POROS™ 20 Micron HQ and PI Perfusion Chromatography™ Bulk Media for Anion Exchange Chromatography

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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™ 20 micron bulk media. The same media are also available in prepacked columns. Contact your Thermo Fisher Scientific representative for more details.

Read this section before you begin

Your new POROS™ media is unique

Thermo Scientific™ POROS™ 20 micron bulk media are made for Perfusion Chromatography™ flow-through particle chromatography—a patented new technology that performs bioseparations 10 to 100 times faster than conventional HPLC or LC without loss in capacity or resolution.

Although columns packed with POROS™ 20 micron media can be operated on standard HPLC and LC instrumentation, they are substantially different from any columns you have used before. You may have to change the way you run, and, to some extent, the way you think about chromatography. In particular, the higher flow rates made possible by Perfusion Chromatography™ allow you to perform experiments you might once have considered a luxury, given the constraints of conventional chromatography's longer run times.

Please read the operating instructions carefully to ensure that you take maximum advantage of the benefits that Perfusion Chromatography™ technology provides.

Increase the flow rate

The largest single difference between POROS™ media and conventional media is the flow rates under which high capacity and resolution are achieved.

Although the media can be operated at flow rates typical of conventional chromatography, you can realize the full benefits of Perfusion Chromatography™ only by increasing the flow rate so that the linear velocity is in the range of at least 2,000 cm/hr.

Linear velocity (cm/hr) is calculated by dividing volumetric flow rate (cm^3/min) by the column cross-sectional area (cm^2) and multiplying by 60 min/hr.

The maximum flow rate is not limited by the media itself (high-resolution separations have been achieved at 10,000 cm/hr), but rather is a function of the system used, the pressure limit on the column selected, and how the column is packed. A typical linear flow rate for POROS™ 20 micron media is 3,600 cm/hr.

The corresponding volumetric flow rates for various column diameters are listed in the following table.

Table 1 Typical flow rates for Perfusion Chromatography™

Column Diameter (mm)	Volumetric Flow Rate (ml/min)	Linear Velocity (cm/hr)
2.1	2.0	3,600
4.6	10.0	3,600
10	47.5	3,600
16	120.0	3,600

The dramatically higher flow rates of POROS™ columns and media introduce new considerations into the design and execution of experiments. This is particularly true when you adapt a method developed on conventional media.

Be sure to read "Guidelines for using Perfusion Chromatography™" on page 4 for a full discussion of these considerations. The Busy Researcher's Guide to Biomolecule Chromatography, available from your Thermo Fisher Scientific technical representative, is another excellent reference.

Reoptimize your method as needed

You may need to reoptimize the separation to account for possible differences in selectivity between POROS™ media and your old media.

The short run times associated with Perfusion Chromatography™ make optimization quick and easy, especially if you are using the VISION™ or BioCad™ Workstation for Perfusion Chromatography™.

Product description

Thermo Scientific™ POROS™ HQ and PI columns are polymeric packings designed for anion exchange chromatography of peptides, proteins, polynucleotides and other biomolecules in the Perfusion Chromatography™ flow-through particle chromatography mode. They consist of cross-linked poly(styrene-divinylbenzene) flow-through particles with a patented bimodal pore size distribution for very rapid mass transport.

POROS™ HQ media is surface coated with fully quaternized polyethyleneimine. It is a strong anion exchanger with complete surface ionization over the pH range 1 to 14.

POROS™ PI media is surface coated with polyethyleneimine. It is a weak anion exchanger for which the surface ionization varies continuously over the pH range of approximately 3 to 9. Different selectivities at different pHs can be used to advantage to develop an optimum separation.

