POROS® HP Glass Columns for Preparative Chromatography

Applied Biosystems

Column Packing Instructions

1 Product Description

Applied Biosystems POROS® HP Glass columns are designed for preparative ion exchange, hydrophobic interaction, and affinity chromatography.

***NOTE**: These columns are not designed for use in reversed-phase applications because some materials are not compatible with high concentrations of organic solvents.*

You can pack POROS HP Glass columns with POROS® Perfusion Chromatography® media or conventional media (purchased separately).

POROS HP Glass columns are designed to take full advantage of the benefits of POROS Perfusion Chromatography media. Benefits include:

- Higher pressure rating than most ordinary glass columns
- Can be operated at the higher linear velocities achieved with high-performance chromatography media like POROS
- Can be used on liquid chromatography workstations such as the BioCAD® family of instruments

The actual flow rates achieved depend on the column used, bed height, media particle size, and configuration of the chromatographic system used.

Column parts and accessories

The following column parts and accessories are provided:

- Precision-bore, flame polished, FEP shielded glass column, 250 mm length
- Two sets of 5 micron polyethylene frits
- Two sets of Viton O-rings
- Two extra-long adjustable Teflon® flow adapters with large diameter adjusting knobs
- Two black acetal end fittings with 0.8 mm ID internal cone
- Two black end plugs

Product characteristics

Table 1 Product Characteristics

Column diameters available	10 mm, 16 mm, and 27 mm		
Column length	Column length 250 mmL Minimum bed height 50 mmL Maximum bed height 220 mmL		
Frits	5 µm polyethylene		
Chromatographic media	POROS media, 20 µm or 50 µm Conventional media 20 to 300 µ		
	Column Diameter (mm)		
	10	**16**	**27**
Minimum bed volume	4 ml	10 ml	28 ml
Maximum bed volume	17 ml	44 ml	126 ml
Pressure limits	600 psi	500 psi	300 psi
Typical flow rates (100 mm bed depth with semi-prep flow cell)			
POROS 50 media	1–20ml/min	1–35mls/min	1–40mls/min
POROS 20 media	1–20ml/min	1–35ml/min	1–35ml/min
Conventional media	1–5ml/min	1–10ml/min	1–15ml/min

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Table 2 Chemical Resistance of Column Parts

pH range	1–14 (up to 5.0 M NaOH, 1M HCl)
Ionic strength	0–5 M, all common salts
Buffer additives	All common agents, including 8 M urea, 6 M guanidine/HCl, ethylene glycol, and detergents.
Solvents	Water, 0–30% alcohols, acetonitrile, other common organic solvents

2 Preparing Slurry and Packing Solvents

Prepare the following before packing:

- Slurry solvent—0.5 M NaCl
- Packing solvent—0.1 to 0.2 M NaCl

3 Preparing the Slurry

3.1 Preparing the Slurry for POROS 20 Columns

Precautions

***WARNING**: POROS media is provided as a dry powder, which may form a light dust. Use one of the following when handling dry POROS media:*

- *NIOSH*/MSHA**-approved respirator with dust cartridge*
- *Fume hood*

** National Institute for Occupational Safety and Health*

*** Mine Safety and Health Administration*

Preparing the Slurry

Follow these steps to form the slurry:

***NOTE**: Do not use a magnetic stirrer. It may abrade the particles and cause fines to form.*

1. Calculate the amount of dry powder needed to give the final bed volume of your column. Use the ratio of dry powder to packed bed volume listed on the product label.

   Example: If the label indicates that 8.3 g of powder gives 25 ml of packed bed, to pack a 10 ml column, weigh out 3.3 g of powder.

2. Add buffer or dilute saline solutions, such as 1 to 3% w/v NaCl for the slurry solvent.

   The volume to add depends on the equipment you are using. In general, the final slurry volume should be a minimum of 2 to 3 times the final packed bed volume.

