S2 to port S3).

Note:

A pressure gauge and a pressure relief valve, or burst disc, must be positioned between the top valve and the column inlet.

- 5 Set a low flow, approximately 5 cm/h, and start the pump to raise the pressure. It is recommended to use design pressure as test pressure.
- When the test pressure is reached, close actuator V2 on the top valve and stop the pump. Wait about 15 minutes until the pressure has stabilized.
- 7 Check the pressure gauge and note the pressure. Wait for 5 minutes.
- 8 Check the pressure on the pressure gauge. The drop in pressure should not exceed 0.1 bar in 5 minutes.

6.7 Cleaning

Regular cleaning

Regular cleaning of BPG columns is essential for reliable results. It is important that the column is kept free from contamination such as microbiological growth and adsorbed proteins. In addition, the column must be kept free from salts and chlorides to avoid corrosion.

Precautions



WARNING

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



NOTICE

Before operation the exterior of the product must be completely dry and clean. Wipe the exterior regularly with a soft damp tissue. Use a mild cleaning agent if necessary.



NOTICE

Make sure that any water used with the column is particle-free (WFI), as particles can block and damage the bed support.

Cleaning agents

All column parts can be cleaned with the most commonly used agents, such as detergents, ethanol, weak acids, sodium hydroxide and high salt concentrations. For special cleaning agents, please refer to Section 8.2 Chemical resistance, on page 137.

In time, even stainless steel might show signs of corrosion, especially if it is in frequent contact with high salt concentrations. Should this occur, repassivation of steel components is recommended and should correct the problem.

Cleaning-in-place (CIP)

One of the most important aspects of production chromatography is the maintenance of the packed column by cleaning-in-place (CIP) procedures. Regular CIP of the packed column between production batches is needed to ensure the proper product quality and the expected resin and equipment life. CIP removes precipitated material, strongly bound substances, and other contaminants from the column bed without dismantling the column.

Recommended CIP procedure

CIP protocols should be included as part of the general process development. CIP of the resin should take place when required, for example after each production batch, when an unusually high back-pressure is generated over the column, or when a change in peak symmetry or discoloration of the resin is observed. See the recommended cleaning procedures in the table below. Refer to the regulatory support files for each cleaning procedure for more detailed information.

After cleaning

After cleaning, restore the column to working conditions using the follow-up procedure. All conditions refer to room temperature unless otherwise stated. If special maintenance routines are required, contact your local Cytiva representative for expert advice. For affinity resin, please contact your local Cytiva representative.

Autoclaving

To ensure effective autoclaving it is recommended that the column is completely disassembled. All plastic components, support net, net, snap plug (BPG 450) and distribution plate (BPG 300 and 450) as well as the wheels and plastic handle caps of the adjuster nut must be disassembled from the column.

After autoclaving it is recommended that all process contact parts that have not been autoclaved are replaced with new parts. Essentially all process contact components such as seals and nets. Any plastic process contact parts that are to be reused must be appropriately cleaned prior to reassembly of the column.



NOTICE

PTFE components can shrink with repeated autoclaving. Remove valves if autoclaving the column.

6.8 Storage

Bacteriostatic solution

BPG columns packed with resin should be stored in the bacteriostatic solution recommended for the resin. Refer to your resin instructions for resin-specific recommendations. Refer also to Section 8.2 Chemical resistance, on page 137. Avoid any solution that may compromise the quality of the resin.

Follow the instructions below to apply bacteriostatic solution:

Step	Action
1	Clean the column before applying bacteriostatic solution, for example with 0.5M NaOH, or other CIP solution recommended for the resin.
2	Rinse the column from all chloride ions by using at least five column volumes (CV) of purified water.
3	Apply the bacteriostatic solution and the column is ready to be stored. See the instructions below for storage of the BPG column.

Store the BPG column

If the column is stored wet for longer periods of time, change the storage solution at regular intervals, at least every 3 months.

