cytiva

# BPG Columns

## Operating Instructions

Original instructions

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# Table of Contents

# 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

# 1.1 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, media and buffer tanks is not covered in this document.

# 1.2 Important user information

## Read this before operating the product

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

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

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

## Intended use of the product

BPG columns are intended for industrial applications that demand high standards of hygiene. BPG columns can be used in conjunction with BioProcess™ Chromatography media. The pressure specifications and low flow resistance make these columns suitable for use with BioProcess media 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.

## 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.

## Notes and tips

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

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

# 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.

## Important

**WARNING**

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

## In this chapter

# 2.1 Safety precautions

## About this section

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



## General precautions

**WARNING**

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

**WARNING**

Only properly trained personnel may operate and maintain the product.

**WARNING**

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

**WARNING**

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

## Flammable liquids and explosive environment

**WARNING**

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

**WARNING**

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

**WARNING**

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

**WARNING**

**EXPLOSION HAZARD!** When connecting the grounding cable, make sure that there is no explosive atmosphere present. Static electricity discharge could occur when connecting the grounding cable.

## Personal protection

**WARNING**

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

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

Always use clean disposable gloves when manually handling parts.

**WARNING**

When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the BPG columns.

## Installing and moving

**WARNING**

Only personnel with appropriate training may move and unpack the delivery crates. All moving and unpacking must be performed according to local regulations.

Even if the safety instructions in the user documentation are followed, it is the customers' responsibility to guarantee the safety of the personnel working with BPG columns.

**WARNING**

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

**WARNING**

Have a minimum of two people on hand, as well as the appropriate lifting equipment for the application, such as a hoist, when moving or lifting a heavier BPG column, i.e., BPG 300 or 450.

**WARNING**

Be careful not to unscrew the wheels too far. If they are unscrewed totally the column may fall and cause injury or damage. Make sure that the wheel support is fully engaged into the stand before start leveling.

**WARNING**

Make sure that the wheel support is fully engaged into the stand before start moving.

**CAUTION**

After moving the column to its resting position, lock the wheel brakes and level the column. Place the column on an even floor area.

**CAUTION**

Make sure that the safety equipment's (for example pressure relief valve or rupture disc) connecting tubing has an unrestricted flow path and connected to waste.

## Operation

**WARNING**

The working pressure of the column should never exceed its design pressure, otherwise there is a risk of personal injury and damage to the column. Always use appropriate safety equipment, for example pressure relief valve or rupture discs.

**WARNING**

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

**WARNING**

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

**CAUTION**

Do not use chemicals with temperatures above the specified limits.

## Maintenance

**WARNING**

Thoroughly check the glass column tube to make sure that it is not cracked or has been 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**. To avoid personnel being exposed to potentially hazardous substances, make sure that the column is properly decontaminated and sanitized before maintenance or service.

**CAUTION**

For continued protection against injury risks due to fluid jets, burst pipes or explosive atmosphere, the piping system must be tested for leakage at maximum pressure when the following situations apply:

- After assembly or maintenance
- Before operation or Cleaning-In-Place (CIP)

**CAUTION**

After assembly, check 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 for slipping accidents.

# 2.2 Symbols and abbreviations

## Introduction

This section describes the information on the system label and other safety or regulatory labels that are attached to the product.

## System label

The system label is located on the back of the equipment. The system label identifies the equipment and shows electrical data, regulatory compliance, and warning symbols.

## Description of symbols on the system label

The following symbols and text may be present on the system label:

| 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. |
|  | **Warning!** Read the user documentation before using the system. |
|  | The equipment complies with applicable European directives.<br>Applicable to BPG 200/950, 300/500, 300/750, 300/950, 450/500, 450/750 and 450/1000. |

## Placement of system label

The system label is placed on the flange on the column.

# 2.3 Emergency procedures

## Precautions

**WARNING**

Pressure may remain in the column and tubing for an extended period after shutdown. Make sure the system 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

# 3.1 Overview

## Introduction

The BPG column can be used conjunction 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 *Column specifications, on page 125*.

All BPG columns are equipped with wheels with brakes, except the BPG 100 column, for which wheel 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 adjuster nut are bolted to the column flange. The 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 µm (PolyPropylene) nets. For media with an average particle diameter <70 µm, change to 10 µm (Polyamide), or 12 µm (PEEK) in both adapters and end-pieces. For big beads use 54 µm nets, see *Recommended net sizes, on page 48*.

## 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.

## 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 may have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.

If potentially explosive liquids will be 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 32*. 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 parts

The image below shows the parts in BPG column 100, 140 or 200.

| Part | Description |
|---|---|
| 1 | Seal adjuster knob |
| 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 |

## Column and adapter


| Part | Description |
|---|---|
| 1 | Column |
| 2 | Adapter |

# 3.3 BPG 300 column

## BPG parts

The image below shows the parts in BPG 300 column.

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

## Column and adapter


| Part | Description |
|---|---|
| 1 | Column |
| 2 | Adapter |

# 3.4 BPG 450 column

## BPG parts

The image below shows the parts in BPG 450 column .


| Part | Description |
|---|---|
| 1 | Seal adjuster knob |
| 2 | Adapter tube, outer |
| 3 | Adapter height adjuster |

| Part | Description |
|---|---|
| 4 | Eyebolt |
| 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 |

## Column and adapter


| Part | Description |
|---|---|
| 1 | Column |
| 2 | Adapter |

# 3.5 Accessories

## Packing extension tube

Packing extension tubes are available for all BPG columns. 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.

## 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.

## 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

# 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.

## Equipment requirements

When transporting and maneuvering BPG columns and adapters the following equipment is recommended:

- Suitable lifting equipment (crane or hoist) for the larger BPG columns, see *Moving the adapter BPG 300 and BPG 450, on page 76*.
- Lifting eyebolts.

## 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.** Make sure that the lifting equipment has the capacity to safely lift the crate weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.

Use proper lifting device 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 start moving.

Be careful not to unscrew the wheels too far before moving. If they are unscrewed totally 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
- Screwdriver
- Suitable wrench or ratchet wrench

## Crate unpacking

**WARNING**

Only personnel with appropriate training may move and unpack the delivery crates. All moving and unpacking must be performed according to local regulations.

Even if the safety instructions in the user documentation are followed, it is the customers' responsibility to guarantee the safety of the personnel working with BPG columns.

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**

Have a minimum of two people on hand, as well as the appropriate lifting equipment for the application, such as a hoist, when moving or lifting a heavier BPG column, i.e., BPG 300 or 450.

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**

Be careful not to unscrew the wheels too far. If they are unscrewed totally the column may fall and cause injury or damage. Make sure that the wheel support is fully engaged into the stand before start leveling.

**CAUTION**

After moving the column to its resting position, lock the wheel brakes and level the column. Place the column on an even floor area.

**NOTICE**

Make sure that the column is located on a level even surface.

## 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 BPG column is operated or maintained in a potentially explosive atmosphere, it **MUST be properly grounded** to avoid static discharge.

**WARNING**

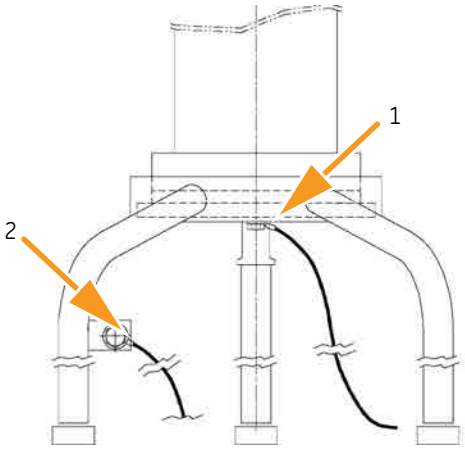
**EXPLOSION HAZARD!** When connecting the grounding cable, make sure that there is no explosive atmosphere present. Static electricity discharge could occur when connecting the grounding cable.

## 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 columns. |
| 2 | Grounding terminal on BPG 300 column. |

# 4.6 Setup

## Precautions

**WARNING**

The working pressure of the column should never exceed its design pressure, otherwise there is a risk of personal injury and damage to the column. Always use appropriate safety equipment, for example pressure relief valve or rupture discs.

**CAUTION**

Make sure that the safety equipment's (for example pressure relief valve or rupture disc) connecting tubing has an unrestricted flow path and connected to waste.

**NOTICE**

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

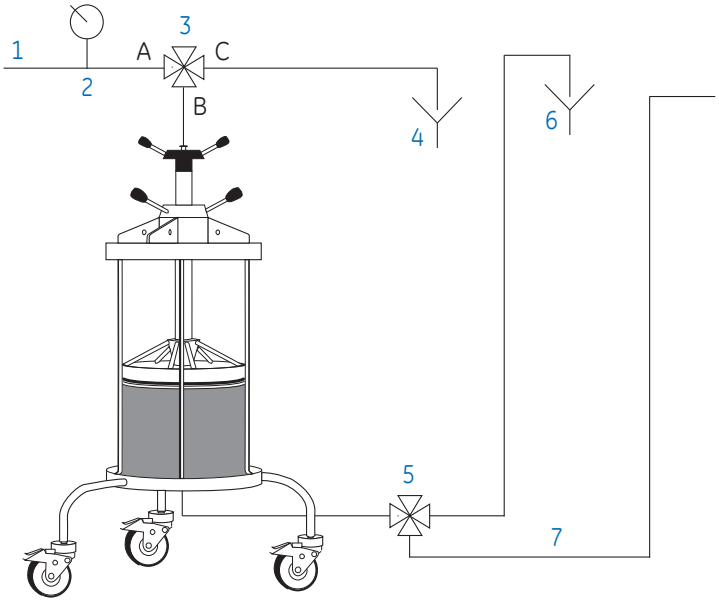
**NOTICE**

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

## Column setup

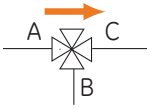
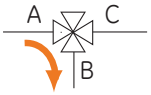
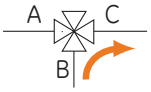
The column is fitted with a 4-port 4-way valve on the adapter inlet (B), and a 4-port 2-way valve on the bottom outlet.

- One port (A) on the top has a pressure gauge followed by tubing connected to a pump. Another port (C) is directed to the waste for purging the pump and column.
- The bottom valve is equipped with tubing on a port perpendicular to the column outlet that will be directing flow to waste, or recycling water back to the tank.




| Flow | Description |
|---|---|
| 1 | From pump |
| 2 | Pressure gauge |
| 3 | 4-port, 4-way valve |
| 4 | Drain |
| 5 | 4-port, 2-way valve |
| 6 | Drain |
| 7 | Return |

## Four-port valve positions

| Flow | Valve position | Description |
|---|---|---|
| A to C |  | Mobile phase from pump to waste |
| A to B |  | Mobile phase to column |
| B to C |  | Column to waste |

# 5 Operation

## About this chapter

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

## In this chapter



## Precautions

**WARNING**

Only properly trained personnel may operate and maintain the product.