Table 2 Product characteristics

Support Matrix	Cross-linked poly(styrene-divinylbenzene)	
Surface Functionality	POROS™ HQ	Quaternized polyethyleneimine
	POROS™ PI	Polyethyleneimine
Dynamic Binding Capacity @ 3600 cm/hr	Bovine serum albumin, pH 8.0	
	POROS™ HQ	60 mg/ml
	POROS™ PI	45 mg/ml
Shrinkage/Swelling	<1% from 1–100% solvent	
Particle Size	20 µm	
Recommended maximum flow rate	10,000 cm/hr	
Maximum pressure drop	170 bar (2500 psi, 17 MPa)	
Permeability	<3 bar at 1000 cm/hr (3 cm bed height)	

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! CHEMICAL HAZARD. Ethylene glycol may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves

Table 3 Chemical resistance

pH Range	1–14 (Up to 5 M NaOH, 1 M HCl)
Ionic Strength	0-5 M, all common salts
BufferAdditives	All common agents suitable, including 8 M urea, 6 M guanidine hydochloride, ethylene glycol, and detergents. Anionic detergents not recommended, as they can bind. **Note:** Do not expose buffer additives 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
Operating Temperature	5–80 °C

Packing the column

POROS™ 20 micron media are mechanically rigid and can therefore be packed effectively both in low-pressure glass columns and in high-pressure PEEK or stainless steel columns. Use column bed supports (frits or screens) with a porosity of 10 µm or less.

Precautions

CAUTION! POROS™ 20 micron bulk media are supplied as a dry powder, which may form a light dust. Avoid inhaling the dust: some surface chemistries are irritants. Keep the media container closed when it is not in use.

Do not exceed 170 bar (2,500 psi) pressure drop across the column during or after packing.

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.

   The packed bed volume specified on the label is based on a packing pressure of 170 bar.
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.

Packing the column

To ensure best results when you pack the column:

- Use a reservoir or adjustable column large enough to hold the entire slurry, so that the bed may be packed all at once.
- Use flow packing techniques. Gravity settling is timeconsuming and usually results in poor performance.

Follow these steps:

1. Gently stir the slurry just before adding it to the column.

   POROS™ beads have a density similar to water, so rapid settling is not usually a problem.
2. Pour the slurry in gradually to minimize the trapping of air bubbles.
3. Tap the column gently to remove air bubbles.
4. Top off the column with the slurry solvent.
5. Connect the column to the packing pump.
6. Pack the column at a flow rate giving a final pressure about 20% to 50% greater than the maximum anticipated operating pressure.
7. Once the bed is formed and the final pressure is reached, pump the column with 10 to 20 bed volumes of slurry solvent to stabilize the bed.

Selecting and preparing the starting buffer

Regardless of the buffer system you choose, always:

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.

- Because POROS™ PI media is a weak anion exchanger, the positive surface charge of the packing increases with decreasing pH while the charge of the protein becomes more positive. These two effects must be balanced for good binding.
- With the strong anion-exchange packing (POROS™ HQ), the charge of the packing itself is essentially unchanged from pH 1 to 14. This allows operation at high pH for binding of proteins with a very high isoelectric point or surface pKa.

Examine the effect of pH in a systematic screening or mapping experiment. For anion exchangers, examine the pH range 6 to 9.

The very high flow rates possible with Perfusion Chromatography™ mean that such pH mapping can be completed quickly. The VISION™ and BioCad™ Workstations have a template feature that automatically performs an entire pH mapping study in less than one hour.

Buffer ions

Table 4 on page 3 lists recommended cationic buffers. Keep this information in mind as you choose your buffer system:

- Buffer ions should be cationic or at least zwitterionic.

- Avoid anionic buffers such as phosphate and borate, because they can bind to the functional groups and cause local changes in pH.
- Keep buffer ion concentration in the range 20 to 50 mM for POROS™ HQ media.
- Use higher buffer concentrations (50 to 100 mM) for POROS™ PI media because of the buffering capacity of the column itself.
- After equilibrating the column, check that the pH of the effluent buffer is at the desired value, especially with POROS™ PI media.

