POROS™ HS Analytical Columns for Cation Exchange Chromatography

Pub. No. 8-0002-40-1093 **Rev.** D

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Note: These instructions cover the specific operational characteristics of POROS™ prepacked columns. POROS™ resins are also available in bulk quantities for direct scale-up of separations developed on prepacked columns. Contact your Thermo Fisher Scientific representative for more details.

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Product description

POROS™ HS columns are used for cation exchange chromatography of peptides, proteins, and other biomolecules. They consist of cross-linked poly(styrene-divinylbenzene) beads allowing for very rapid mass transport. The particles are surface coated with a polyhydroxylated polymer functionalized with sulfopropyl (POROS™ HS) groups.

POROS™ HS resin is a strong cation exchanger, with complete surface ionization over the pH range 1 to 14.

POROS™ HS column packages include the following items:

- Packed column, with sealing end caps
- *Product Information Sheet*
- Column Test Certificate
- EZ™ Grip stainless steel fittings

POROS™ column sizes

POROS™ cation-exchange columns are available in different sizes. Check that the column you have purchased is appropriate for your type of application and is compatible with the pressure limits of the system you are using (see Table 2 on page 1). See "Account for system pressure" on page 3 for a detailed discussion of pressure considerations for running POROS™ HS columns on conventional systems.

Table 1 Classification of POROS™ cation exchange columns

Particle Size	Maximum Pressure Drop
POROS™ 10 micron	170 bar (2,500 psi, 17 MPa)
POROS™ 20 micron	170 bar (2,500 psi, 17 MPa)

Table 2 Product characteristics

Support Matrix	Cross-linked poly(styrene-divinylbenzene)	
Surface Functionality	POROS™ HS	Sulfopropyl ($-CH_2CH_2CH_2SO_3^-$)
Dynamic Binding Capacity @ 3,600 cm/hr	(lysozyme, pH 6.2) POROS™ HS	75 mg/ml
Counterion as Supplied	Na^+	
Shipping Solvent	0.1 M Na_2SO_4, 30% methanol	
Packing Density	0.35 g/ml	
Shrinkage/Swelling	<1% from 0–100% solvent	

Table 3 Chemical resistance

pH Range	1–14 (Up to 1.0 M NaOH, 1 M HCl)
Ionic Strength	0–5 M, all common salts
Buffer Additives	All common agents, including 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents. Cationic detergents not recommended, as they can bind. **WARNING! CHEMICAL HAZARD. Guanidine hydrochloride may be** harmful if swallowed or absorbed through the skin. Exposure may cause eye, skin, and respiratory tract irritation and adverse effects on the central nervous system and bone marrow. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **WARNING!** Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric) or strong reducing agents (such as sulfite).
Solvents	Water, 0–100% alcohols, acetonitrile, other common organic solvents **WARNING! CHEMICAL HAZARD. Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Operating Temperature	2–30 °C

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Connecting and preparing the column

POROS™ columns come with EZ™ Grip stainless steel fittings that are designed to be tightened by hand.

IMPORTANT! For PEEK™ columns, do not use standard steel fittings that require tightening with a wrench. Overtightening can strip the threads of the column.

Extra EZ™ Grip fittings are available from **thermofisher.com**.

Connect the column

Columns can be connected to M-6 metric fitting systems.

1. Connect the short tubing section to the column using the red Fingertight fittings.
2. Slip the black metric nuts over the other end of the tubing, followed by a blue ferrule, with the conical end *pointing toward the nut*.
3. Connect the nut to a female M-6 fitting.

The columns can also be connected to 1/4-28 fitting systems using the green 1/4-28 male nut instead of the black M-6 nut.

Prepare the column

POROS™ cation-exchange columns are shipped in 0.1 M Na_2SO_4, 30% methanol. Before you use the column for the first time, pump the column with 5 to 10 column volumes of water to remove the methanol.

To prepare the column for a routine injection:

1. Pump 5 to 10 column volumes of high-strength eluent buffer.
2. Equilibrate with 10 to 15 column volumes of starting buffer.

Unlike the lower flow rates of conventional chromatography, the high flow rates possible with POROS™ beads allow a thorough column equilibration cycle in a matter of minutes.

Selecting and preparing the starting buffer

Regardless of the buffer system you choose, it is always important to:

1. Use buffers of the highest purity practical.
2. Degas and filter (0.22 or 0.45 µm) all buffers prior to use.

Buffer pH

The buffer pH is the most critical variable in ion exchange chromatography. With the strong cation exchange packings, the charge of the packing itself is unchanged from pH 1 to 14. This unchanged charge allows operation at low pH for binding of proteins with low isoelectric points or surface pKas.