**WARNING**

Thoroughly check the glass column tube to make sure that it is not cracked or has been 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 should NEVER be air or gas under pressure in the column.

**WARNING**

The working pressure of the column should never exceed its design pressure, otherwise there is a risk of personal injury and damage to the column. Always use appropriate safety equipment, for example pressure relief valve or rupture discs.

**CAUTION**

Do not use chemicals with temperatures above the specified limits.

**NOTICE**

Make sure that any water is WFI, since particles can block and damage the nets.

**NOTICE**

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

# 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.

## In this section



## 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

**NOTICE**

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

## Materials

The following materials are suitable for the column packing:

- BPG column with suitable net for the media
- Media about 50% slurry in water
- WFI for packing

## Recommended net sizes

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

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

[1] Polyamide
[2] Polyetheretherketone
[3] Polypropylene

## Packing extension tube

If a packing extension tube is used it will either remain in place for very long bed heights, or be removed during the packing procedure when the media is below the top of the column tube.

## 5.1.1 Column flow packing method

### Packing flow rate

Packing flow rates should be found in the leaflet supplied with the media. The optimum packing flow rates is dependent on temperature, type of media,concentration and the packing method. Consequently, the optimum flow rate or rates must be determined empirically by producing a pressure/flow rate curve.

It is advisable to do a pressure flow curve with a similar volume media that will be packed in the column.

### Method description

Constant flow packing is a two step method that is applicable to all types of media. 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.

1. In the first step, 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 second step, the flow rate is increased to a predetermined optimal value to obtain the desired packing density. When flow is stopped, the proximity of the adapter to the bed minimizes the time necessary to successfully reposition the adapter to stabilize the packed bed.

# 5.1.2 Slurry preparation

## Slurry concentration

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

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 *Instruction 29112530*.

## Compression factor

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

*Compression Factor CF = (gravity settled bed height)/(packed bed height)*

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

## Amount of media and slurry volume

The amount of media 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 media needed, liter

$\pi = 3.14$

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

$H$ = bed height in column, cm

$Cf$ = Compression factor of media

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

## Media quantities required per cm bed height

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

| BPG column | Column diameter (mm) | (π × r2 × H × Cf/1000) | Amount of media[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 media[1] per cm of packed bed |
|---|---|---|---|
| BPG 200 | 200 | 314 × 1.15 | 361 ml |
| BPG 300 | 296 | 0.707 x 1.15 | 812 ml |
| BPG 450 | 446 | 1.59 x 1.15 | 1.83 l |

[1] 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

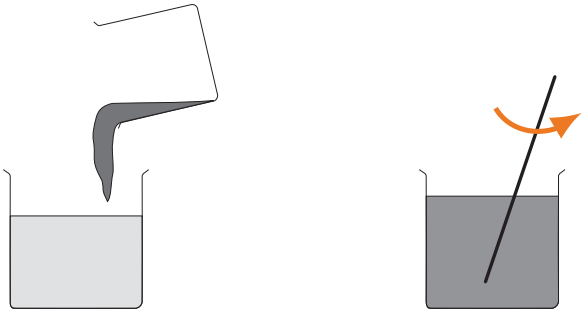
The Sephadex™ G25 media swell 4 to 5 times (1 g = 4-5 ml of media). The table shows the amount of media per centimeter of packed bed.

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

| Media | Amount of media per liter of packed column (g) | Pack factor (PF) (l) | Amount of media per cm of packed bed (g) | | | | |
|---|---|---|---|---|---|---|---|
| | | | BPG 100 | BPG 140 | BPG 200 | BPG 300 | BPG 450 |
| Sephadex G-25 C and M grades | 250 | 1.15 l | 22.6 | 43.9 | 90.3 | 202.4 | 453 |
| Sephadex G-25 grade SF | 280 | 1.15 l | 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 | Make sure that the slurry is homogeneous. |
| 2 | Pour the slurry into a suitable container for pouring into the column. |
| |  |
| 3 | Check the slurry concentration. |

# 5.2 Column packing

## About this section

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

## In this section

# 5.2.1 Preparing the column

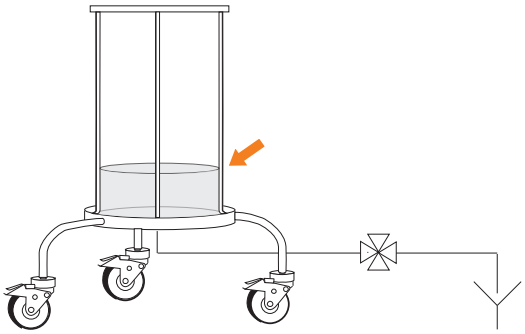
## Column preparation

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.

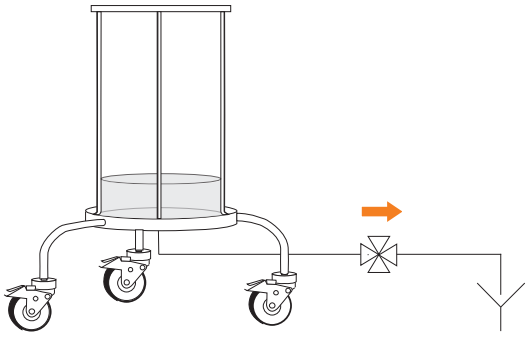
## Remove air from bottom net

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.

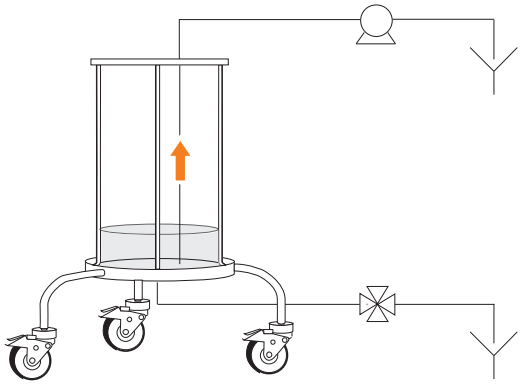
| Step | Action |
|---|---|
| 1 | Make sure that the column is level, see *Section 4.4 How to level the column, on page 38*. |
| 2 | Make sure that the column is grounded, see *Section 4.5 Grounding kit, on page 40*. |
| 3 | Wet the column net with 20% ethanol and pour 2 to 3 cm of packing buffer into the column with the bottom valve closed and the outlet to drain. |



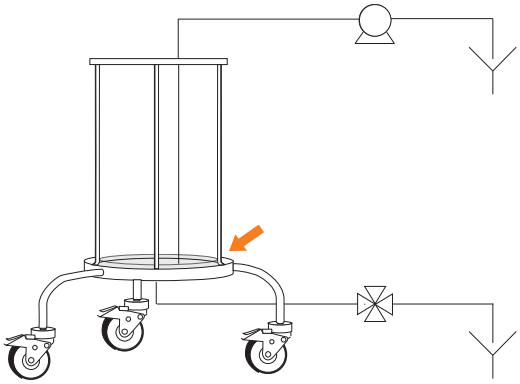
| Step | Action |
|---|---|
| 4 | Open the outlet valve at the bottom of the column and allow liquid and air to drain from the bottom distributor plate. |



| | |
|---|---|
| 5 | Close the bottom valve. |
| 6 | Connect a pipe to a pump, or a vacuum line, and lower the pipe down onto the surface of the bottom net. |
| 7 | Start the suction to remove any remaining air from under or on the surface of the bottom net. |



| Step | Action |
|---|---|
| 8 | Continue until there is only 1 cm of buffer in the column. |

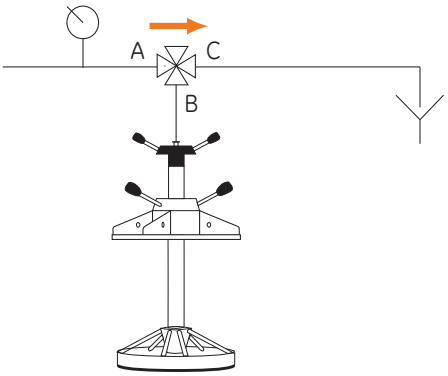


*Result:*
The column tube is now ready for packing.

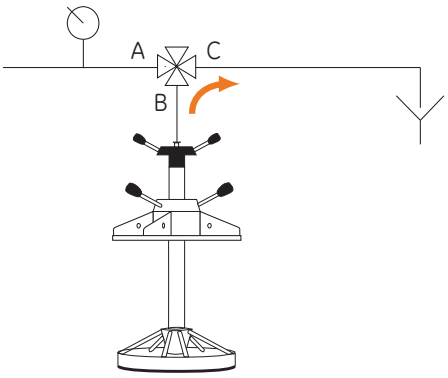
## 5.2.2 Preparing the adapter

### Position and seal the adapter

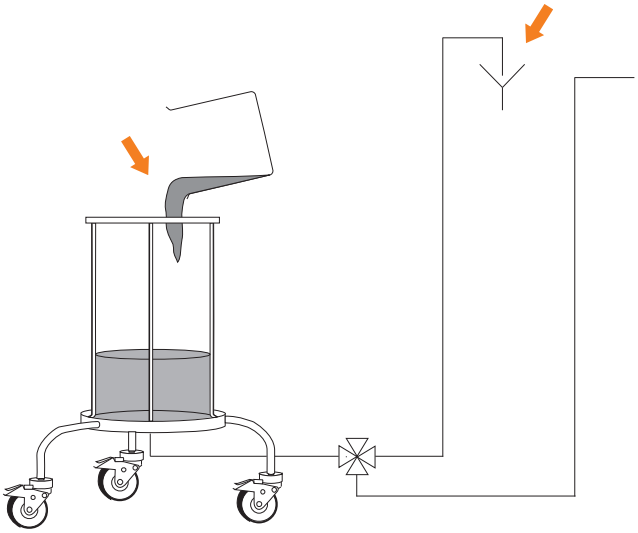
| Step | Action |
|---|---|
| 1 | Connect the 4-port, 4-way valve, to the top of the adapter. |
| 2 | Position the valve from pump to waste (A to C) to purge air and start the pump. |