Avoid storage conditions that expose the column to extreme changes in temperature. Follow the instructions below to store the BPG column:

Step	Action
1	Disassemble the column.
2	Perform routine maintenance, i.e., replace O-rings etc.
3	Thoroughly dry all column components.
4	Assemble the column.
5	Seal all inlets and outlets as indicated in wet storage protocol above.
6	Cover the column with a plastic sheet to prevent exposure to dust and other airborne contaminants.
7	Store the column at controlled ambient temperatures in a clean and dry environment.

After storage of empty column

When the BPG column is going to be taken into operation after storage, follow the instructions below:

Step	Action
1	Disassemble the column.
2	Perform routine maintenance, i.e., inspect or replace O-rings etc.
3	Thoroughly dry all column components.
4	Assemble the column and perform an integrity test.

After storage of packed column

When a packed BPG column is taken into operation after storage, follow the instructions below:

Step	Action
1	Perform a column performance test.
2	Perform CIP.
3	Equilibrate to clean, hold, or storage solution before processing.

7 Troubleshooting

About this chapter

This chapter provides information to operators and service personnel to identify and correct problems that can occur when operating BPG columns.

If the suggested actions in this guide do not solve the problems, or if the problem is not covered by the this guide, contact your Cytiva representative for advice.

Air in the column

If air has entered the column, the column can be run at a high velocity in the opposite direction until de-aired. Place a flow restrictor on the outlet to generate a higher back pressure over the bed. However, if only a small amount of air has been trapped between the column net and the adapter head, it is possible to remove the air by pumping a solution, with a temperature a few degrees higher than the resin, through the column, in the opposite direction. After reverse pumping, test the column again prior to use, see Section 5.4 Performance evaluation of the column, on page 77.

Troubleshooting guide

Problem	Cause	Action
High-back pres- sure	The valves between the pump and the collection vessel are closed.	Open the valves.
	The valves are blocked.	Verify that all valves are clean and free from internal blockage.
	The resin is packed too hard.	Repack the resin.
	The column nets are blocked. There can be air trapped in the net.	Change nets and make sure proper air removal for successive packs.
	The support net is blocked.	Clean or change the support net.
	Flow valve selected is not correct.	Change flow valve.
	Flow cell used not the correct selected.	Select adequate size of flow cell. Verify if the sample and collection vessels are at approximately the same level as, or above, the pump.

Problem	Cause	Action
	There are differences in internal diameter between the tubing on the column, the tubing from the pump, or anywhere else in the system.	Change tubing to the same diameter.
	The pressure gauge is showing faulty values.	Change or calibrate the pressure gauge.
Column leakage	No leakage test is performed.	Perform a leakage test, see Section 6.6 Leakage test, on page 119.
	Possible damages on seals or sealing surfaces.	Inspect seals and sealing surfaces.
	The adapter seal is not properly engaged.	Make sure that the adapter seal is properly engaged.
	The end-piece and/or flange is not properly positioned with respect to the tube.	Disassemble and re-assemble the column.
	The solvents used are not compatible with the O-rings and gaskets.	Check material chemical compatibility, see Section 8.2 Chemical resistance, on page 137.
	The tubing is not compatible with all the solvents.	Check material chemical compatibility and take appropriate adaptation actions.
	The connectors have not the same dimensions.	Change connectors and perform a leakage test.
	Air leaks into the column from the suction side of the pump.	Check that the connections to and from the pump are correct and tightened.
	The torque is not correct on the rods or the flange.	Check that the correct torque specification has been used during column assembly.
Chromatogram	The linear velocity is not as expected.	Check sample.
not as expected	Back-mixing has occurred somewhere in the column set-up.	Check connections.
	The sample could have passed through an air trap.	Check connections or test method.

