

POROS[®] MabCapture[™] A Perfusion Chromatography[®] Media

Protocol

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Preface

This preface covers:

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Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below.

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

 **CAUTION** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

 **WARNING** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

 **DANGER** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

 **WARNING CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “[About MSDSs](#)” on [page vi](#).)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to <https://docs.appliedbiosystems.com/msdssearch.html>
2. In the Search field of the MSDS Search page:
 - a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
 - b. Select the language of your choice.
 - c. Click **Search**.

3. To view, download, or print the document of interest:
 - a. Right-click the document title.
 - b. Select:
 - **Open** – To view the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose
 - **Print Target** – To print the document
4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
 - a. Select **Fax** or **Email** below the document title.
 - b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
 - c. Enter the required information.
 - d. Click **View/Deliver Selected Documents Now**.

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Waste Hazards



CAUTION HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.



WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.



WARNING CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety



WARNING

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; <http://bmb1.od.nih.gov>)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

<http://www.cdc.gov>

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

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How to Obtain Support

For the latest services and support information for all locations, go to <http://www.appliedbiosystems.com>, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

POROS[®] MabCapture[™] A Perfusion Chromatography[®] Media Protocol

Overview

Applied Biosystems POROS[®] MabCapture[™] A media enables you to employ Perfusion Chromatography[®] media technology. Chromatographic separations of biomolecules can be performed considerably faster than conventional liquid chromatography separations while maintaining high dynamic binding capacity. With POROS MabCapture A media, you can perform separations at operating flow rates up to 1000 cm/hr.

Product Description

POROS MabCapture A media is a polymeric media designed for preparative purification of monoclonal antibodies. The media consists of rigid cross-linked poly(styrene-divinylbenzene) flow-through particles with pore structure optimized for very rapid mass transport. The particle surface is coated with a cross-linked polyhydroxylated polymer. This coating is further derivatized by covalent immobilization of a recombinant Protein A.

Table 1 Product characteristics

Parameter	Description
Support Matrix	Cross-linked poly(styrene-divinylbenzene)
Immobilized Ligand	Recombinant Protein A
Dynamic Binding Capacity @300 cm/hr in 20 cm bed length	Human IgG, pH 7.5, > 45 mg/ml 5% breakthrough capacity
Shrinkage/Swelling	< 1% from 1–100% organic solvent
Particle Size	45 µm
Maximum flow rate in 20 cm bed length	2,000 cm/hr

Table 1 Product characteristics (continued)

Maximum operating pressure	100 bar (1,500 psi, 10 MPa)
Media backpressure (see Figure 1)	< 2.5 bar at 700 cm/hr (20 cm bed height)
Protein A leaching	< 50 ppm

POROS MabCapture A media is mechanically stable up to backpressures of 1,500 psi. Bed compression at high flow rates is therefore not a concern. The pressure-flow properties of POROS MabCapture A media ([Figure 1](#)) allow the media to be packed and run using conventional low pressure columns and systems.

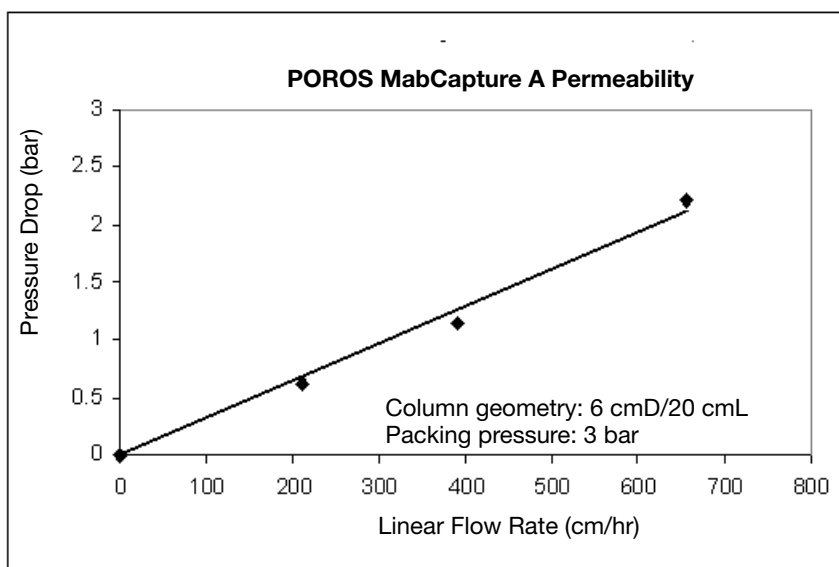


Figure 1 Pressure-flow properties of POROS MabCapture A media

POROS MabCapture A media's chemical stability characteristics make it compatible with a variety of solvents and buffer salts, shown in [Table 2](#).