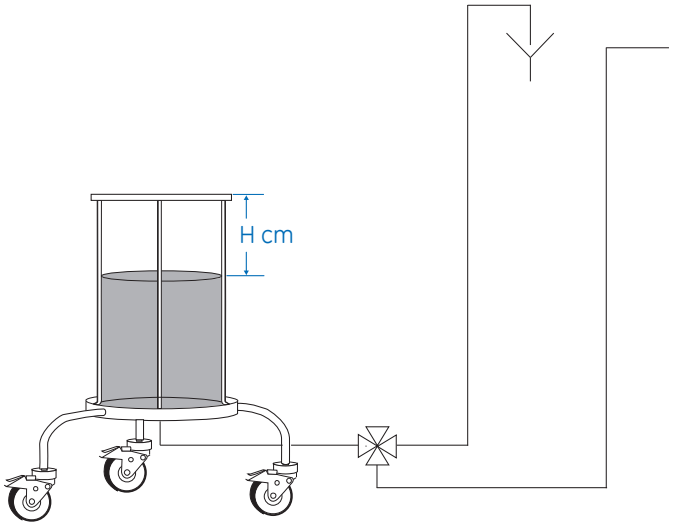

| | |
|---|---|
| 3 | Stop the pump once all air is removed. |
| 4 | Position the valve from adapter to waste (B to C). |



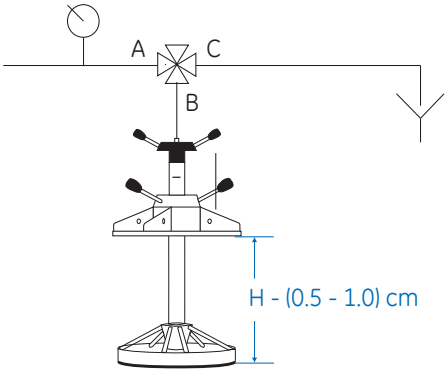
| Step | Action |
|---|---|
| 5 | Carefully pour the homogenous slurry into the column. Make sure that no air bubbles are trapped within the slurry and the bottom outlet is higher than the column. |



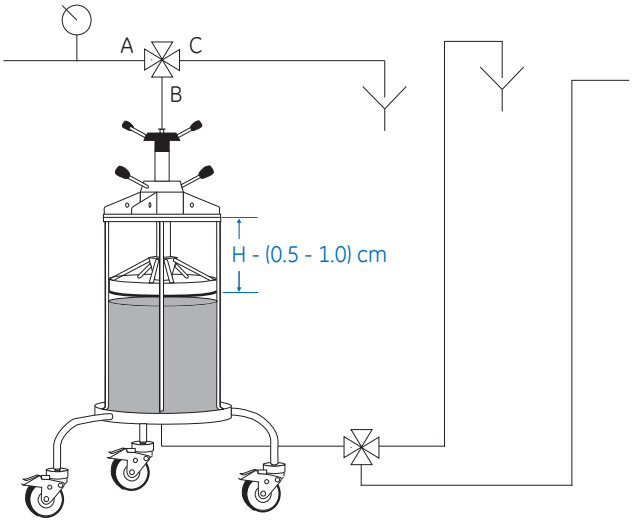
| | |
|---|---|
| 6 | Measure the height (H) from the top of the column to the surface of the slurry. |



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




| | |
|---|---|
| 8 | Insert the adapter in the column just above the slurry level in the column with the seal fully relaxed. |




**WARNING**

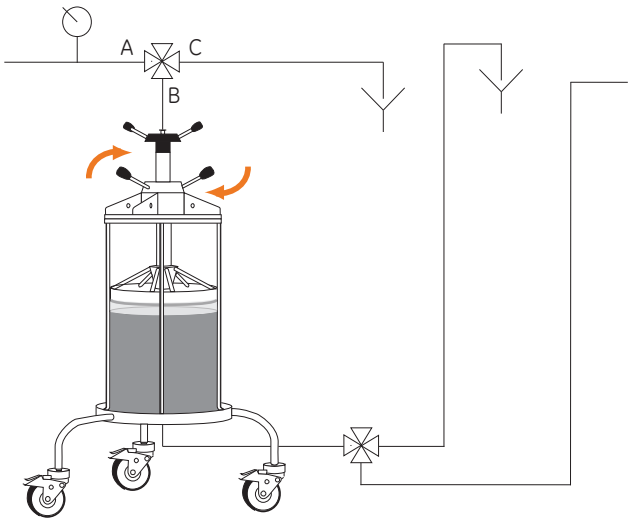
Pay extra attention when fitting the adapter to the column as there is a risk of crushing your hands.

| Step | Action |
|---|---|
| 9 | Secure the column adapter in place. |

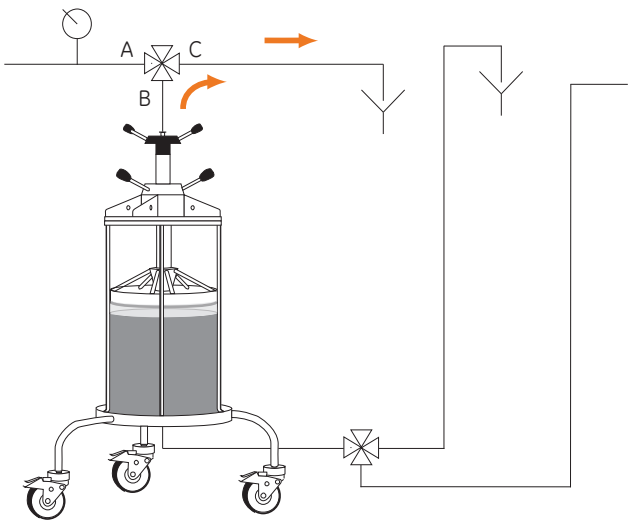
| | |
|---|---|
| 10 | Seal the adapter O-ring by turning the seal adjuster knob or handle on the top of the adapter, clockwise. |

| | |
|---|---|
| 11 | Wait until a small clear zone is visible above the slurry. |

| Step | Action |
|---|---|
| 12 | Move the height adjuster handle clockwise to position the adapter 0.1 to 0.5 cm below the top surface of the liquid. |




| | |
|---|---|
| 13 | *Result:*<br>Air, and then liquid, is pushed up through the adapter and out to waste as the adapter is pushed downwards. |



# 5.2.3 Packing the column - first step

## Description of the first step

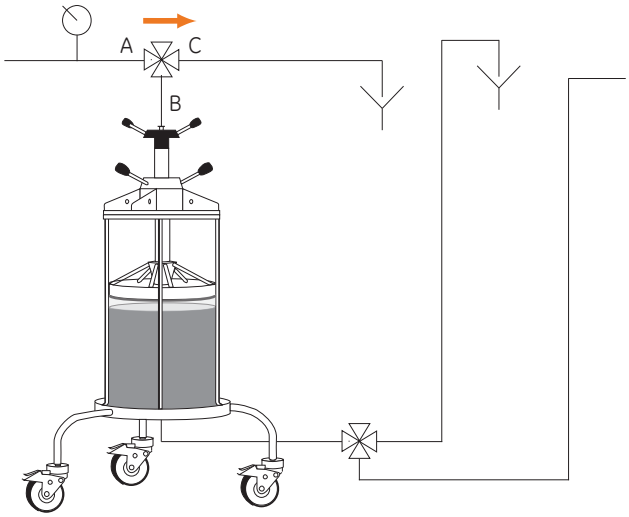
The first step of constant flow packing is to form a consolidated bed at a low flow rate (20 – 30 cm) and position the adapter near the final bed height. The top valve is placed in position to allow buffer to exit from the column up through the adapter to waste. The adapter is again lowered until air under the net and in the adapter shaft has been purged. The pump is started and the tubing to the column also purged of air without changing the valve position.

The flow should be quickly adjusted to approximately that desired for the first step and then the top valve turned to place the column inline with the pump. The bottom valve is immediately opened to waste or recycle, and the flow rate adjusted as needed. After the bed height of the media stabilizes in one to two column volumes (1 to 2 CVs). The adapter O-ring is loosened so buffer will pass above it, and the adapter lowered to 0.5-1.0 cm above the top of the bed.

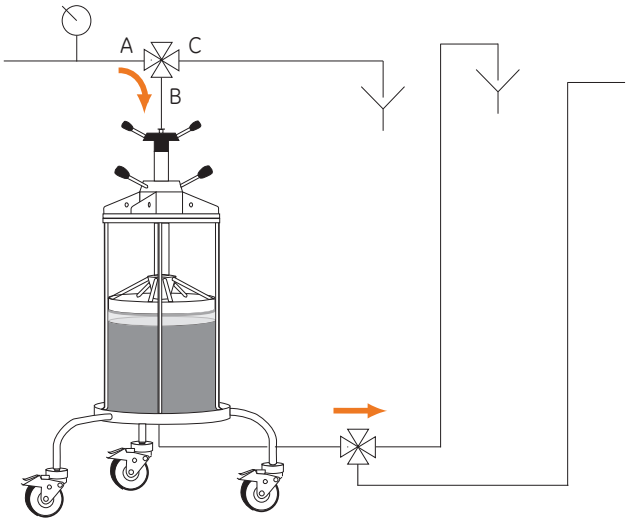
The O-ring is retightened and the adapter lowered to 0.1 to 0.2 cm above the bed with buffer passing out the top valve to waste. The top valve should be turned to place the pump in line with the column again and the bottom valve opened. The user should proceed immediately to the second packing step.

## First step

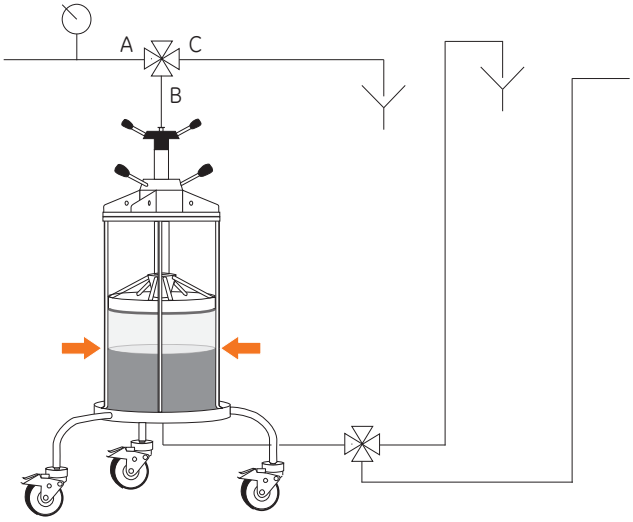
| Step | Action |
|---|---|
| 1 | Remove any air in the hoses by positioning the valve from system to waste (A to C) and start the pump. |



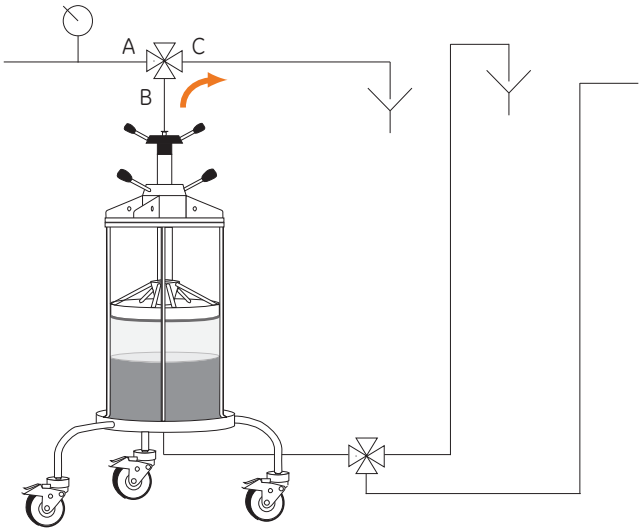
| Step | Action |
|---|---|
| 2 | Position the valve to flow from the pump to the column (A to B) through the inlet on the adapter, and at the same time open the bottom valve to waste. |