Problem	Cause	Action	
	The adapter is not correctly positioned in close contact with the bed.	Repack the column to appropriate compression to have the adapter within the bed.	
	The column packing has not been evaluated and is eventually not optimal.	Determine the performance of the packed bed.	
	The column has not been correctly equilibrated.	Equilibrate the column.	
	Old sample mixed with new.	Check sample and sample handling.	
	There have been any changes to the following: • Preparation of buffers/solutions • Preparation of system and tubing	 Check the dilution, filtration, temperature, and preparation of the sample and buffers. Check all dead volumes were correctly filled with appropriate buffers and sample before running. 	
Infected and/or pyrogen contami- nated column	Column bed shows signs of bacterial growth.	CIP.Prepare new buffers/sample.	
	The starting material is contaminated.	Use clean material.	
	The in-going components (such as water, serum components in growth resin, etc.) are not ok.	Check material.	
	The column is not cleaned.	Clean the column, see Recom- mended CIP procedure, on page 123.	
	The NaOH has been removed, in case that positive LAL tests were seen after a CIP cycle with NaOH.	Repeat cleaning, but also consider a more thorough cleaning process.	
Air trapped in the column	The buffers have not the same temperature as the column.	Check the buffers are at the same temperature as the column.	
	The connections are not correctly fitted together and leaks air into the stream.	Check the connections.	
	The valves are leaking or are not shut tight enough.	Check the valves and close them.	
	The volume of the air trap is too small or large.	Fill air trap with buffer.Change air trap.	

8 Reference information

About this chapter

This chapter contains specifications of BPG columns. It also contains a list that specifies the chemical resistance of BPG columns to different chemicals.

In this chapter

Section		See page
8.1	Specifications	130
8.2	Chemical resistance	137
8.3	Recycling information	140
8.4	Regulatory information	141

8.1 Specifications

In this section

Section		See page
8.1.1	BPG 100	132
8.1.2	BPG 140	133
8.1.3	BPG 200	134
8.1.4	BPG 300	135
8.1.5	BPG 450	136

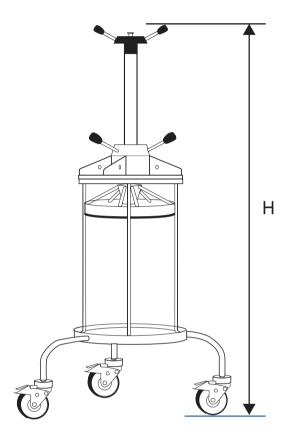
Column specifications

Bed volumes and bed heights are based on a slurry concentration of 75% and a packing compression of 15%. The compression is the difference in volume between a sedimented bed and a flow compressed bed.

Note: The optimal slurry concentration for column packing is close to 50%.

Height

Height (H) used in this section refers to a BPG column with the adapter in its most upper position.



8.1.1 BPG 100

Parameter	Unit	BPG 100		
Tube height	mm	500	750	950
Column inner diameter	mm		100	
Cross sectional area	cm ²		78.5	
Max column volume	liter	3.2	5.1	6.7
Max column volume with extension	liter	6.2	8.1	9.7
Min bed height	cm	1	26	46
Bed height ¹	cm	26	42	55
Bed height with extension ²	cm	35	56	74
Bed height with extension ³	cm	45	67	N/A
Min bed volume	liter	0.1	2.0	3.6
Bed volume ¹	liter	2.0	3.3	4.3
Bed volume with extension ²	liter	2.7	4.4	5.8
Bed volume with extension ³	liter	3.5	5.3	N/A
Max pressure	bar(g)		8	
Total weight (tare mass)	kg	15	16	17
Total weight (tare mass) with extension	kg	21	22	23
Adapter weight	kg	7		
Overall dimension	D×W×H cm	48×48×127	48×48×152	48×48×172
Height with extension tube	cm	165	190	210
Operating temperature	°C	4 to 40		

¹ Without an extension tube.

² These bed heights and volumes are achievable using a packing extension during consolidation of the bed (sedimentation). Total amount of 75% slurry must fit into column + extension with adapter.

The bed height and volume are achievable when retaining the extension tube during the final packing step. Adapter must seal at least 5 cm into the column tube to avoid high tensions on glass tube.

