Operating Instructions

NOTE: These instructions cover the specific operational characteristics of POROS prepacked columns. POROS media are also available in bulk quantities for direct scale-up of separations developed on prepacked columns. Contact your Applied Biosystems representative for more details.

Your New POROS Column Is Unique!

Read this section before doing anything!

Applied Biosystems POROS columns 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 POROS columns can be operated on standard HPLC 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 run the column to its full capability and take maximum advantage of the benefits that Perfusion Chromatography technology provides.

Increase the Flow Rate

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

While the column can be operated at flow rates typical of conventional chromatography, the full benefits of Perfusion Chromatography can be realized only by increasing the flow rate so that the linear velocity is in the range of at least 1,000 to 5,000 cm/hr. Higher flow rates are possible but may not be practical, depending on the system you are using, the viscosity of your solvents, or the pressure rating on the column.

Linear velocity (cm/hr) is calculated by dividing volumetric flow rate (cm³/min) by the column cross-sectional area (cm²) and multiplying by 60 min/hr.

NOTE: If you are operating with a peristaltic pump, you may need to run at lower flow rates to keep within the pressure rating of the pump.

Table 1 Typical Flow Rates for Perfusion Chromatography

<table>
<thead>
<tr>
<th>Column Diameter (mm)</th>
<th>Flow Rate Range (ml/min)</th>
<th>Linear Velocity (cm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>0.5–5</td>
<td>900–8,500</td>
</tr>
<tr>
<td>4.6</td>
<td>3–15</td>
<td>1,000–5,300</td>
</tr>
<tr>
<td>10</td>
<td>15–75</td>
<td>1,100–5,700</td>
</tr>
<tr>
<td>16</td>
<td>30–150</td>
<td>900–4,500</td>
</tr>
</tbody>
</table>

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 Section 8, Guidelines for Using Perfusion Chromatography, for a full discussion of these considerations. Another excellent reference is The Busy Researcher’s Guide to Biomolecule Chromatography, available from your Applied Biosystems Technical Representative

Reoptimize Your Method as Needed

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

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

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<tr>
<td>4</td>
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<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
</tr>
</tbody>
</table>

1. Product Description
2. Connecting and Preparing the Column
3. Selecting and Preparing the Starting/Wash Buffer
4. Preparing and Loading the Sample
5. Wash/Elution Protocols
6. Cleaning Up and Regenerating the Column
7. Storing the Column
8. Guidelines for Using Perfusion Chromatography
9. Standard Test Protocols
10. Accessories, Spare Parts, and Ordering Information
11. Technical Support
1 Product Description

POROS A and G columns are designed for analytical and preparative affinity purification of antibodies using Perfusion Chromatography technology. The packings consist of cross-linked poly(styrene-divinylbenzene) flow-through particles with a patented bimodal pore size distribution for very rapid mass transport. The particle surface is coated with a cross-linked polyhydroxylated polymer. This coating is further derivatized with recombinant protein A (POROS A) or protein G (POROS G).

POROS A and G column packages include the following items:

- Packed column, with sealing end caps
- Product Operating Instructions
- Column Test Certificate
- EZ™ Grip stainless steel fittings

1.1 POROS Column Sizes

POROS affinity 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). Refer to Section 8.1, Account for System Pressure, for a more detailed discussion of pressure considerations for running Perfusion Chromatography columns on conventional systems.

Table 2 Classification of POROS A and G Columns

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Maximum Pressure</th>
<th>Type of Separation</th>
<th>Chromatography System</th>
</tr>
</thead>
<tbody>
<tr>
<td>POROS 20 micron</td>
<td>170 bar (2,500 psi, 17 MPa)</td>
<td>Analytical or Preparative</td>
<td>BioCAD Workstation, or conventional HPLC instrumentation</td>
</tr>
</tbody>
</table>

Table 3 Product Characteristics

<table>
<thead>
<tr>
<th>Support Matrix</th>
<th>Cross-linked poly(styrene-divinylbenzene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilized Ligand</td>
<td></td>
</tr>
<tr>
<td>POROS A</td>
<td>Recombinant protein A</td>
</tr>
<tr>
<td>POROS G</td>
<td>Recombinant protein G</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dynamic Binding Capacity @ 3,600 cm/hr</th>
<th>30 mg/ml (human IgG), pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>POROS A</td>
<td>15 mg/ml, pH 7.5</td>
</tr>
<tr>
<td>POROS G</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shipping Solvent</th>
<th>0.1 M phosphate pH 7.0, 0.05% sodium azide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrinkage/Swelling</td>
<td>&lt;1% from 1–100% solvent</td>
</tr>
</tbody>
</table>