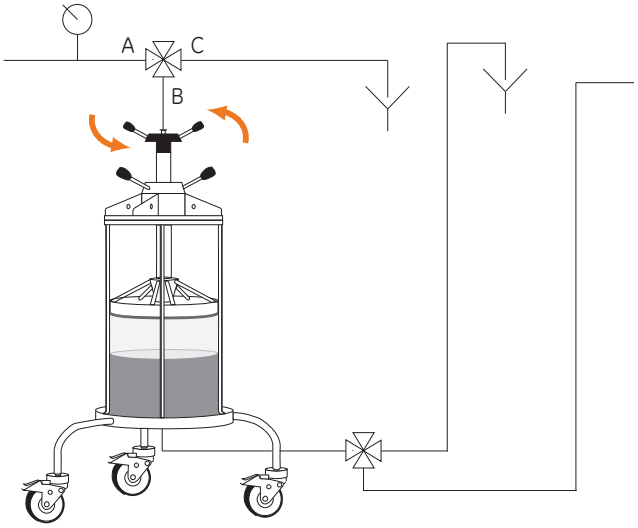

| | |
|---|---|
| 3 | Check the packing flow rate and adjust as necessary. |
| 4 | Stop the pump when the bed has settled, normally one to two column volumes. |



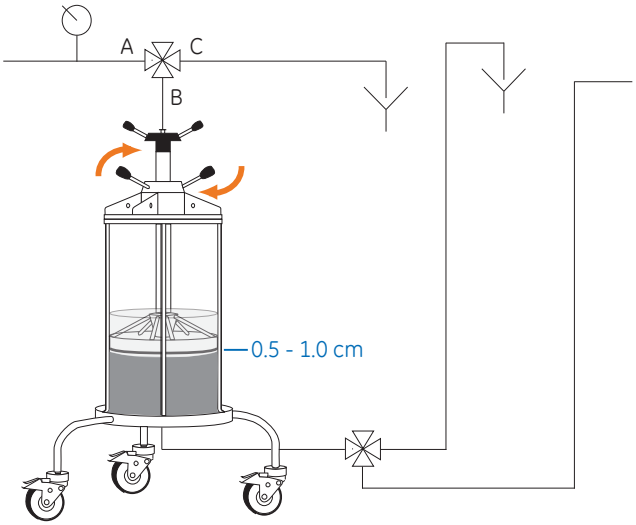
| Step | Action |
|---|---|
| 5 | Close the bottom valve when the pressure in the column has returned to zero and set the top valve to waste (B to C). |



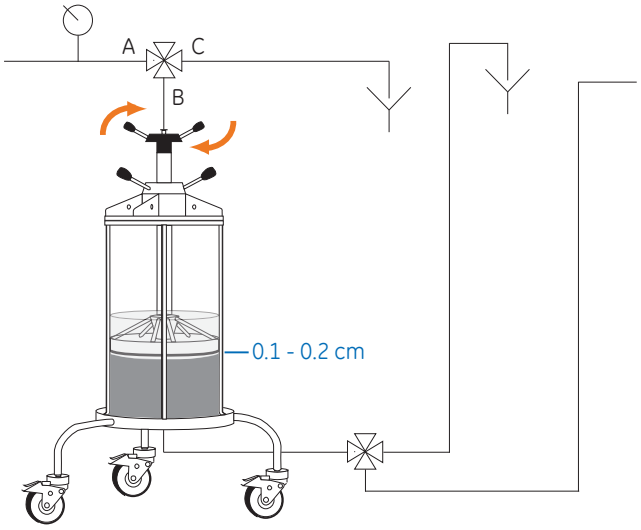

| | |
|---|---|
| 6 | Loosen the adapter O-ring seal by turning the adjuster knob, or the seal adjuster handle, on the top of the column counter-clockwise. |

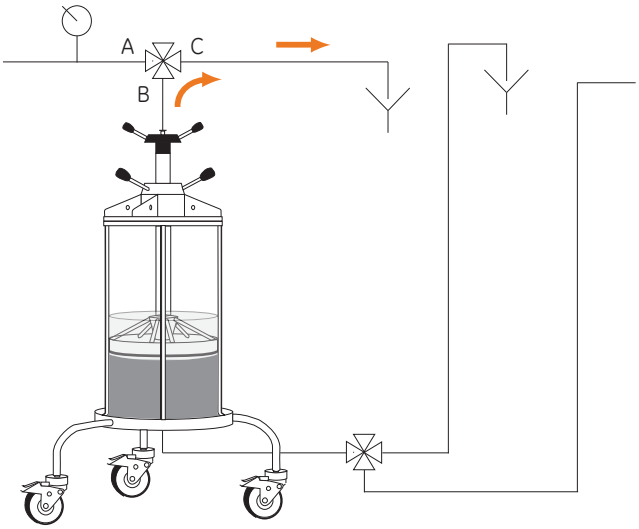


| Step | Action |
|---|---|
| 7 | Lower the adapter quickly by turning the height adjuster handle clockwise. The buffer between the sedimented bed and the adapter passes between the adapter O-ring and the column wall. During this operation the bed will start to rise. Stop lowering the adapter when it is 0.5 to 1.0 cm above the bed. |




| | |
|---|---|
| 8 | Seal the adapter O-ring by turning the knob, or seal adjuster handle, clockwise and lower the adapter to 0.1 or 0.2 cm above the bed. |



| Step | Action |
|---|---|
| 9 | *Result:*<br>The buffer is passing out the top valve to waste (B to C).<br>  |
| 10 | Close the top valve. |

# 5.2.4 Packing the column - second step

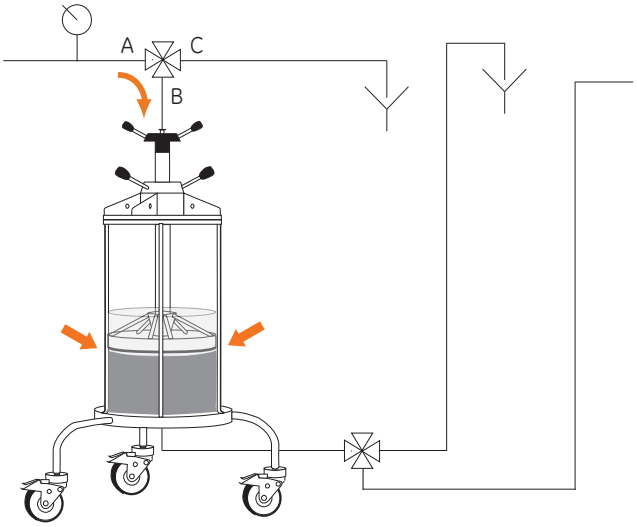
## Description of the second step

The second step of flow packing is to compress the bed using a flow rate predetermined from the pressure flow curve data, and rapidly set the adapter position to maintain the packed bed density. The pump is adjusted quickly to the desired flow rate to obtain the final bed height, and the integrity of the adapter O-ring seal checked and further tightened if necessary. When the bed has stabilized in about 1 CV, the bed height is marked on the column tube. The top valve is turned to place the pump out of line with the column at the same time as the bottom column valve is closed.

The adapter must be rapidly lowered into place with minimal disturbance of the packed bed. This should be done with the adapter O-ring just loose enough that buffer exits out of the top valve and not around the O-ring. Once the adapter is in place, the O-ring should be retightened immediately. If the packed bed decompresses significantly above the marked bed height before the adapter can be lowered in place, this second packing step can be repeated once. Wait at least 5 minutes for the bed to cure before moving the column.

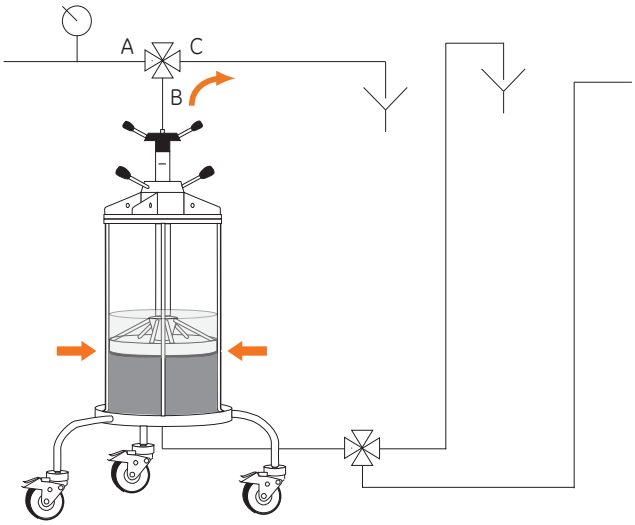
## Second step

| Step | Action |
|---|---|
| 1 | Open the bottom valve and put the top valve from pump to column (A to B) and start the pump. This will move the bed down and once stabilized the level should be marked on the tube. |




| | |
|---|---|
| 2 | Stop the pump. |

| Step | Action |
|---|---|
| 3 | When the pressure reaches zero, position the top valve to waste (B to C), and move the adapter down, pushing into the media until the adapter reaches the mark on the tube. |



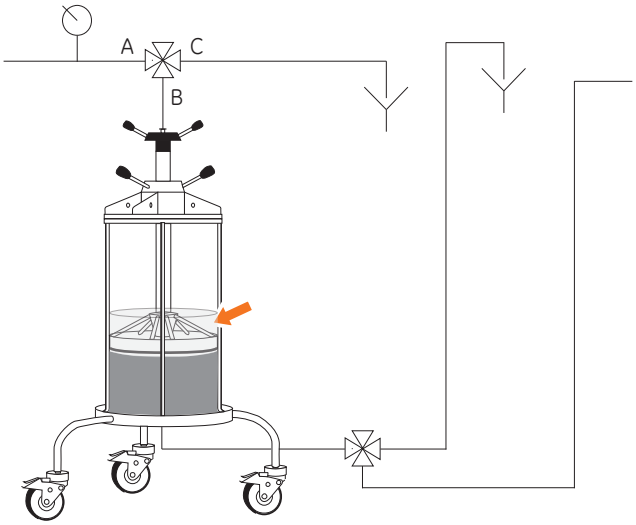

| | |
|---|---|
| 4 | Close the top valve and the bottom valve. |
| 5 | *Result:*<br>The column is now packed. |
| 6 | Test the packed column, see *Section 5.3 Performance evaluation of the column, on page 72*. |

## Remove buffer from adapter

The design of BPG columns allows buffer to be on top of the adapter while packing. When packing is completed 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 132*.