8.1.2 BPG 140

Parameter	Unit	BPG 140		
Tube height	mm	500	750	950
Column inner diameter	mm		140	
Cross sectional area	cm ²		154	
Max column volume	liter	6.2	10.1	13.1
Max column volume with extension	liter	12.1	15.9	19
Min bed height	cm	1	26	46
Bed height ¹	cm	26	42	55
Bed height with extension ²	cm	35	55	74
Bed height with extension ³	cm	45	67	N/A
Min bed volume	liter	0.2	4.0	7.1
Bed volume ¹	liter	4.0	6.5	8.5
Bed volume with extension ²	liter	5.4	8.5	11.4
Bed volume with extension ³	liter	6.9	10.3	N/A
Max pressure	bar(g)		6	
Total weight (tare mass)	kg	25	26	27
Total weight (tare mass) with extension	kg	34	35	36
Adapter weight	kg	11		
Overall dimension	D×W×H cm	59×59×127	59×59×152	59×59×172
Height with extension tube	cm	165	190	210
Operating temperature	°C	4 to 40		

¹ Without an extension tube.

² These bed heights and volumes are achievable using a packing extension during consolidation of the bed (sedimentation). Total amount of 75% slurry must fit into column + extension with adapter.

The bed height and volume are achievable when retaining the extension tube during the final packing step. Adapter must seal at least 5 cm into the column tube to avoid high tensions on glass tube.

8.1.3 BPG 200

Parameter	Unit		BPG 200	
Tube height	mm	500	750	950
Column inner diameter	mm	200		
Cross sectional area	cm ²		314	
Max column volume	liter	12.7	20.5	26.8
Max column volume with extension	liter	24.6	32.5	38.8
Min bed height	cm	1	26	46
Bed height ¹	cm	26	42	55
Bed height with extension ²	cm	35	56	74
Bed height with extension ³	cm	45	67	N/A
Min bed volume	liter	0.3	8.2	14.5
Bed volume ¹	liter	8.2	13.2	17.3
Bed volume with extension ²	liter	11	17.6	23.2
Bed volume with extension ³	liter	14.1	21.0	N/A
Max pressure	bar(g)	6		
Total weight (tare mass)	kg	34	36	39
Total weight (tare mass) with extension	kg	45	47	50
Adapter weight	kg	13		
Overall dimension	D×W×H cm	59×59×127	59×59×152	59×59×172
Height with extension tube	cm	165	190	210
Operating temperature	°C	4 to 40		

¹ Without an extension tube.

² These bed heights and volumes are achievable using a packing extension during consolidation of the bed (sedimentation). Total amount of 75% slurry must fit into column + extension with adapter.

The bed height and volume are achievable when retaining the extension tube during the final packing step. Adapter must seal at least 5 cm into the column tube to avoid high tensions on glass tube.

8.1.4 BPG 300

Parameter	Unit		BPG 300	
Tube height	mm	500	750	950
Column inner diameter	mm		296	
Cross sectional area	cm ²		688	
Max column volume	liter	26.6	43.8	57.5
Max column volume with extension	liter	52.7	69.9	83.7
Min bed height	cm	3	28	48
Bed height ¹	cm	25	41	54
Bed height with extension ²	cm	33	55	72
Bed height with extension ³	cm	45	66	N/A
Min bed volume	liter	2.1	19.3	33.0
Bed volume ¹	liter	17.2	28.2	37.2
Bed volume with extension ²	liter	22.7	37.8	49.5
Bed volume with extension ³	liter	31	45.4	N/A
Max pressure	bar(g)		4	
Total weight (tare mass)	kg	68	73	78
Total weight (tare mass) with extension	kg	89	94	99
Adapter weight	kg	29		
Overall dimension	D×W×H cm	69×69×133	69×69×158	69×69×178
Height with extension tube	cm	171	196	216
Operating temperature	°C	4 to 40		

¹ Without an extension tube.

² These bed heights and volumes are achievable using a packing extension during consolidation of the bed (sedimentation). Total amount of 75% slurry must fit into column + extension with adapter.

The bed height and volume are achievable when retaining the extension tube during the final packing step. Adapter must seal at least 5 cm into the column tube to avoid high tensions on glass tube.