3. Mix the slurry gently.

3.2 Preparing the Slurry for POROS 50 Columns

POROS 50 media comes as a slurry containing 20% ethanol as a bacteriostat and gives the final packed bed volume indicated on the label. Calculate the total volume of slurry needed to pack your own column by keeping in mind that the ratio of slurry volume to packed bed volume is 1.8:1.

Example: 18 ml of slurry contains enough media for a 10 ml packed bed volume. The packed bed volume specified on the label is based on a packing pressure of 3 bar.

To prepare the slurry for packing:

***NOTE**: Do not use a magnetic stirrer. It may abrade the particles and cause fines to form.*

1. Allow the media to settle for 30 to 60 minutes.
2. Pour off the supernatant.
3. Resuspend the media in 0.5 M NaCl.

   The volume of 0.5 M NaCl to add depends on the column equipment you use. In general, the final slurry volume should be 2 to 3 times the final packed bed volume.

3.3 Preparing the Slurry for Conventional Media

When packing columns with media other than POROS Perfusion Chromatography media, please refer to the slurry and packing conditions provided by the vendor.

4 Packing the Column

This section describes:

- Packing recommendations
- Using column adjustment knobs
- Adding slurry to the column
- Flow packing the column
- Testing the column

Packing recommendations

To ensure the best results when packing POROS HP Glass columns:

- Add the entire slurry volume to the column before packing.
- Use flow packing techniques as outlined below.

Using column adjustment knobs

Before packing, read this section to understand how to position the flow adapters in the column (Figure 1).

Each flow adapter includes a large-diameter adjusting knob and locking nut.

- To adjust the position of the flow adapter, loosen the locking nut so that the adjusting knob can turn easily
- To withdraw the bottom or top flow adapter from the column, turn the adjustment knob clockwise
- To push the bottom or top flow adapter into the column, turn the adjustment knob counterclockwise
- To lock the flow adapter in place after positioning, turn the locking nut clockwise until finger-tight

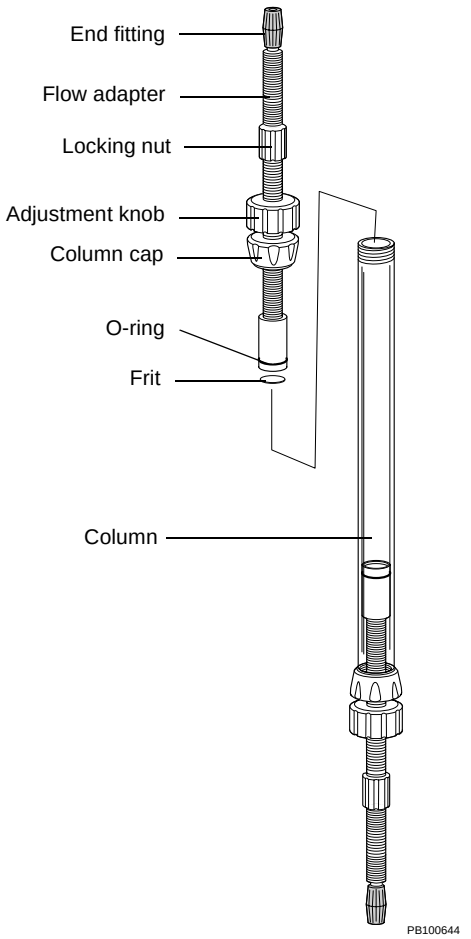

Figure 1 Column Parts

Adding slurry to the column

1. Remove one of the flow adapters from the column. There is no direction of flow, so you can remove either end.
2. Turn the adjustment knob to adjust the position of the bottom flow adapter for the bed height you are packing. You may need to loosen the column cap to allow you to adjust the position. Turn the locking nut to lock the adapter in place.
3. Clamp the glass column to a ring stand with the open end up.
4. Screw an end plug (not shown in Figure 1) into the bottom end fitting to cap the column outlet.
5. Pour about 5 ml of packing solvent into the column to cover the frit. This prevents bubbles from becoming trapped on the bottom edges and frit.
6. Add the entire slurry to the column.
7. Place a beaker under the column outlet.
8. Remove the bottom end plug to allow packing solvent to flow by gravity.
9. Gently tap the sides of the column to remove air bubbles trapped on the sides of the column.
10. When packing solvent has flowed out of the column and there is enough room at the top of the column for the top flow adapter, wet the O-ring of the flow adapter and insert it into the column.
11. Screw an end plug into the bottom end fitting to cap the column outlet.
12. Slowly push the flow adapter onto the top of the slurry.
13. Place the column cap on the column and turn it 3 or 4 turns to hold the flow adapter in place.