Follow the instructions below to remove buffer from adapter:

| Step | Action |
|---|---|
| 1 | Siphon or pump out the buffer. |




| | |
|---|---|
| 2 | Wash, if necessary. |
| 3 | Repeat. |

# 5.2.5 Storage and unpacking of packed columns

## Storage

Both outlets of the column should be sealed after equilibrating the column in storage buffer. The column must be stored in a temperature-controlled environment with water or 20% ethanol above the adapter O-ring to prevent it from drying out and cracking. Covering the column with a clean plastic bag helps prevent dust and debris from accumulating on exterior surfaces.

## Unpacking

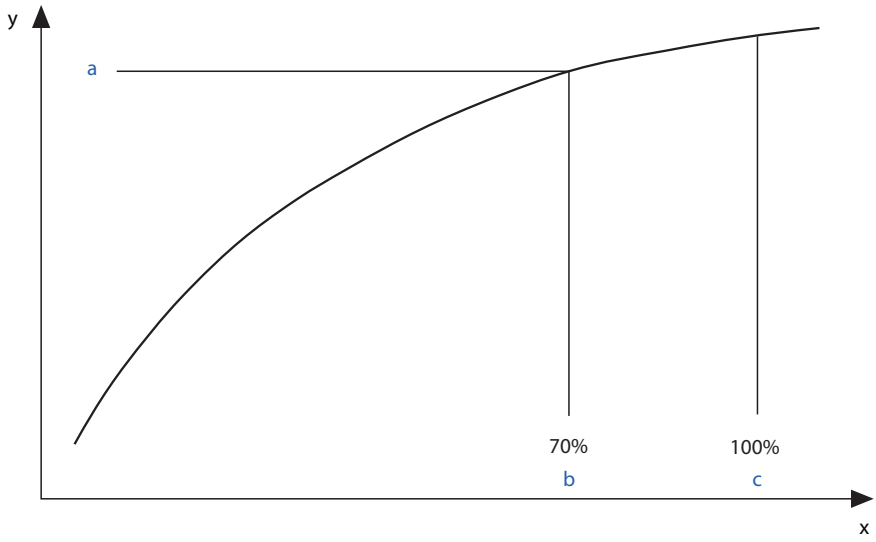
Place several centimeters of buffer or water above the adapter O-ring of the BPG column and fully loosen the wing nut which seals the adapter O-ring. Make sure the adapter outlet is open to waste. If the adapter is not easily raised, try slightly lowering it to break the O-ring seal on the glass tube. If the O-ring rolls, tighten the wing nut a bit to prevent this 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 will help to lift and disperse the media more rapidly and clear the bottom net.

## 5.2.6 Pressure flow curve

### Plot the result

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 media there will be no plateau, in those cases use the flow rate at maximum allowable pressure for the column. The figure below shows the pressure flow curve.




The description of the axis are:

x = Pressure

y = Flow rate

The characters in the table are described below:

| Value | Meaning |
|---|---|
| a | Packing flow rate |
| b | Packing pressure |
| c | Max pressure |

# 5.3 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 media giving you all relevant information you need.

## 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 media should be repacked or replaced.

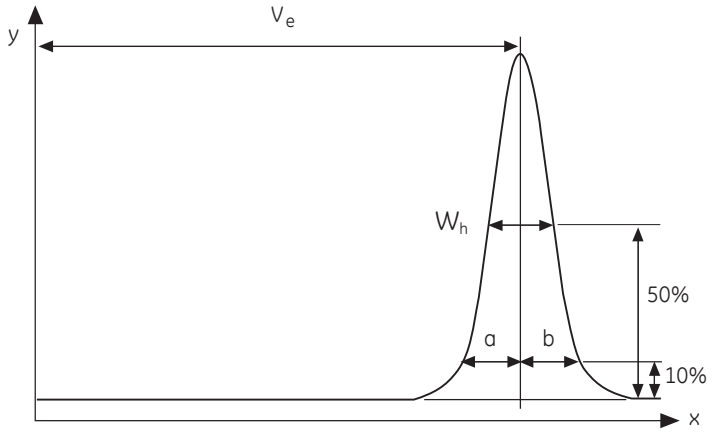
A widely used method for expressing the efficiency of a packed bed is in terms of the height equivalent to a theoretical plate (HETP) and the peak asymmetry factor ($A_S$). These values can easily be determined by applying a sample of for example acetone or NaCl solution to the column.

## Measuring HETP and $A_S$

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 ensure best column performance. Follow the specific instructions for the packed media 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 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

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.

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.

$A_S = b/a$

# 6 Maintenance

## About this chapter

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

## In this chapter



## Precautions

**WARNING**

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

**WARNING**

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

## Moving the adapter BPG 300 and BPG 450

**WARNING**

Have a minimum of two people on hand, as well as the appropriate lifting equipment for the application, such as a hoist, when moving or lifting a heavier BPG column, i.e., BPG 300 or 450.

The adapters on BPG 300 and 450 columns are heavy. For safety reasons, it is recommended that the adapter is lifted with an adapter handling unit or ceiling hoist connected to the eyebolts on the adapter top plate.

# 6.1 Service and preventive maintenance

## Precautions

**WARNING**

Only properly trained personnel may operate and maintain the product.

**WARNING**

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

**NOTICE**

If the column tube or the rods are replaced, the column assembly must be checked in accordance with local authority instructions.

## 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 ensure the protection and 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.

Please complete the checklist in the *On Site Service Health and Safety Declaration Form* or the *Health and Safety Declaration Form for Product Return or Servicing*, depending on whether the instrument is going to be serviced on site or returned for service, respectively.

## Health and safety declaration forms

Health and safety declaration forms are available for copying or printing in the *Reference information* chapter of this manual, or on 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 Replacements of nets and O-rings

## In this section



## 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 sizes, on page 48*.
- 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 once each year. It may 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.*

## Liquids required for changing nets and O-rings

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

## 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 |
|  | Spanners |
|  | Torque wrenches for hexagonal socket fittings and torque settings of 4, 5, 6, 6.5, 8 Nm |
|  | 12-point opening sockets |
|  | Screwdrivers |

## 6.2.1 Replacements in 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 |  | Snap plug | N/A | No | Yes |
| 5 |  | Support net | Yes | Yes | Yes |
| 6 |  | Net | Yes | Yes | Yes |

## 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 |
|---|---|
| 1 | Turn the handles on the adjusting handle (or knob) several turns to loosen the adapter O-ring 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 according to the illustration below.

| Step | Action |
|---|---|


**NOTICE**

Do not loosen each nut completely in one movement. Three to four turns per nut is enough to loosen and remove the nuts.

3

**CAUTION**

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

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

| Step | Action |
| --- | --- |
| |  |

## 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.*

| | |
|---|---|
| 3 | 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 81*. |

| Step | Action |
|---|---|

5 Wet the net with 20% ethanol to remove all air bubbles trapped in the net. If the net is tight, warm it in 50°C water.

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

## Replace the O-ring in the adapter

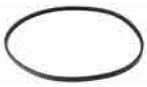
**NOTICE**
Make sure not to scratch any steel surfaces of the column when removing seals and O-rings. If scratched, the sealing of the column may be compromised and leakage may result.

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. |

## 6.2.2 Replacements in the column

### 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 |  | Snap plug | N/A | N/A | Yes |
| 7 |  | Distribution plate | N/A | Yes | Yes |
| 8 | | End-piece | Yes | Yes | Yes |

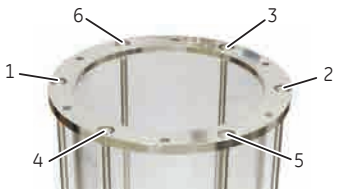
## Torque wrench

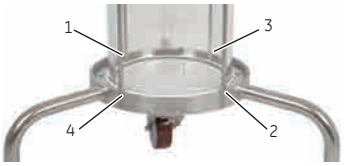
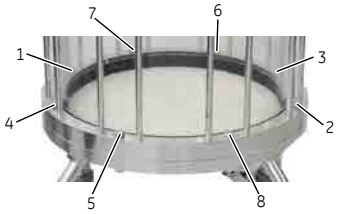
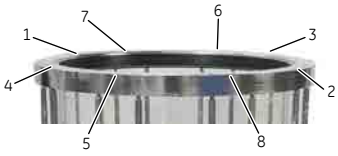
Use the torque wrench listed in the following table when securing the column tube to the end-piece. The torque apply for both ends of rods for BPG 450. For BPG 100 to 300 the rod is threaded into the bottom flange and the same torque apply as for the top.

Tighten the bolts in crosswise sequence according to figures below.

**NOTICE**

Tighten the nuts and bolts close to the given torque. But do not exceed the given torque, this could damage the column. Always use a torque wrench.

| BPG | Description | 100 | 140 | 200 | 300 | 450 |
|---|---|---|---|---|---|---|
|  | Support rod nuts (4) | 4 Nm | 5 Nm | 5 Nm | N/A | N/A |
|  | Support rod nuts (6) | N/A | N/A | N/A | 6 Nm | N/A |
|  | Adapter top plate bolts | N/A | N/A | N/A | 6.5 Nm | N/A |

| BPG | Description | 100 | 140 | 200 | 300 | 450 |
|---|---|---|---|---|---|---|
|  | Support rod nuts (4) | 4 Nm | 5 Nm | 5 Nm | 6 Nm | N/A |
|  | End-piece domed nuts | N/A | N/A | N/A | N/A | 8 Nm |
|  | Support rod bolts top and bottom. | N/A | N/A | N/A | N/A | 4 Nm |