8.1.5 BPG 450

Parameter	Unit		BPG 450	
Tube height	mm	500	750	1000
Column inner diameter	mm	446		
Cross sectional area	cm ²		1562	
Max column volume	liter	55.6	94.7	133.7
Max column volume with extension	liter	102.5	141.5	180.6
Min bed height	cm	12	28	53
Bed height ¹	cm	23	39	55
Bed height with extension ²	cm	30	52	74
Bed height with extension ³	cm	42	59	N/A
Min bed volume	liter	18.7	43.7	82.8
Bed volume ¹	liter	35.9	60.9	85.9
Bed volume with extension ²	liter	46.9	81.2	115.6
Bed volume with extension ³	liter	65.6	92.1	N/A
Max pressure	bar(g)	2.5		
Total weight (tare mass)	kg	200	215	230
Total weight (tare mass) with extension	kg	225	240	255
Adapter weight	kg	100		
Overall dimension	D×W×H cm	80×80×140	80×80×165	80×80×190
Height with extension tube	cm	170	195	220
Operating temperature	°C		4 to 40	

¹ Without an extension tube.

² These bed heights and volumes are achievable using a packing extension during consolidation of the bed (sedimentation). Total amount of 75% slurry must fit into column + extension with adapter.

The bed height and volume are achievable when retaining the extension tube during the final packing step. Adapter must seal at least 5 cm into the column tube to avoid high tensions on glass tube.

8.2 Chemical resistance

Introduction

This section provides general chemical resistance guidelines for various chemicals that may be used with BPG columns.

Chemicals to be avoided

In general, the use of the following chemicals in BPG columns should be avoided:

- Extreme oxidizers (such as peroxides)
- · Halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters (such as acetates)
- Aromatic hydrocarbons (such as toluene)
- · High concentration of strong acids
- Chloroform
- THF
- Toluene
- Methylenechloride

Chemical resistance guide



NOTICE

Only use chemicals that have been proven not to be harmful to the wetted parts of the product.

Note:

Avoid salt in combination with pH below 4.5. Always wash the column with at least two column volumes of pH-neutral solution between the use of salt and low pH buffers.

The table below is intended as a guide to the chemical resistance of the materials used in BPG columns. The information has been compiled from published material from several sources, not from individual tests on the components of the column. Consequently, the user should use this list only as a guide to the level of chemical resistance exhibited by each material. It should be noted that the effects of a chemical are more severe at higher temperatures and pressures, and that combined effects have not been taken into consideration.

Table 8.1: General guideline to chemical resistance for BPG 50 to 200 columns.

Chemical ¹	Concentration ²	Time/cycle restrictions	Comments	CAS No.3
Acetic acid	25%	3 h	CIP	64-19-7
Acetone	2%	1 h	Function test	67-64-1
Ammonium sulfate	2 M ⁴	5 h	Adsorption	7783-20-2
Benzyl alcohol	2%	12 months	Storage	100-51-6
Ethanol	20%	12 months max. 0.5 bar	Storage	64-17-5
Ethanol	70% ⁵	3 h	CIP	64-17-5
Ethanol/acetic acid	20%/10%	3 h	CIP	64-17-5/ 64-19-7
Guanidine hydro- chloride	6 M ⁶	5 h	CIP	50-01-1
Hydrochloric acid	0.1 M (pH=1) ⁷	1 h	CIP	7647-01-0
Isopropanol	30%	1h	CIP	67-63-0
Peracetic acid	100 mM	24 h	CIP	79-21-0
Phosphoric acid	5%	8 h	Passivation of SS bed supports	7664-38-2
Sodium chloride	0 to 3 M ^{5,7,8}	3 h	Purification, CIP	7647-14-5
Sodium hydroxide ⁹	1 M (pH 14)	24 h	CIP	1310-73-2
Sodium hydroxide / ethanol	1 M / 20%	3 h	CIP	1310-73-2/ 64-17-5
Sodium sulfate	1 M ⁴	3 h	Adsorption	7757-82-6
Urea	8 M ⁴	5 h	Purification, CIP	57-13-6
Commonly used aqueous buffers for chromatographic use	10 to 250 mM pH 3 to 10	24 h	Equilibration, adsorption, elution	N/A

 $^{^{1} \ \}text{If nothing else stated, the recommended temperature for usage is } 2^{\circ}\text{C to 40}^{\circ}\text{C for all chemicals listed in this table.}$

When a concentration is given as a percentage, this is v/v.