Table 2 Chemical resistance

Parameter	Compatibility
pH Range (routine use)	pH 2–10
Ionic Strength Range	0–5 M, all common salts
Cleaning Agents	All common agents, including 0.1 N sodium hydroxide (NaOH), 8 M urea, 2–6 M guanidine/hydrochloric acid (HCl), ethylene glycol, and detergents. Agents that may degrade the protein ligand are not recommended.
Solvents	Water, 0–100% alcohols, acetonitrile, other common organic solvents. Note: Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric) or strong reducing agents (such as sulfite). See “Column Cleaning” on page 10 and “Column Sanitizing” on page 12 for guidelines on using NaOH.

Packing the Column

This section discusses:

- Packing solvents
- Preparing the slurry
- Packing procedures

POROS MabCapture A media is mechanically rigid and can be packed effectively at low or high pressure. The recommended screen (frit) size is 10 to 20 μm .

Packing Solvents

Use these solutions:

- Slurry buffer: 0.5 M sodium chloride (NaCl)
- Packing buffer: 0.1 M NaCl

Preparing the Slurry

POROS MabCapture A media is supplied as a slurry containing 20% buffered ethanol as a bacteriostat. See the label for the lot-specific volume of media, based on a packing pressure of 3 bar.

The volume of slurry needed to pack your own column is $1.8 \times$ the packed bed volume. (The packed bed volume is 56% of the volume of slurry needed.)

Example: At a packing pressure of 3 bar, a column with 10 mL packed bed volume requires 18 mL of slurry.

To prepare the slurry for packing:



WARNING

CHEMICAL HAZARD. POROS MabCapture A Perfusion Chromatography Media Slurry containing ethanol is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry the skin. It contains material that may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Allow the media to settle for 3 to 5 hours.
2. Pour off the supernatant.

3. Resuspend the media in 0.5 M NaCl with gentle agitation.

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.

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

Packing Procedures

To ensure best results when you pack the column, use:

- A large enough reservoir or adjustable column to contain the entire slurry, so that the bed may be packed all at once.
- Flow packing or pressure packing techniques.

Flow Packing

IMPORTANT! When delivering the slurry and adjusting the flow rate, work efficiently to avoid media settling via gravity. While adjusting the flow rate and forming the bed, you may observe some fine material in the eluent as packing begins. This will clear as packing proceeds and 2 to 3 bed volumes of packing buffer pass through the column.

1. Deliver the slurry:
 - a. Gently mix the slurry just before adding it to the column.

Note: POROS MabCapture A beads have a skeletal density similar to the density of water, so rapid settling is not a problem.
 - b. Pour the slurry into the column gradually to minimize the trapping of air bubbles.
 - c. Tap the column gently to remove air bubbles.
 - d. Top off the column with the slurry buffer.
2. Connect the top adjuster to the column.
3. Adjusting the flow rate:
 - a. Start the flow until a clear space between the column top adjuster and the slurry forms.
 - b. Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used.

Note: The final packing flow rate should be at least 20% greater than the maximum anticipated operating flow rate.

4. Once the bed is formed and the final flow rate is reached, bring the column top adjuster into contact with the top of the bed.
5. Restart the flow for 3 to 5 bed volumes to stabilize the bed. POROS MabCapture A media does not shrink or swell, so an open “head space” is not recommended.
6. Equilibrate the column with 3 to 5 bed volumes each of:
 - a. 100 mM phosphate buffered saline (PBS), pH 7.5
 - b. 100 mM glycine HCl, pH 3.0
 - c. 100 mM PBS, pH 7.5The column is ready for operation.
7. Test the column. For HETP and asymmetry determinations, recommended test solutions are conductivity or UV-based solutions. Avoid using acetone for these measurements.

Axial Compression Pressure Packing

1. Deliver the slurry:
 - a. Gently mix the slurry just before adding it to the column.
Note: POROS MabCapture A beads have a skeletal density similar to the density of water, so rapid settling is not a problem.
 - b. Pour the slurry into the column gradually to minimize the trapping of air bubbles.
 - c. Tap the column gently to remove air bubbles.
 - d. Top off the column with the slurry buffer.
2. Connect the top adjuster.
3. Apply pressure:
 - a. Expel trapped air from the column.
 - b. Select the packing pressure on the pressure gauge.
4. Pack the column.

You may observe some fine material in the eluent as packing begins. This material will clear as packing proceeds and 2 to 3 bed volumes of packing buffer pass through the column.
5. Apply flow for 3 to 5 bed volumes to stabilize column bed.
Note: The flow rate should generate no more than 80% of the packing pressure.