## Disassemble the column

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.*

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

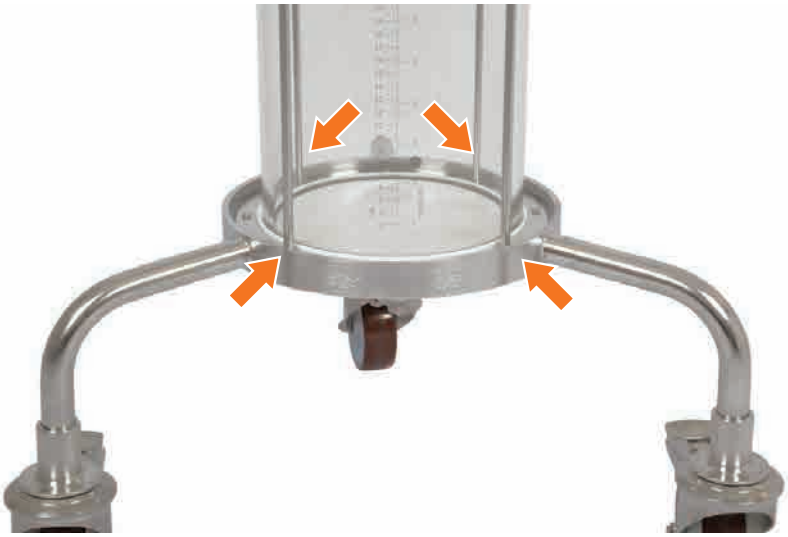

| | |
|---|---|
| 2 | Remove the flange carefully. |

**NOTICE**

When the flange is 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. |

| Step | Action |
|---|---|



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

5 Remove the bolts and washers securing the end-piece to the stand using a spanner.

| Step | Action |
|---|---|
| 6 | Remove the nets and O-ring.<br><br>***Tip:***<br>*To be able to remove the O-rings without damaging other parts, spray 20% ethanol over the flange or seal, or if that does not work, pour 50°C hot water into the column and let stand for 15 to 30 minutes. If solvents are not compatible with EPDM seals, change to seals in FEP/PFR.*<br><br>**NOTICE**<br>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. |

## 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 to other BPG columns.*

| Step | Action |
|---|---|
| 1 | Fit new end-piece items according the order in table *End-piece items, on page 87*.<br><br>***Note:***<br><br>*Wet the net with 20% ethanol to eliminate trapped air.*<br><br>***Note:***<br><br>*BPG 450: Carefully place the column tube on the U-shaped seal. Make sure the flange is centered on the U-shaped seal.* |
| 2 | Secure the end-piece to the stand using the bolts and washers.<br><br>  |

## Change top and bottom flange O-rings in the column

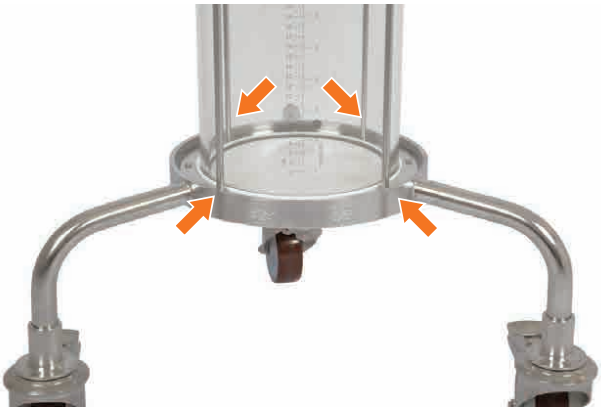
Follow the instructions below to change the top and bottom flange O-rings in the column.

| Step | Action |
|---|---|
| 1 | Disassemble the column according to *Disassemble the column, on page 90*. |
| 2 | Position and fit the bottom flange O-ring. |
| 3 | Put back the glass tube. |
| 4 | Position and fit the new top O-ring. |
| 5 | Assemble the column according to *Assemble the column, on page 95*. |

## Assemble the column

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

***Note:*** *The instructions shows a BPG 140 column but is applicable to other BPG columns.*

| 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.<br><br>***Note:***<br>*BPG 450: Carefully place the column tube on the U-shaped seal. Make sure the flange is centered on the U-shaped seal.*<br><br>**NOTICE**<br>The glass tube can crack if touching any stainless steel. |
| 2 | Screw the support rods into the end-piece, see *Torque wrench, on page 88*. |
| 3 | Fit the column tube to the end-piece with the bolts.<br>  |
| 4 | Position the flange so that the support rods fit into the appropriate holes. |

| Step | Action |
|---|---|

5 Make sure that the O-ring on the flange is aligned with the glass tube.

**NOTICE**
Twisted or deformed O-rings will cause leakage.

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

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 wrench, on page 88*.

**NOTICE**
Do not exceed the given torque, this could damage the column.

9 Tighten the nuts in cross-wise sequence evenly as shown in *Torque wrench, on page 88*.

**CAUTION**
After assembly, check that the column is tight and not leaking.

| Step | Action |
|---|---|
| |  **NOTICE** Failure to use cross and stepwise tightening can damage the column tube. |

# 6.3 Replacement sealing washer

## Replace the PTFE sealing washer in valves

Follow the instructions below to replace the sealing washer.

| Step | Action |
|---|---|
| 1 | Unscrew the retaining nut at the bottom of the valve with help of the handle.  |
| 2 | Insert the new PTFE sealing washer in the positioning disc. |
| 3 | Insert the positioning disc in the square of the tapered plug (the PTFE sealing washer has to touch the body). |
| 4 | Insert the dished washer (the larger diameter has to touch the positioning disc). |
| 5 | Tighten the retaining nut until the dished washer is flat. |
| 6 | Tighten the retaining nut up 1/4 turn. |
| 7 | Stabilize the valve for 24 hours, minimum (as PTFE is a floating material). |
| 8 | Screw the retaining nut off 3/8 turn (the tightening torque of the retaining nut is 1.5 Nm). |

# 6.4 Fitting a packing extension tube

## Precautions

**NOTICE**

Do not pack the column to bed heights higher than the length of the column, i.e., the adapter should seal against the column wall, NOT against the extension tube wall. A tube length of 10 cm is required when installing the adapter onto the column tube 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.

| 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. |
| 3 | Use the washers and domed nuts from the column lid to secure the extension tube to the column. |
| 4 | The extended column is now ready for the first packing step in two-step packing procedures. |

## BPG 300 and 450 columns

The extension tube is assembled when delivered.

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

| Step | Action |
|---|---|
| 1 | Remove the adapter from the column and place the extension tube onto the upper flange. |
| 2 | Make sure that the thin O-ring is properly positioned in the flange. |
| 3 | Use the bolts delivered with the extension tube to secure the extension tube to the column. |
| 4 | The extended column is now ready for the first packing step in two-step packing procedures. |

# 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.

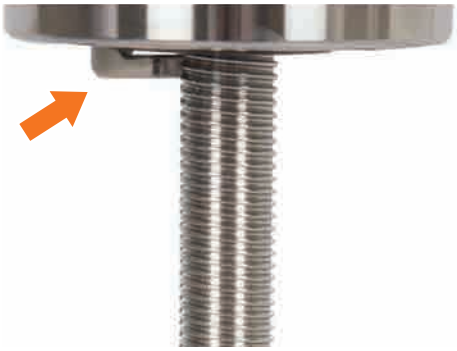
***Note:*** *The instructions show a BPG 300 column but is applicable to other BPG columns.*

| Step | Action |
|---|---|
| 1 | Remove the adapter from the column tube according to instructions in *Remove the adapter, on page 82*. |
| 2 | Remove the adapter screw bolts. |

| | |
|---|---|
| 3 | Lift off the adapter top plate and adapter screws. Take care not to bend the inlet tube. |
| 4 | The top plate assembly can be disassembled by removing the screws. |

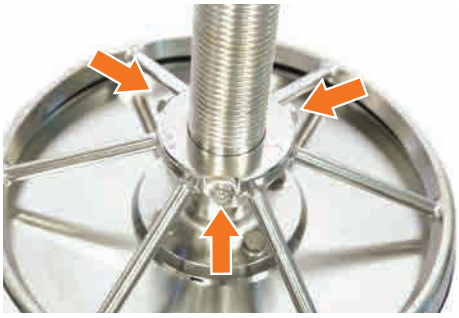
| Step | Action |
|---|---|
| 5 | Unscrew and remove the cap screws that secure the stopper on the adapter top plate with the hex key. |

| | |
|---|---|
| 6 | Remove the stopper. |



| Step | Action |
|---|---|
| 7 | Remove the stop screw and locking nut from the top plate. |

| | |
|---|---|
| 8 | Unscrew the bolts on the upper ring of the sealing unit. This will release the sealing unit and the inner adapter tube. |



| Step | Action |
|---|---|
| 9 | Carefully unscrew and remove the adjusting knob. This releases the tension on the spring situated under the inner adapter tube. |

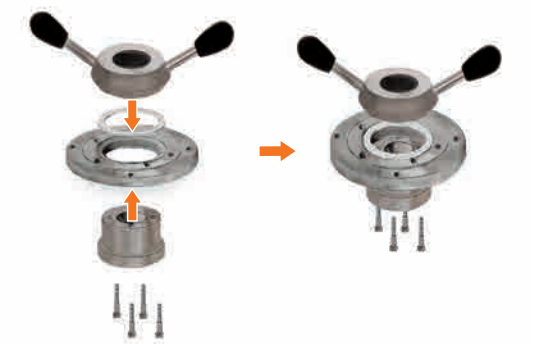
| | |
|---|---|
| 10 | Remove the top-plate assembly by turning it in a clockwise direction. |

| Step | Action |
|---|---|
| 11 | Remove the sealing unit and the adapter O-ring. |

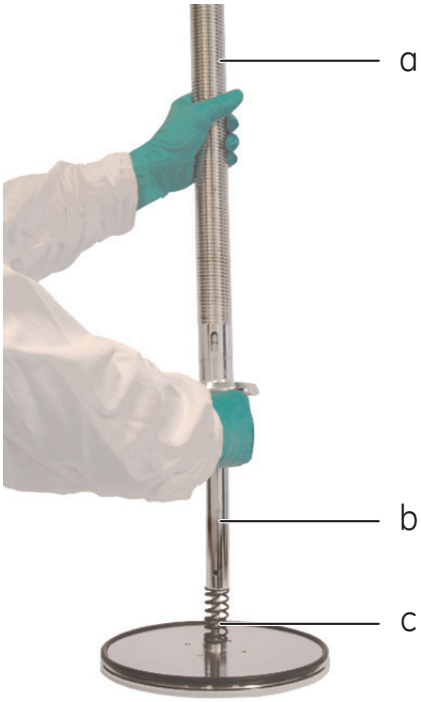
## Assemble the adapter

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.<br>***Note:***<br>*This image shows BPG 140 column but is applicable to other BPG columns.*<br>  |
| 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.<br>***Note:***<br>*This image shows BPG 140 column but is applicable to other BPG columns.*<br>  |
| 3 | Slide the spring (c), inner adapter tube (b) and outer adapter tube (a) onto the adapter plate. |

| Step | Action |
|---|---|




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

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

| Step | Action |
|---|---|

Torque:

| | |
|---|---|
| BPG 100 | 4Nm |
| BPG 200 | 4Nm |
| BPG 300 | 8Nm |
| BPG 450 | 8Nm |

6 Slide the sealing unit onto the adapter tube.

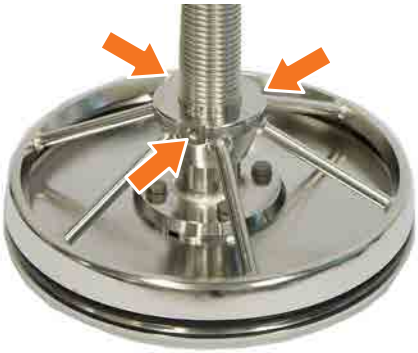
7 In a counter clockwise direction, screw down the top plate assembly 5 cm along the outer adapter tube.

| Step | Action |
|---|---|

8 Turn the adjusting knob 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 18* for respective size of the adjusting knob.)