³ CAS No. is the registration number assigned by the Chemical Abstract Services (CAS), American Chemical Safety. Refer to Material safety data Sheet (MSDS) for more information.

 $^{^4\,}$ pH in these solutions depends on the pH of the liquid, which can vary between pH 3 to 13.

⁵ Glass and stainless steel columns.

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

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.

⁹ Room temperature to 30°C

Chemical resistance to sodium chloride

BPG columns are equipped with wetted components of stainless steel and must therefore be appropriately maintained when exposed to NaCl during chromatographic processes. If a process incorporates the use of NaCl solutions it is essential that the column be thoroughly rinsed to remove any residual NaCl. It is recommended that a water rinse of at least five column volumes be used. Water is the preferred rinse solution due to the solubility properties of NaCl in water verses other possible rinsing solutions like ethanol solution.

The rinsing protocol should use at least five column volumes of purified water to assure optimal removal of residual chloride ions, which can be corrosive to stainless steel over time.

8.3 Recycling information

Decontamination

BPG columns shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.



WARNING

LOCK OUT / TAG OUT (LOTO). Make sure that the following actions have been performed before any decommissioning or maintenance work is performed:

- The column is empty and depressurized.
- Relevant energy sources and process feed are disconnected.
- The column is clearly tagged as taken out of operation.
- All process wetted areas are clean and decontaminated.



WARNING

Decommissioning. Decontaminate before decommissioning to make sure that hazardous residues are removed.

Disposal

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

Recycling of hazardous substances

BPG columns may contain hazardous substances. Make sure that there are no hazardous substances left in the column prior to recycling.

8.4 Regulatory information

Introduction

This section lists the regulations and standards that apply to the product. Your product is marked or listed according to the applicable regulatory requirements for your region. Local language translations are only provided according to regulatory requirements.

In this section

Section		See page
8.4.1	Contact information	142
8.4.2	European Union and European Economic Area	143
8.4.3	Great Britain	144
8.4.4	Other regulations and standards	145

8.4.1 Contact information

Contact information for support

To find local contact information for support and sending troubleshooting reports, visit *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

8.4.2 European Union and European Economic Area

Introduction

This section describes the information that applies to the product in the European Union and European Economic Area.

Conformity with EU Directives

Refer to the EU Declaration of Conformity for the directives and regulations that apply for the CE marking.

If not included with the product, a copy of the EU Declaration of Conformity is available on request.

CE marking



The CE marking and the corresponding EU Declaration of Conformity is valid for the product 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 Great Britain

Introduction

This section describes the information that applies to the product in Great Britain.

Conformity with UK Regulations

Refer to the UK Declaration of Conformity for the regulations that apply for the UKCA marking.

If not included with the product, a copy of the UK Declaration of Conformity is available on request.

UKCA marking



The UKCA marking and the corresponding UK Declaration of Conformity are valid for the product 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.

8.4.4 Other regulations and standards

Introduction

This section describes the additional 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 or ISO 10993-6, -10, -11	USP <88> Biological Reactivity Test In VivoClass VI. ISO 10993 Biological evaluation of medical devices parts 6, 10 and 11.