6. Equilibrate the column with 3 to 5 bed volumes each of:
 - a. 100 mM PBS, pH 7.5
 - b. 100 mM glycine HCl, pH 3.0
 - c. 100 mM PBS, pH 7.5

The column is ready for operation.

7. Test the column. For HETP and asymmetry determinations, recommended test solutions are conductivity or UV-based solutions. Avoid using acetone for these measurements.

Selecting and Preparing the Starting/Wash Buffer

Regardless of the buffer system you choose:

- Use buffers of the highest purity practical.
- Filter (0.22 or 0.45 μm) all buffers prior to use.

Follow these guidelines when you select and prepare the starting/wash buffer:

- In most cases, use 100 mM PBS pH 7.0 to 7.5.
- For murine IgG1 or antibodies that exhibit low affinity for Protein A in low ionic strength buffers, a buffer consisting of 3 M NaCl or KCl, 100 mM glycine, pH 8.5 to 9.0 may give improved binding.

Preparing and Loading the Sample

To ensure efficient binding and prevent column plugging:

- Centrifuge or filter samples (0.22 or 0.45 μm) prior to injection.
- Delipidate samples, if possible. Lipids can cause fouling (see [“Column Cleaning” on page 10](#)).
- Lower the operating flow rate when you work with viscous solutions (example: loading an antibody in high salt at 4° C). The lower flow rate compensates for the increased pressures generated by the viscosity of the solution.

Determining the Sample Load

Consider the following factors as you determine sample load:

- The dynamic binding capacity of POROS MabCapture A beads for human IgG is listed in Table 1.
- The binding capacity for other antibodies depends upon the antibody source and subclass, but is generally lower than the capacity for human IgG.

Dilute Feed Capture

POROS MabCapture A media can concentrate very dilute samples, such as cell culture supernatants, with very high throughput (up to 50 column volumes per hour). If you use POROS MabCapture A media as the first step to recover an antibody from a dilute feed, the high throughput capability of the media can allow you to eliminate an ultrafiltration (UF) concentration step on scale-up. In designing such a process, use a membrane filtration (MF) step to clarify the feed and apply the filtrate directly to the POROS MabCapture A column.

Wash/Elution Protocols

The eluent used to recover bound antibody can vary. To elute most antibodies, reduce the pH to the range of pH 2.0 to 3.0. Use any of these eluents:

- Acetic acid (2 to 20% v/v, pH 2.0 to 3.0)
- 0.01 to 0.1 M glycine (pH 2.0 to 3.0)
- 0.1 M citrate (pH 3.0)

Because antibodies differ by both species and subclass in their binding/elution behavior, the best elution conditions are determined experimentally. A good eluent to start with is 0.1 M glycine, pH 3.

Eluting the Sample

To elute the sample:

1. Wash unbound material from the column with the starting/wash buffer.

Generally a 5 to 10 column volume wash is sufficient to remove all unbound proteins from the column. Samples with high impurity levels may require a longer wash to return to a stable baseline.
2. Use 5 column volume steps to elute with the chosen eluent and to re-equilibrate with the starting/wash buffer. Generally, antibodies elute from POROS MabCapture A media in approximately 2 column volumes.
3. Immediately neutralize the eluted antibody to prevent denaturation of some antibodies at low pH.

Column Cleaning

During normal column operation, column fouling can occur due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities. These symptoms indicate column fouling:

- Increased bandspreading
- Loss of binding capacity
- Loss of recovery
- Increased pressure drop
- Trace or “ghost” peaks different from the usual refractive index peak during blank runs

To avoid contaminant buildup and to ensure long column lifetime, clean the column in place (CIP) at least every third cycle. Depending on the expected cause of fouling, the following CIP procedures are recommended:

Protein Precipitation

Use acetic acid as the cleaning agent for routine CIP when protein precipitation is the expected cause of fouling:

- Expose the column to 1M acetic acid at a low flow rate (50 to 100 cm/hr) for at least 2 minutes or at least 5 column volumes.
- Re-equilibrate the column with 5 to 10 bed volumes of 100 mM PBS, pH 7.5 until the desired pH is reached.

Lipid Binding

Use acid alcohol as the cleaning agent for routine CIP when lipid is the expected cause of fouling:

1. Expose the column to a solution containing 1 M acetic acid, 20% ethanol in water at a reduced flow rate (50 to 100 cm/hr) for at least 5 minutes or at least 10 column volumes. If possible, run the column in reverse mode.
2. Re-equilibrate the column with 5 to 10 bed volumes of 100 mM PBS, pH 7.5 until the desired pH is reached.

Note: 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 bed. Also, slow the flow rate to give several minutes' exposure to the regeneration solution at each step of the cleaning protocol.