Examine the effect of pH carefully in a systematic screening or mapping experiment. For cation exchangers, examine a pH range of 4 to 8.

Buffer ions

Keep this information in mind as you choose a buffer system:

- Buffer ions should be anionic or at least zwitterionic.
- Avoid cationic buffers such as Tris, because they can bind to the functional groups and cause local changes in pH.
- Keep buffer ion concentration in the range of 20 to 50 mM for POROS™ HS columns.
- After equilibrating the column, check that the pH of the effluent buffer is at the desired value.

Table 4 Recommended anionic buffers

pH Range	Buffer Ion	pKa
1.5–2.5	Maleic acid	2.00
2.4–3.4	Malonic acid	2.88
2.6–3.6	Citric acid	3.13
3.6–4.3	Lactic acid	3.81
3.8–4.3	Formic acid	3.75
4.3–4.8	Butanedioic acid	4.21
4.8–5.2	Acetic acid	4.76
5.0–6.0	Malonic acid	5.66
5.5–6.7	MES	6.15
6.7–7.6	Phosphate	7.2
7.6–8.2	HEPES	7.55
8.2–8.7	BICINE	8.35

Preparing and loading the sample

To ensure efficient binding and prevent column plugging, it is important to:

1. Dissolve or exchange samples in the starting buffer, if possible.
2. Centrifuge or filter samples (0.22 or 0.45 µm) prior to injection.
3. Delipidate samples, if possible. Lipids can cause irreversible fouling.

Determine the sample load

The dynamic binding capacities of POROS™ HS are listed in "POROS™ column sizes" on page 1. In general, high-resolution separations are achieved at 20% or less of the total binding capacity.

However, the maximum loading at which a given resolution can be obtained (the loadability) depends on several factors, including sample solubility, column selectivity, and so on.

Perform a loadability experiment:

1. Determine the most effective elution conditions (eluent, gradient, and flow) at low loading.
2. Gradually increase the sample load (either through increasing injection volume or sample concentration or both) until the required resolution no longer occurs.

Note: As the loading is increased, the peaks may elute earlier on the gradient, possibly requiring reoptimization of the gradient.

Eluting the sample

To elute, increase the ionic strength of the starting buffer.

- NaCl and KCl are the most commonly used salts for elution, although sulfate, formate or acetate salts also can be used.
- Up to 1.0 M ionic strength elutes most proteins.
- Use up to 2.0 M ionic strength for removal of tightly bound proteins or for column cleanup (see below).
- Ionic strength changes may be either by step or continuous gradients.
- Gradient volumes of 10 to 20 column volumes provide a good compromise between resolution and peak dilution.

Note that with the very high flow rates possible with POROS™ HS columns, increased gradient volumes (with decreased slope and therefore increased resolution) are possible without the excessive times normally experienced with conventional resin.

Cleaning up and regenerating the column

In some applications, sample molecules may not fully elute or may precipitate on the column. Regenerate the column if these symptoms appear:

- Loss of resolution
- Loss of binding capacity
- Loss of recovery
- Increased pressure drop

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- "Ghost" peaks occurring during blank gradient runs.

IMPORTANT! In any cleanup method, reversing the flow direction is recommended to help flush out particulates and to prevent contamination of the lower part of the column bed. Also, slow the flow rate to give several minutes' exposure time to the regeneration solution at each step of the cleaning protocol.

Simple regeneration

Wash with 3 to 5 column volumes of 1–2 M of the salt that was used for elution. If simple regeneration does not restore column performance, a more complex regeneration is needed.

Complex regeneration

To regenerate more completely:

1. Wash with 3 to 5 column volumes of 1.0 M acetic or hydrochloric acid (or 1.0% trifluoroacetic acid).
2. Wash with water to remove the acid.
3. Wash with 3 to 5 column volumes of 1.0-M NaCl, 1.0-M NaOH.
4. Wash with water to remove base.
5. Reequilibrate the column with starting buffer.

Removing lipids and lipoproteins

To remove fouling lipids or lipoproteins, try one of these methods:

- Use a mixture of 50% methanol, isopropanol, or acetonitrile with the acid or base.
- Use a mixture of 50% methanol and 50% 3 M guanidine thiocyanate.