Index

Numerics	CE, 143
100 140 200 200 saluman 102	conformity, 143
100, 140, 200, 300 column, <i>103</i>	marking, 143
assembly, 103	Chemical resistance, 137
450 column, <i>105</i> assembly, <i>105</i>	Chromatogram, 78, 127
assembly, 105	CIP, 123 protocol, 123
Δ.	Cleaning, 34, 122
A	agents, 122
Abbreviations, 7	Cleaning-in-place, 123
Adapter, <i>84</i> , <i>91</i> , <i>107</i> , <i>111</i>	Column, 19, 34, 36, 58, 77, 87, 90,
assembly, 111	91,93,98,99,124,128
disassembly, 107	assembly, 58
net, 91	blockage, 91
remove, <i>84</i>	change O-ring, 100-300, <i>98</i>
Air, 52, 126	change O-ring, 450, 99
remove from column, 126	contaminated, 128
remove from top of adapter,	disassembly, 87, 90
52	leveling, 36
Air trapped, 128	material, 19
A _s , 77	moving, <i>34</i>
ATEX directive, 20	nets, 93
Autoclaving, 123	performance evaluation, 77
	storage, 124
В	Column leakage, 127
	Columns, 19, 31
Bacteriostatic solution, 124	floor load, 31
Bed height, 48	net, 19
BPG 100-140-200 columns illus-	space requirements, 31
trations, 21	Compression factor, 48
BPG 300 columns illustrations, 23	Connections, 46
BPG 450 columns illustrations, 25	arrange, 46
BPG columns, 5, 18, 19, 28, 130	
accessories, 28	D
design pressure, 18	
intended use, 5	Decontamination, 140
material, 19	Disassembling the column, 84
specifications, 130	Disposal, 140
tubing, 19	general instructions, 140
volumes, 18	Drain, 41
	symbol, 41
C	_
Calculation, 78, 79	E
assymetry, 79	Emergency 16
HETP, 78	Emergency, 16 restart, 16
11211,70	shutdown, 16
	Silucusvii, 10

Emergency stop, 16 End-piece items, 93 Environment, 31 Evaluation, 77 column performance, 77	replacement, 95 Nets, 19, 97 column, 97 Notes and tips, 6
Explosive atmosphere, 20 Explosive environment, 10	0
precautions, 10 Extension tube, 28, 42 fitting, 42	O-rings, 89, 95, 98, 99 replacement, 89 replacement adapter, 95 replacement in 100-300 columns, 98, 99
F	Operation, 12
Flammable liquids, 10 precautions, 10	precautions, 12
Flow rate, 64	P
G Grounding, 37 Grounding kit, 38	Packing column, 65, 72, 75 compression phase, 72 remove buffer from adapter, 75
H	settling phase, 65 Packing extension tube, 28, 47
HETP, 77, 78	Packing flow rate, 64 plot the result, 64
High-back pressure, 126	Passivation, 83
	Personal protection, 11
1	Pressure flow curve, 64
Important user information F	Pressure gauge, 40
Important user information, 5 Installing, 11	Purpose of this manual, 6
precautions, 11	R
L	Recycling, 140
	hazardous substance, 140
Leakage test, 119 Location, 46	Regulatory information, 141
Location, 40	Remove buffer, 75 Replacement, 94
M	tools, 94
141	Restart, 16
Maintenance, 13,82	·
precautions, 13	S
Manufacturing information, 142 Moving, 11	Cafabanatiana 0
precautions, 11	Safety notices, 9 Safety precautions, 9
precautions, 11	introduction, 9
N	Service, 82
	frequency, 82
Nameplate, 14	Service agreements, 82
Net, 47, 95	Setup, 40
adapter, 95	Slurry, 48, 50
end-piece, 47	concentration, 48

preparation, 50 Slurry volume, 48 Stand, 28 Storage, 124 System operation, 12 precautions, 12

Т

Three-port valve, bottom valve, 41 positions, 41

Three-port valve, top valve, 41 positions, 41

Tools, 33, 94 replacement, 94 unpacking, 33

Top and bottom valves, 28

Torque specifications, 101

Transport, 32 crate, 32

Troubleshooting, 126

Typographical conventions, 6

U

UK, 144 conformity, 144 UKCA, 144 marking, 144 Unpacking, 33 instructions, 33

W

Wheel, 35 lock, 35







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