9 Use a flashlight and a screwdriver through one hole to lock the inner tube in position. Then two of the adapter head screws can fasten the sealing unit in place.

| Step | Action |
|---|---|

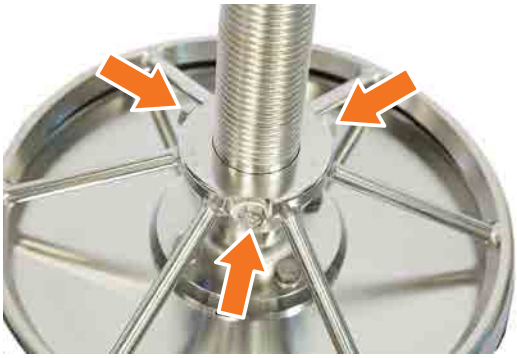


10 Level the hole in the outer adapter tube with the inner adapter tube with the help of a fine screwdriver.

11 Remove the screwdriver and fit the third screw.

| Step | Action |
|---|---|

12 Tighten the screws with a spanner.



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

| Step | Action |
|---|---|

14 Turn the stop screw until it engages the groove in the outer adaptor tube, preventing sideways movement of the tube without preventing vertical movement.

15 Lock the stop screw in position with the locking nut.

16 Attach the nets on the adapter plate.

# 6.6 Leakage test

## Precautions

**CAUTION**

For continued protection against injury risks due to fluid jets, burst pipes or explosive atmosphere, the piping system must be tested for leakage at maximum pressure when the following situations apply:

- After assembly or maintenance
- Before operation or Cleaning-In-Place (CIP)

## Perform leakage test

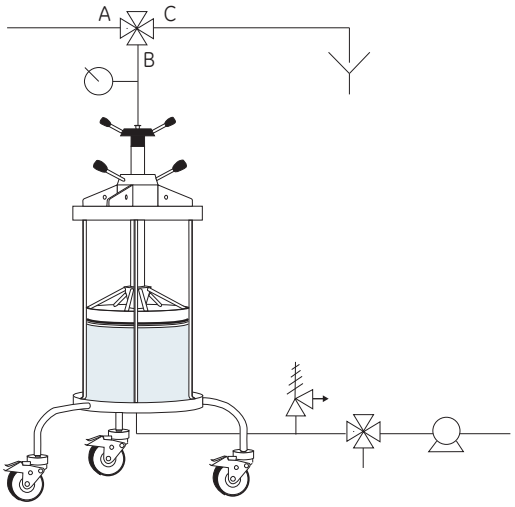
Follow the instructions below to test for leakage across the adapter.

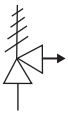
| Step | Action |
|---|---|
| 1 | Fill the column with water or sanitizing solution. |
| 2 | Position the adapter in the column to the same height as you intend to run the column. |
| 3 | Seal the adapter with the seal adjuster. |
| 4 | Remove the air in the column. |

**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.

| Step | Action |
|---|---|
| 5 | Close the column outlet valve. |



Pressure relief valve

| | |
|---|---|
| 6 | Raise the pressure to maximum working pressure. Note that pressure spikes may cause poor packing (cracking).<br>A pressure relief valve should be placed between the pump and column. |

**WARNING**

The working pressure of the column should never exceed its design pressure, otherwise there is a risk of personal injury and damage to the column. Always use appropriate safety equipment, for example pressure relief valve or rupture discs.

| | |
|---|---|
| 7 | Wait about 15 minutes until the pressure has stabilized. |
| 8 | Check the pressure drop on the pressure gauge. The pressure drop should not exceed 0.1 bar during 5 minutes. A recorder linked to a pressure controller gives more precise figures. |

# 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.

## Precautions

**WARNING**

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

**NOTICE**

**Cleaning**. Keep the exterior of the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

**NOTICE**

Make sure that any water is WFI, since particles can block and damage the nets.

**NOTICE**

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

## 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 132*.

In time, even electropolished 316L stainless steel may 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 media 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. CIP of the media 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 media is observed. See the recommended cleaning procedures in the table below.

| Media | Contaminants | Suggested procedure | Follow-up |
|---|---|---|---|
| Sepharose Fast Flow and Sepharose High Performance based media | Soluble proteins/ electrostatic interaction. | Wash with 0.5 column volumes of filtered 2 M NaCl for a contact time 10 to 15 minutes. | Regenerate to start conditions. See instructions for respective media. |
| | Soluble proteins/ hydrophobic interaction. | Wash with 2 to 3 column volumes of filtered low ionic strength buffer or water for a contact time of 10 to 15 minutes, or<br>wash with 1 column volume of 30% isopropanol for a contact time of 10 to 15 minutes, or<br>wash with 2 to 4 column volumes of 0.5 to 1 M NaOH for a contact time of 1 to 2 hours. | Regenerate to start conditions. See instructions for respective media. |
| | Precipitated proteins | Wash with 2 to 4 column volumes of 1 M NaOH for a contact time of 1 to 2 hours. Use reversed flow, or<br>wash with 2 to 4 column volumes of a mixture of 1 M NaCl/1 M NaOH for a contact time of 1 to 2hours. Use reversed flow. | If necessary, wash with 1.5 column volumes of water followed by 1 column volume of 2 M NaCl (not HIC).Regenerate to start conditions, see instructions for respective media.<br>Regenerate to start conditions, see instructions for respective media. |

| Media | Contaminants | Suggested procedure | Follow-up |
|---|---|---|---|
| | Lipids | Wash with 2 to 4 column volumes of 0.5 to 2 M NaOH for a contact time of 1 to 2 hours. Use reversed flow, or<br>wash with 2 to 4 column volumes of 0.1% to 0.5% non-ionic detergent for a contact time of 1 to 2 hours. Use reversed flow, or<br>wash with 2 to 4 column volumes of 30% isopropanol or 70% ethanol for a contact time of 1 to 2 hours. Use reversed flow. | Regenerate to start conditions. See instructions for respective media. |
| Sephacryl High Resolution, Sephadex G-25 | Precipitated proteins/lipids. | Wash with 0.5 to 1 column volumes of 0.5 M NaOH for a contact time of 0.5 to 1 h. Use reversed flow, or<br>wash with 2 to 4 column volumes of 0.5% non-ionic detergent for a contact time of 1 to 2 hours. Use reversed flow. | Wash immediately with at least 2 column volumes of working buffer. |
| Protein A Sepharose 4 Fast Flow,<br>Protein G Sepharose 4 Fast Flow | Precipitated or hydrophobically bound substances | Wash with 1 to 2 column volumes of 6 M Guanidine-HCl for a contact time of 1 to 2 hours. Use reversed flow, or<br>wash with 2 to 4 column volumes of 0.1% to 0.5% non-ionic detergent for a contact time of 1 to 2 hours. Use reversed flow. | Wash immediately with at least 5 column volumes of working buffer. |
| Superdex 75 and 200 prep grade | Precipitated proteins/lipids. | Wash with 0.5 to 1 column volume of 0.5 M NaOH for a contact time of 0.5 to 1 hour. Use reversed flow, or<br>wash with 2 to 4 column volumes of 0.1% to 0.5%non-ionic detergent for a contact time of 1 to 2 hours. Use reversed flow. | Wash immediately with at least 2 column volumes of working buffer. |

## 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 media, 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 seals and nets. Any plastic process contact parts that are to be reused must be appropriately cleaned prior to reassembly of the column.

**NOTICE**

The accessory valves have bodies in PTFE, which can shrink, and thereby cause leakage after repeated autoclaving. Therefore, exclude the valves if autoclaving the column.

# 6.8 Storage

## Bacteriostatic solution

BPG columns packed with media should be stored in the bacteriostatic solution recommended for the media. Refer to your media instructions for media-specific recommendations. Refer also to *Section 8.2 Chemical resistance, on page 132*. Avoid any solution that may compromise the quality of the media.

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 media. |
| 2 | Rinse the column from all chloride ions. |
| 3 | Rinse with purified water after the CIP for at least five column volumes (CV). |
| 4 | 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

Avoid storage conditions that could expose the column to extreme changes in temperature.

If the column is intended to be stored for longer periods of time, change the storage solution at regular intervals, for example every 3 months.

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 | Wrap column in plastic to prevent exposure to dust and other airborne contaminants. |
| 7 | Store column at controlled ambient temperatures in a clean and dry environment. |

## After storage

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., replace O-rings etc. |
| 3 | Thoroughly dry all column components. |
| 4 | Assemble the column. |

# 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 media, through the column, in the opposite direction. After reverse pumping, test the column again prior to use, refer to *Section 5.3 Performance evaluation of the column, on page 72*.

## Troubleshooting guide

| Problem | Cause | Action |
|---|---|---|
| High-back pressure | The valves between the pump and the collection vessel are closed. | Open the valves. |
| | The valves are blocked. | Check that all valves are clean and free from internal blockage. |
| | The medium is packed too hard. | Repack the medium. |
| | The column nets are blocked and there can be air trapped in the net. | Change nets. |
| | 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.<br>• Check to see 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 | 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 solvent, see *Section 8.2 Chemical resistance, on page 132*. |
| | The tubing is not compatible with all the solvents. | Change solvent. |
| | The connectors have not the same dimensions. | Change connectors. |
| | 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 correcton the rods or the flange. | Check that the correct torque specification has been used during column assembly. |
| Chromatogram not as expected | The linear velocity is not as expected. | Check sample. |
| | Back-mixing has occurred anywhere in the column set-up. | Check connections. |
| | The sample could have passed through an air trap. | Check connections or test method. |
| | The adapter is not correctly positioned in close contact with the bed. | • Move the adaptor down 5 mm into the bed.<br>• Disassemble and assemble the column. |
| | The column packing has not been checked. | Check the column packing. |
| | The column has not been correctly equilibrated. | Equilibrate the column. |
| | Old sample mixed with new. | Check sample and sample handling. |

| Problem | Cause | Action |
|---|---|---|
| | There have been any changes to the following:<br>• Preparation of buffers/solutions<br>• Preparation of system and tubing | • Check the dilution, filtration, temperature and preparation of the sample and buffers are considered.<br>• Check all dead volumes were correctly filled with appropriate buffers and sample before running. |
| Infected and/or pyrogen contaminated column | Column bed shows signs of bacterial growth. | • CIP.<br>• Prepare new buffers/sample. |
| | The starting material is contaminated. | Use clean material. |
| | The in going components (such as water, serum components in growth media, etc.) are not ok. | Check material. |
| | The column is not cleaned. | Clean the column, refer to *Recommended CIP procedure, on page 116*. |
| | The NaOH has been removed, in case that positive LAL tests were seen after a CIP cycle with NaOH. | Repeat cleaning. |
| 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. | Check the connections. |
| | The valves are not shut tight enough. | Check the valves and close them. |
| | The volume of the trap is too small or large. | • Fill air trap with buffer.<br>• Change air trap. |