NOTE: *Do not completely tighten the column cap. Doing so will make adjustment of the flow adapter very difficult. Tighten the adapter locking nut completely after the flow adapter is in the correct and final position.*

14. Turn the adjustment knob to lower the flow adapter down onto the bed until a small volume of buffer is expelled from the top of the column. This removes air on the top of the column and in the flow adapter before flow packing.
15. Tighten the column cap and locking nut to lock the adapter in place.

Flow packing the column

1. Connect the outlet of the packing pump to the top flow adapter using the Fingertight fittings and ferrules provided.
2. Place a waste beaker under the column outlet.
3. Remove the bottom end plug.
4. Install the packing solvent on the packing pump.
5. Start the packing pump at the maximum packing flow rate that generates a backpressure that is:
   - Close to the pressure limits of the column
   - At least 25% higher than the pressure the column will experience during normal operation.

   Packing at a high flow rate and pressure packs the media completely and prevents the bed from compressing during use, which can result in a head space at the top of the column.

NOTE: *You may observe some fine material in the eluent as packing begins. This will clear as packing proceeds and 2 to 3 column volumes have passed through the column.*

6. Allow 2 to 5 column volumes of packing solvent to flow through the column into the waste beaker to ensure that fine media particles are not introduced into the chromatographic system.
7. Stop the pump flow.
8. Turn the adjustment knob to lower the top flow adapter until it touches the top of the bed.
9. Turn the adjustment knob to lower the flow adapter an additional 0.5 to 1 cm so that the frit is in complete contact with the top of the bed.
10. Start the packing pump at the maximum packing flow rate (flow rate used in step 5) and flow packing solvent through the column for a minimum of 10 column volumes.
11. Stop the pump flow.
12. Tighten the adapter locking nut on the top flow adapter.

The column is ready for operation.

Testing the column

After packing, run a protein test standard compatible with your application to ensure correct performance of the column.

If column performance is not acceptable, repack the column.

5 Column Operating Considerations

All systems

Note the following when using POROS HP Glass columns on any chromatographic system:

- Make sure that the LC components downstream of the column (for example, UV flow cell, fraction collector) do not add significant pressure at the maximum operating flow rates. Additional pressure may exceeded the maximum pressure limits of the column.
- Most LC systems require 10 to 15 psi backpressure for proper check valve operation. Add a backpressure regulator to your system if the column does not generate 15 psi backpressure at the operating flow rate.

BioCAD® 700E, VISION™, SPRINT™, and INTEGRAL® 100Q systems

Note the following when using POROS HP Glass columns on BioCAD 700E, VISION, SPRINT, or INTEGRAL 100Q systems:

- To reduce system pressure, plumb system with green PEEK tubing and a semi-prep or preparative flow cell
- To reduce the tubing used, plumb the system in single column or low dispersion configurations
- When configuring the column pressure limit, include the system pressure upstream of the column (to determine the pressure upstream, disconnect the column inlet tubing and place in a waste beaker, flow the system at operating flow rate, and note the system pressure on the Control Panel.)
- Program methods in column volumes and linear velocity. This will allow the methods to be updated automatically when larger columns are configured.

6 Unpacking a Column

Unpacking

To unpack a column:

1. Remove one of the flow adapters.
2. Connect the inlet end of the column to the pump outlet tubing of your LC system.
3. Position a beaker under the column.
4. Start the flow rate at 5 to 20 ml/min.
5. As soon as the media is pushed out of the column into the beaker, stop the flow.
6. Remove the other flow adapter and rinse all fittings, frits, and the column tube in water.