Table 4 Recommended cationic buffers

pH Range	Buffer Ion	pKa
4.5–5.0	N-methylpiperazine	4.75
5.0–6.0	Piperazine	5.68
5.8–6.4	Bis-Tris	6.50
6.4–7.3	Bis-Tris propane	6.80
7.3–7.7	Triethanolamine	7.77
7.5–8.0	Tris	8.16
8.0–8.5	N-methyldiethanolamine	8.54
8.4–8.8	Diethanolamine	8.88
8.5–9.0	1,3-diaminopropane	8.64
9.0–9.5	Ethanolamine	9.50
9.5–9.8	Piperazine	9.73
9.8–10.3	1,3-diaminopropane	10.47

Additives

You can use POROS™ HQ and PI media with most additives such as urea, ethylene glycol and nonionic or cationic detergents.

Avoid anionic detergents, because they bind tightly to the packing.

Adding up to 30% alcohol or acetonitrile can be a very useful technique for peptides or small proteins that may not be fully soluble or that bind by mixed ionic/hydrophobic interactions in 100% aqueous eluents.

Preparing and loading the sample

To ensure efficient binding and prevent column plugging:

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.

Determining the sample load

The dynamic binding capacities of POROS™ HQ and PI are listed in Table 2.

In general, high resolution separations are generally achieved at 20% of the total binding capacity or less. However, the maximum loading at which a given resolution can be obtained (the loadability) is dependent upon a number of factors, including sample solubility, column selectivity, and so on.

The long run times associated with conventional chromatography usually prohibit a systematic determination of loadability. Perfusion Chromatography™'s short run times make it easy to perform this determination.

Perform a loadability experiment as follows:

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.
3. If you are using a VISION™ or BioCad™ Workstation, take advantage of the loading study template designed specifically for this purpose.

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

Again, short run times made possible by Perfusion Chromatography™ make reoptimization quick and easy, especially if you use the template features of the VISION™ and BioCad™ Workstations.

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 can also be used.
- Up to 1.0 M ionic strength elutes most proteins.
- Use up to 2.0 M ionic strength to remove very 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 normally provide a good compromise between resolution and peak dilution.

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

Cleaning up and regenerating the media

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
- "Ghost" peaks during blank gradient runs

Note: In the cleanup method, reverse the flow direction to help flush out particulates and to prevent contamination of the lower part of the bed. Also, slow the flow rate to expose the column to the regeneration solution for several minutes at each step of the cleaning protocol.

Simple regeneration

Wash with 1 to 5 column volumes of 1 to 2 M of the salt used for elution.

If simple regeneration does not restore column performance, a more complex regeneration is required.

Complex regeneration

To regenerate more completely:

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

Removing lipids and lipoproteins

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.

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.

WARNING! CHEMICAL HAZARD. Methanol is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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

- Use a mixture of 50% methanol or acetonitrile with the acid or base.
- Use a mixture of 50% methanol and 50% 3 M guanidine thiocyanate.
- 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 exposes the column to the regeneration solution for several minutes.

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 media

Store the dry powder at room temperature.

To store a packed column:

- Carefully seal the ends of the column to prevent drying. Drying results in decreased chromatographic efficiency.
- Store the column between 5 and 30 °C.

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.

CAUTION! Sodium azide is toxic. Follow precautions and decontamination procedures recommended by the National Institute for Occupational Safety and Health.

Guidelines for using Perfusion Chromatography™

When you make the transition to Perfusion Chromatography™, consider factors related to:

The Chromatography System:

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

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 Perfusion Chromatography™ 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 required to take full advantage of Perfusion Chromatography™ technology.

It is important to isolate the relative contribution of 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).

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 as well as 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.

On the BioCad™ Workstation, gradient performance is tracked on each run with the internal conductivity detector. If there is any question about gradient performance on other systems, the gradient can be visualized as follows:

1. Connect a union in place of the column.
2. Form a gradient with water as the A solvent and 0.5% acetone in water as the B solvent with detection at 280 nm.