Column Sanitizing

Perform column sanitizing when one of the following occurs:

- Contamination of a process stream with microorganisms
- Increasing levels of microorganisms in a POROS MabCapture A column

For a sanitization procedure for POROS MabCapture A media, use the NaOH method.

NaOH Method

1. Clean the column with up to 0.1 N NaOH for up to 30 minutes per cycle.
2. Re-equilibrate with neutral pH buffer such as 100 mM PBS, pH 7.5.

Figure 2 demonstrates the stability of POROS MabCapture A media upon exposure to NaOH.

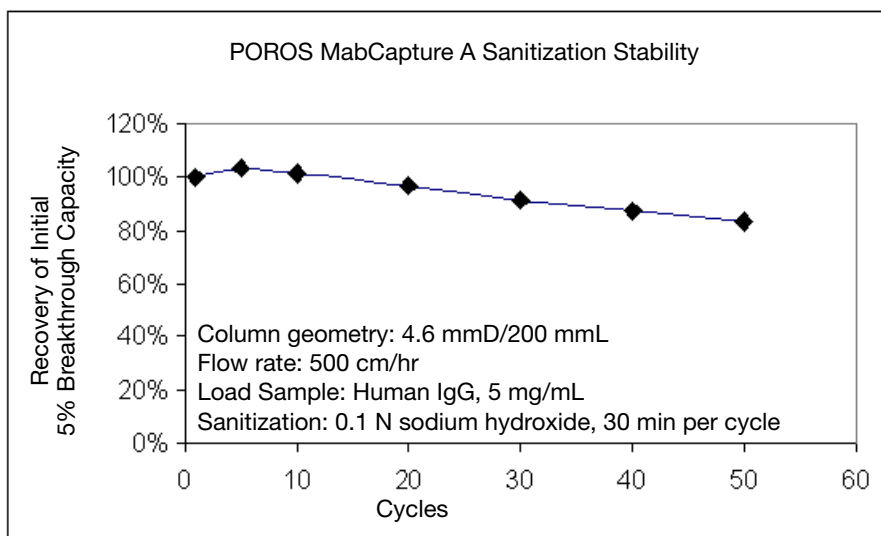


Figure 2 Effect of sanitization cycles on binding capacity

Storing the Media

- Store POROS MabCapture A media refrigerated at 2 to 8 °C.

IMPORTANT! Do not freeze.

- Store packed columns with the endcaps in place, carefully sealed to prevent drying. Drying can result in decreased chromatographic efficiency.

Before you store the column for longer than 1 week, put the column through a cleaning-in-place cycle (see [“Column Cleaning” on page 10](#)). Equilibrate and store the column in 100 mM PBS, pH 7.5 in 20% ethanol or 0.02% sodium azide.



DANGER

CHEMICAL HAZARD. Sodium azide is a poison. It may be fatal if inhaled, swallowed, or absorbed through the skin. Exposure may cause nerve and heart damage. Contact with acids liberates toxic gases. DO NOT ADD acids to any liquid wastes containing sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Scaling Up

Follow the guidelines below during methods development to ensure easy scale up to preparative and production volumes:

- Program your chromatographic system in column volumes. If programming your system is not possible, make sure that gradient volumes and linear velocities do not change out of proportion to column volume.
- Keep sample loading proportionally the same as column size increases.
- Keep column bed height the same as you scale up. If keeping the column bed height the same is not practical as you increase column size, make sure that you do not reduce sample residence time as scale increases.
- As column volume increases, if the column maximum pressure limit is reduced, thereby forcing changes in packing pressures or flows, test the effectiveness of packing by measuring HETP and asymmetry using a suitable probe molecule. Protocols are available from your Applied Biosystems Technical Representative.

Guidelines for Using Perfusion Chromatography Media

Guidelines related to the chromatography system:

- Account for system pressure
- Check the gradient system
- Adjust the data collection system
- Maintain your column and system

Guidelines related to experimental design:

- Develop method steps in terms of column volumes not time
- Adjust the sample load
- Measure recovery properly

Account for System Pressure

The high flow rates used with Perfusion Chromatography media 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. 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:
 - a. Connecting a union in place of the column
 - b. 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.
3. Ensure that the system and column pressures do not exceed the column operating pressure.

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.

Note: In some systems, excessive system pressure can preclude the high flow rates required to take full advantage of Perfusion Chromatography media.

It is important to isolate the relative contribution of column and instrument when pressures approach the maximum column pressure (measured pressure = column pressure drop + system pressure).

**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 constant.

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Headquarters

850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Technical Support

In North America, call +1 800.899.5858.
Outside North America, see our Web site at **www.appliedbiosystems.com**.

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