# 8 Reference information

## About this chapter

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

## In this chapter

# 8.1 Specifications

## In this section



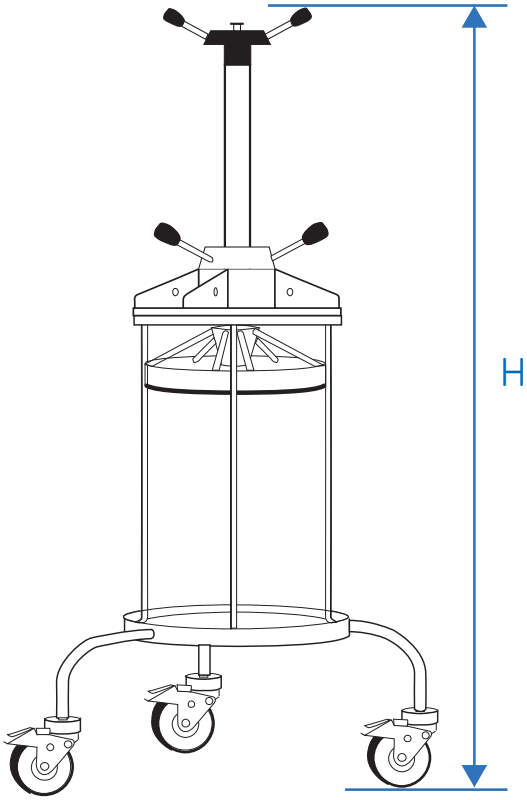
## Column specifications

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 pressurized bed.

***Note:*** *The optimal slurry concentration 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 | l | 3.2 | 5.2 | 6.2 |
| Max column volume with extension | l | 6.2 | 8.2 | 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 | l | 0.3 | 2.0 | 3.6 |
| Bed volume[1] | l | 2.0 | 3.2 | 4.3 |
| Bed volume with extension[2] | l | 2.7 | 4.3 | 5.8 |
| Bed volume with extension[3] | l | 3.5 | 5.2 | N/A |
| Max pressure[4] | 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.
[3] 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.
[4] Use a pressure gauge to monitor the pressure.

## 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 | l | 6.3 | 10 | 13 |
| Max column volume with extension | l | 12 | 16 | 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 | l | 0.1 | 4.0 | 7.0 |
| Bed volume[1] | l | 4.0 | 6.4 | 8.4 |
| Bed volume with extension[2] | l | 5.3 | 8.4 | 11.3 |
| Bed volume with extension[3] | l | 6.9 | 10.3 | N/A |
| Max pressure[4] | 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.
[3] 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.
[4] Use a pressure gauge to monitor the pressure.

## 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 | l | 13 | 21 | 27 |
| Max column volume with extension | l | 25 | 33 | 39 |
| 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 | l | 0.3 | 8.1 | 14.4 |
| Bed volume[1] | l | 8.1 | 13.1 | 17.2 |
| Bed volume with extension[2] | l | 10.9 | 17.5 | 23.2 |
| Bed volume with extension[3] | l | 14.1 | 21.0 | N/A |
| Max pressure[4] | 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.
[3] 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.
[4] Use a pressure gauge to monitor the pressure.

## 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 | l | 27 | 44 | 58 |
| Max column volume with extension | l | 53 | 70 | 84 |
| 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 | l | 0.6 | 19.2 | 33.0 |
| Bed volume[1] | l | 17.2 | 28.2 | 37.1 |
| Bed volume with extension[2] | l | 22.7 | 37.8 | 49.5 |
| Bed volume with extension[3] | l | 30.9 | 45.4 | N/A |
| Max pressure[4] | 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.
[3] 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.
[4] Use a pressure gauge to monitor the pressure.

## 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 | l | 56 | 95 | 134 |
| Max column volume with extension | l | 103 | 142 | 181 |
| Min bed height | cm | 16 | 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 | l | 25.0 | 43.7 | 82.8 |
| Bed volume[1] | l | 35.9 | 60.9 | 85.9 |
| Bed volume with extension[2] | l | 46.8 | 81.2 | 115.6 |
| Bed volume with extension[3] | l | 65.6 | 92.1 | N/A |
| Max pressure[4] | 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.
[3] 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.
[4] Use a pressure gauge to monitor the pressure.

# 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 listed in the Chemical Resistance information.

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

***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 solvent 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 will be more severe at higher temperatures and pressures, and that combined effects have not been taken into consideration.

| Substance | Concentration by volume | 60–90 days[1] |
|---|---|---|
| Acetic acid | 10% | Not recommended to be used with PA nets. |
| Acetic acid | 25% | Not recommended to be used with PA nets. |
| Acetonitrile | 5% | EPDM rubber changes characteristics. For repetitive and long term use, use FEP/PFR/PTFE O-rings and gaskets. |
| Acetonitrile | 50% | Change to FEP/PFR/PTFE O-rings and gaskets.<br>Polypropylene plastic resistance is fair. |
| Acetone | 50% | OK |
| Cyclohexane | 100% | Change to FEP/PFR/PTFE O-rings and gaskets.<br>Polypropylene plastic resistance is fair. |
| Ethanol | 100% | EPDM rubber changes characteristics. For repetitive and long term use, use FEP/PFR/PTFE O-rings and gaskets. |
| Ethyl acetate | 100% | Polypropylene plastic resistance is fair. |
| Ethylene glycol | 50% | OK |
| Glycerol | 100% | OK |
| Hexane | 100% | EPDM rubber changes characteristics. For repetitive and long term use, use FEP/PFR/PTFE O-rings and gaskets.<br>Not recommended to be used with PA nets. |
| Hydrochloric acid | 0.1 M | Not longer than 4 hours.<br>Not recommended to be used with PA nets. |
| Isopropyl alcohol | 100% | EPDM rubber changes characteristics. For repetitive and long term use, use FEP/PFR/PTFE O-rings and gaskets. |
| Methanol | 100% | OK |
| Nitric acid | 0.1 M | Not recommended to be used with PA nets. |
| n-Propanol | 100% | OK |

| Substance | Concentration by volume | 60–90 days[1] |
|---|---|---|
| Sodium chloride | 2 M | Can be used under normal running conditions. Do not use NaCl in storage solutions. Please note that NaCl can cause corrosion on stainless steel in acid solutions (pH below 4,5). |
| Sodium hydroxide | 2 M | OK |
| Trifluoroacetic acid | 0.1% | Not recommended to be used with PA nets.<br>FEP changes characteristics. Thus, use EPDM rubber. |
| Detergent | 100% | OK |
| Tween™/Tri-n-butyl phosphate | 1% / 0.3% | OK |
| UREA | 8 M | OK |

[1] The test does not include PVC tubing.

## 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 Abbreviations

The abbreviations used in this manual are defined as follows:

| Abbreviation | Description | Translation |
|---|---|---|
| $A_s$ | Peak asymmetry factor | Peak asymmetry factor |
| CIP | Cleaning-in-place | Cleaning-in-place |
| CV | Column volume | Column volume |
| EPDM | Ethylenepropylenedienemonomer rubber | Ethylenepropylenedienemonomer 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 |

# 8.4 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**

Before decommissioning work is performed on the column, make sure that:

- the column is empty and depressurized.
- relevant energy sources and process feed are disconnected.
- all process wetted areas are clean and decontaminated.
- the column cannot be accidentally re-energized, refilled or re-contaminated during maintenance.

**WARNING**

**Decommissioning**. Decontaminate the equipment before decommissioning to ensure 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.

# 8.5 Regulatory information

## Introduction

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

## In this section

# 8.5.1 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<br>Björkgatan 30<br>SE 751 84 Uppsala<br>Sweden |
| Telephone number of manufacturer | + 46 771 400 600 |

# 8.5.2 European Union and European Economic Area

## Introduction

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

## Conformity with EU Directives

See 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 instrument when it is:

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

# 8.6 Health and Safety Declaration Form

## On site service

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### On Site Service Health & Safety Declaration Form

| | |
|---|---|
| **Service Ticket #:** | |

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

| Yes | No | Review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below. |
|---|---|---|
| ○ | ○ | **Instrument has been cleaned of hazardous substances**. Rinse tubing or piping, wipe down scanner surfaces, or otherwise make sure removal of any dangerous residue. Make sure the area around the instrument is clean. If radioactivity has been used, perform a wipe test or other suitable survey. |
| ○ | ○ | **Adequate space and clearance is provided to allow safe access** for instrument service, repair or installation. In some cases this may require customer to move equipment from normal operating location prior to Cytiva arrival. |
| ○ | ○ | **Consumables, such as columns or gels, have been removed or isolated from the instrument and from any area that may impede access to the instrument.** |
| ○ | ○ | **All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.** |
| **Provide explanation for any "No" answers here:** | | |

| | | | |
|---|---|---|---|
| **Equipment type / Product No:** | | **Serial No:** | |
| I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible. | | | |
| **Name:** | | **Company or institution:** | |
| **Position or job title:** | | **Date (YYYY/MM/DD):** | |
| **Signed:** | | | |

# Product return or servicing

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## Health & Safety Declaration Form for Product Return or Servicing

| | | | |
|---|---|---|---|
| **Return authorization number:** | | *and/or* **Service Ticket/Request:** | |

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

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

| **Yes** | **No** | **Specify if the equipment has been in contact with any of the following:** | |
|---|---|---|---|
| ◯ | ◯ | Radioactivity (specify) | |
| ◯ | ◯ | Infectious or hazardous biological substances (specify) | |
| ◯ | ◯ | Other Hazardous Chemicals (specify) | |

**Equipment must be decontaminated prior to service / return. Provide a telephone number where Cytiva can contact you for additional information concerning the system / equipment.**

| | | |
|---|---|---|
| **Telephone No:** | | |
| **Liquid and/or gas in equipment is:** | ☐ | **Water** |
| | ☐ | **Ethanol** |
| | ☐ | **None, empty** |
| | ☐ | **Argon, Helium, Nitrogen** |
| | ☐ | **Liquid Nitrogen** |
| | **Other, specify** | |

| | | | |
|---|---|---|---|
| **Equipment type / Product No:** | | **Serial No:** | |

**I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.**

| | | | |
|---|---|---|---|
| **Name:** | | **Company or institution:** | |
| **Position or job title:** | | **Date (YYYY/MM/DD)** | |
| **Signed:** | | | |



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

# Index

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For local office contact information, visit cytiva.com/contact

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