You can reuse the media to pack a new column.

NOTE: *As with any chromatographic media, repacking media may result in increased backpressure in the packed column. Backpressure is caused by particulate matter generated when you unpack and repack the column. Do not repack your column more than five times.*

You can reduce backpressure by removing all particulate matter each time you unpack the column.

Removing particulate matter

To remove particulate matter:

1. Suspend the unpacked media in slurry solvent.
2. Let the slurry settle for 30 to 60 minutes.
3. Draw off the supernatant, which contains particulate matter.

Reusing media

To reuse media:

1. After removing particulate matter, add enough fresh media to make up for the media lost during removal of the particulate matter.
2. Slurry the entire mixture thoroughly and use it to repack the column.

7 Storing the Column

When you store the column, always be sure to:

- Store the column between 5 and 30°C.
- Store the column with the end plugs in place, carefully sealed to prevent drying. Drying results in decreased chromatographic efficiency.

Short-term storage

Store the column for short periods in any appropriate buffer.

Long-term storage

Flush the column with 1 M NaCl, followed by water with either 0.02% sodium azide or 30% alcohol.

WARNING*: Sodium azide is toxic. Follow precautions and decontamination procedures recommended by the National Institute for Occupational Safety and Health.*

8 Accessories, Spare Parts, and Ordering Information

These accessories are available for POROS HP Glass columns:

Table 3 POROS HP Glass Column Accessories

Description	Quantity	Part Number
Glass Columns, complete, includes glass column body, frits, O-rings, flow adapters, end fittings, end plugs, and Fingertight fittings and ferrules		
10 mmD	1	1-9545-48
16 mmD	1	1-9545-57
27 mmD	1	1-9545-72
Packing Funnel		
10 mmD	1	1-9152-48-0000
16 mmD	1	1-9152-57-0000
27 mmD	1	1-9152-72-0000
10 mmD Column Accessories		
10 mmD flow adapter	1	1-9153-48-0000
10 mmD glass column body	1	1-9154-48-0000
10 mmD frits and O-rings	4 each	1-9155-48-0000
16 mmD Column Accessories		
16 mmD flow adapter	1	1-9153-57-0000
16mmD glass column body	1	1-9154-57-0000
16mmD frits and O-rings	4 each	1-9155-57-0000
27 mmD Column Accessories		
27 mmD flow adapter	1	1-9153-72-0000
27 mmD glass column body	1	1-9154-72-0000
27 mmD frits and O-rings	4 each	1-9155-72-0000
Fittings and Ferrules		
Ferrule, 1/16", blue	10	P5-1025-06-0006
Nut, 1/16", black	10	P5-1025-06-1250
Fitting, LP Union Tefzel®, 1/4”	2	P5-1028-01-0025

For POROS media part numbers and pricing, consult the POROS price list, or contact Applied Biosystems.

9 Technical Support

Applied Biosystems is dedicated to helping you use Perfusion Chromatography and POROS media to the fullest extent possible. Our biochromatographers, bioprocess engineers, and applications development laboratories are available for support ranging from telephone consultation to full-scale method development.

Applied Biosystems also offers a full line of other POROS media for Perfusion Chromatography in the reversed-phase, ion exchange, affinity, and other chromatographic modes. Please contact your Applied Biosystems representative for technical and ordering information.

Applied Biosystems publishes a continuing series of Application and Technical Notes, highlighting specific purification problems and technical aspects of Perfusion Chromatography. Contact Applied Biosystems directly for a publication list.

For further details or for answers to questions on POROS media and columns, Perfusion Chromatography, or other products, contact Applied Biosystems. Refer to the back page of this document for contact information.

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Subtractive Assay technology, enabled by the use of ImmunoDetection (ID) Sensor Cartridges and the INTEGRAL Micro-Analytical Workstation, is covered by U.S. patent 5,234,586. Other patents pending.

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