   The UV absorbance is directly proportional to the concentration of B solvent and can be compared to the programmed gradient.

Consult your system vendor for serious gradient problems.

Adjust the data collection system

Because Perfusion Chromatography™ runs are much shorter than conventional chromatography runs, you may need to adjust your data collection system as follows:

- To obtain high-definition chromatograms, use a shorter total run time and higher data collection rate (or lower average peak width parameter). A typical data collection rate is 10 points/second.

- If you use a chart recorder, increase the chart speed in proportion to the flow rate increase.

Maintain your column and system

With Perfusion Chromatography™, you can perform runs more quickly than other chromatography techniques. For this reason, perform maintenance tasks such as replacing filters or regenerating columns after a certain number of runs, rather than after a set period 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 used for Perfusion Chromatography™, the gradient times are dramatically shorter than what you are accustomed to working with. To convert a method to Perfusion Chromatography™, keep the gradient volume constant in terms of column volumes and adjust the time of the gradient according to the new flow rate.

When you work routinely with Perfusion Chromatography™, always think of gradients in terms of column volumes, since a slight change in gradient time may 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.

The VISION™ and BioCad™ Workstations allow you to program by column volumes.

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.

Measure recovery properly

Quantitation (recovery) measurements using peak integration are comparable run to run, only if the conditions are kept nearly 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 may lead to the incorrect conclusion that recovery is less 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 method can give invalid results.
- Using peak height can give a general comparison, but bandspreading differences due to different efficiencies or gradient slopes cause errors.
- Recovery may be compared accurately using 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 Perfusion Chromatography™.*
- 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 very likely that recovery is high.

Run the test standard

Run a standard to verify that your system and column are running properly. For details, see “Standard test protocols“ on page 5.

Standard test protocols

Use these standard test protocols for POROS™ HQ and PI media to:

- Confirm that the column and hardware are functioning properly in the Perfusion Chromatography™ mode
- Obtain working experience with Perfusion Chromatography™ before running a real sample
- Troubleshoot

Protein separation

For the sample, use the Anion Exchange Protein Test Standard available from **thermofisher.com**. Run the separation with a linear gradient from 0 to 0.5 M NaCI. Run conditions are described below.

The test consists of these steps:

1. Dissolve the lyophilized sample mixture in 1 ml of Eluent A (5 mg/ml ovalbumin, 5 mg/ml bovine serum albumin).
2. Filter the sample after thorough mixing.
3. Store the reconstituted test mix frozen. Do not store it for long periods at room temperature.
4. Run the sample.

Run conditions

Table 5 Protocol

Conditions Common to All Column Sizes	
Eluent A	50 mM Tris Hydrochloride pH 8.5
Eluent B	50 mM Tris Hydrochloride pH 8.5, 0.5 M NaCl
Flow rate	2000 cm/hr
Sample	1–2% of column bed volume
Gradient	0–100% B in 5 minutes
Detection	280 nm

Results

The standard chromatogram shown in Figure 1 on page 5 was generated on a 4.6-100 mm column packed with POROS™ 20 micron PI. The retention times and bandspreading may be different with different packings, column sizes, and gradient systems, but the general profile should be similar.

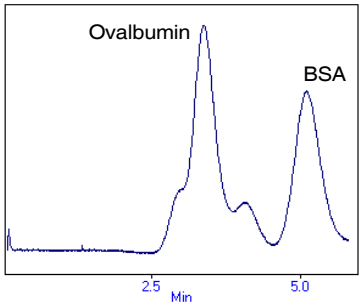

Fig. 1 POROS™ 20 micron PI chromatogram

Ordering information

Protein test standards are available from **thermofisher.com**.

Table 6 Test standard ordering information

Description	Quantity	Part Number
Anion Exchange Protein Test Standards	Package of 5 vials	1-9002-05

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
C	10 January 2017	Baseline for this revision history.

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