DANGER! CHEMICAL HAZARD. Guanidine thiocyanate causes eye burns and can cause skin and respiratory tract irritation. It is harmful if absorbed through the skin or swallowed. Contact with acids and bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing guanidine thiocyanate. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

- Store the column overnight in 1 mg/ml pepsin, DNAse, or other enzymes.

Multiple injections

It is possible to use multiple injections of regeneration solutions instead of pumping them directly. This method is recommended for very aggressive or very viscous solvents. To clean by injections:

- Make the injection volume as large as possible.
- Use a low flow rate that allows at least several minutes' exposure time to the regeneration solution.

Note: Backpressure increase is sometimes caused by a plugged inlet frit. If backflushing the column does not solve the problem, replace the inlet frit.

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.

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.

Guidelines for using POROS™ chromatography

There are a few simple but important things to keep in mind when you make the transition to POROS™ chromatography. They can be grouped into two general categories:

Related to the chromatography system:

- Account for System Pressure
- Check the Gradient System
- Maintain Your Column and System

Related to experimental design:

- Think in Terms of Column Volumes
- Adjust the Sample Load
- Measure Recovery Properly

Account for system pressure

The high flow rates used with POROS™ cause a higher-than-usual system pressure (resulting from the chromatography hardware itself). In some cases, this system pressure can be equal to or even greater than the column pressure.

Therefore, when you use your POROS™ column, you cannot simply set the upper pressure limit of the system at the pressure rating of the column. Instead:

1. Determine the system pressure by:
   - Connecting a union in place of the column
   - Pumping the highest salt concentration to be used at the planned flow rate
2. Set the upper pressure limit by adding the system pressure observed above to the column pressure rating.

If the system pressure is too high:

1. Check carefully for plugged or crimped tubing or other restrictions in your plumbing.
2. Use larger-ID or shorter tubing.
3. Use a larger detector flow cell.

In some systems, excessive system pressure can prevent the high flow rates that are required to take full advantage of POROS™ resin.

It is important to isolate the relative contribution of the column and instrument when pressures approach the maximum column pressure. Take the column out of line to determine those contributions (measured pressure = column pressure drop + system pressure). The maximum allowable pressure drops of POROS™ columns are listed in "POROS™ column sizes" on page 1.

Typical pressure drops of POROS™ columns (at 2,000 cm/hr with water as the mobile phase) are shown in Table 6 on page 3. Use this table to verify column performance or to help you decide if column regeneration is required.

Table 5 Typical column pressure drops

Pressure: Bar (psi) (1 bar = 0.1 MPa)

Column Type	30 mmL	50 mmL	100 mmL
POROS™ 10	40 (600)	60 (900)	80 (1,200)
POROS™ 20	20 (300)	30 (450)	40 (600)

Check the gradient system

High flow rates and short run times can expose both operational and design problems in gradient blending systems. Gradient system problems can affect step changes and linear gradients. Most problems come from one of two sources:

- Excessive delay (dwell) or mixing volume can cause both delay in the start of the gradient at the column and rounding or distortion of the edges of the gradient. Mixing or delay volume can be reduced by using a smaller mixer and shortening the tubing between the mixer and sample injector.
- Poor gradient proportioning can cause either short-term fluctuations or long-term inaccuracies. Adding a mixer can sometimes help.

Consult your system vendor for serious gradient problems.

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Maintain your column and system

POROS™ chromatography enables you to perform runs more quickly than other chromatography technologies. For this reason, you can perform maintenance tasks such as replacing filters or regenerating columns after a specified number of runs, instead of after a specified amount of time. You can reduce the frequency of such maintenance by always filtering the sample and eluent.

Think in terms of column volumes

In any chromatographic separation, as flow rate increases, gradient time must decrease to maintain constant gradient volume. At the flow rates that are used for POROS™ chromatography, the gradient times are dramatically shorter than those you are accustomed to working with. To convert a method, keep the gradient volume constant in terms of column volumes and adjust the time of the gradient according to the new flow rate. Table 7 on page 4 provides bed volumes of POROS™ columns to help you make the necessary calculations.

Table 6 POROS™ prepacked columns

Diameter (mmD)	Length (mmL)	Column Bed Volume (ml)
2.1	30	0.10
2.1	100	0.35
4.6	50	0.8
4.6	100	1.7
10	100	7.9
16	100	20.1

Always think of gradients in terms of column volumes, because a slight change in gradient time can result in a dramatic difference in gradient volume and column performance.

For example, on a conventional 4.6 × 250-mm analytical column (volume 4.25 ml), a 45-minute run at 1 ml/min represents a 10.6-column volume gradient.

On a POROS™ 4.6mmD/100mmL column (volume 1.7 ml), a 5-ml/min flow rate translates into 3-column volumes/min (5/1.7). Therefore, a 10.6-column volume gradient would be completed in 3.5 minutes.

Adjust the sample load

If the volume of your POROS™ column is different from the column you are currently using, adjust the sample volume or mass proportionally to keep the same load per unit volume of column. See "Think in terms of column volumes" on page 4 for POROS™ column bed volumes to help you make the necessary calculations.

Measure recovery properly

Quantitation (recovery) measurements using peak integration are comparable run to run only if the conditions are kept almost constant.

Flow rate affects the value of the integrated peak area for a given mass recovered, because the amount of material recovered in a peak is equal to the concentration (absorbance) times the volume. However, an integrator (or integration software) quantitates peaks by summing absorbance measurements over *time.* A change in flow rate is a change in the amount of volume over time.

Therefore, time integration does not allow comparison of recovery at different flow rates. Direct comparison of your POROS™ results with previous results on conventional chromatography can lead to the incorrect conclusion that recovery is lower on POROS™ columns.

There are several ways to compensate for limitations in time-based integration:

- Multiply the peak area by the flow rate. Unfortunately, because integrators often vary the data rate with expected peak width, this approach can give invalid results.
- Use peak height for a general comparison, but bandspreading differences due to different efficiencies or gradient slopes causes errors.
- Use peak integration at the same flow rate, but *doing this may not show important effects such as a recovery increase due to shorter time on the column with POROS™ resins.*
- Collect the peaks carefully and analyze using spectrophotometry or other methods. Make sure to develop blanks, especially when UV-absorbing components are present in the eluent.
- If peak collection is not feasible, generate standard curves by injecting increasing amounts of calibration standards of known concentration. If the measured peak area increases linearly with load, and the standard curve passes through the origin, it is likely that recovery is high.

Run the test standard

Run a standard to verify that your system and column are running properly. See "Standard test protocols" on page 4 for details.

Standard test protocols

Use these standard test protocols for POROS™ HS columns to troubleshoot column performance, whenever in doubt.

Chromatographic efficiency

The Column Test Certificate that is supplied with the column lists the chromatographic efficiency, asymmetry, and permeability of the column. The test uses a small, nonretained molecule run at low flow rate, which gives the most sensitive measurement of the packed column bed. Determine plate count with the half-height method.

Conditions

Table 7 Protocol

These conditions are common to all column sizes:				
Eluent A	20 mM MES pH 6.2			
Eluent B	0.5 M NaCl in Eluent A			
Gradient	0–100% B in 5 minutes			
Detection	280 nm			
Flow rate and sample size depend on column diameter and are listed below:				
Column (mmD)	2.1	4.6	10	16
Flow (ml/min)	1	5	25	50
Sample (µl)	5	20	100	200

Results

The standard chromatogram shown in Figure 1 on page 4 was generated on a 4.6mmD POROS™ 20 micron HS column. The retention times and bandspreading vary somewhat between chemistries, particle size, column size, and systems. The general profile should be similar, however.

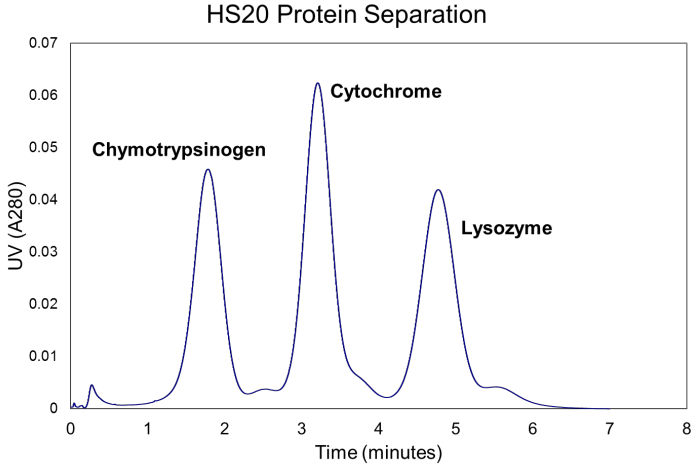

Fig. 1 POROS™ 20 micron HS chromatogram

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Support

For service and technical support, go to **thermofisher.com/poros** or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**, or contact you local Thermo Fisher Scientific representative.

Limited product warranty

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Revision	Date	Description
D	20 September 2017	POROS S products were discontinued and removed from the product insert.
C	10 January 2017	Baseline for this revision history.

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