Table 4 Chemical Resistance

<table>
<thead>
<tr>
<th>pH Range</th>
<th>POROS A</th>
<th>POROS G</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–10</td>
<td>2–9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ionic Strength Range</th>
<th>POROS A</th>
<th>POROS G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 M, all common salts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Buffer Additives</th>
<th>POROS A</th>
<th>POROS G</th>
</tr>
</thead>
<tbody>
<tr>
<td>All common agents, including 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents. Agents that may degrade the protein ligands not recommended.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solvents</th>
<th>POROS A</th>
<th>POROS G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, 0–100% alcohols, acetonitrile, other common organic solvents.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

NOTE: Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric) or strong reducing agents (such as sulfite).

Operating Temperature | 5–40 °C

2 Connecting and Preparing the Column

The column fitting is an Upchurch Scientific® 10-32 female fitting.

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

NOTE: 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 Applied Biosystems (see Section 10, Accessories, Spare Parts, and Ordering Information).

Connecting the Column

Columns can be connected to M-6 metric fitting systems (such as the Pharmacia FPLC® system) by using the fitting adaptor kit (see Table 8 on page 7).

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 in place of the black M-6 nut.
Preparing the Column
1. POROS A and G columns are shipped in 0.1 M phosphate, pH 7.0, 0.05% sodium azide. Before you use the column for the first time, pump the column with 5 to 10 column volumes of eluent buffer through the column.
2. Equilibrate with 10 to 15 column volumes of starting/wash buffer. Unlike the lower flow rates of conventional chromatography, the high flow rates possible with Perfusion Chromatography allow a thorough column equilibration cycle in a matter of minutes.

3 Selecting and Preparing the Starting/Wash 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.
Follow these guidelines when you select and prepare the starting/wash buffer:
• In most cases, simple buffers such as 10 to 25 mM phosphate or Tris can be used.
• The starting/wash buffer pH can range from 6.0 to 9.0, although binding is usually strongest in the higher pH range.
• Add some salt (0.1 to 0.2 M NaCl or KCl) to prevent nonspecific adsorption due to protein/protein interactions.

4 Preparing and Loading the Sample
To ensure efficient binding and prevent column plugging:
• Dissolve or exchange samples into the starting/wash buffer. This is particularly important for large samples (greater than 25% of the column volume).
• Centrifuge or filter samples (0.22 or 0.45 µm) prior to injection.
• Heat-treat serum samples (56 °C for 30 minutes) to remove residual fibrinogen that can clog the column on multiple runs.
• Delipidate samples, if possible. Lipids can cause irreversible fouling.

Determining the Sample Load
Consider the following factors as you determine sample load:
• The dynamic binding capacities of POROS A and G columns are listed in Table 3.
• The binding capacity for other antibodies depends on the antibody source and subclass and the ligand used, but is generally lower than the capacity for IgG listed in Table 3.
• In analytical applications, maximum load is determined by the linearity of the standard curve. Approximately 100 µg is the maximum recommended analytical load for 2.1 mmD/30 mmL columns.

The long run times associated with conventional chromatography usually prohibit a systematic determination of loadability. Your POROS column’s shorter run times make it easy to perform this determination.

Concentrating Dilute Samples
The binding of antibody to POROS A and G 2.1 mmD/30 mmL columns and the resulting elution peak depend on the total mass, not the concentration, in the sample. This is because of the very high binding constants inherent in the antibody-ligand interaction. Therefore, POROS A and G columns can concentrate very dilute samples such as cell culture supernatants.

Because of the high flow rates possible with Perfusion Chromatography, concentration of a dilute sample occurs quickly. On analytical 2.1 × 30 columns, sample sizes can be as large as 2 to 3 ml (20 to 30 column volumes) or more and still give good results in 2 to 5 minutes or less.

5 Wash/Elution Protocols
The eluent used to remove bound antibody can vary. Follow these guidelines:
• To remove most antibodies, reduce the pH to between 2 and 3. Acetic acid (2 to 20% v/v) and 0.01 to 0.1 M glycine (pH 2 to 2.5) are most commonly used.
• 6 to 12 mM HCl, 0.15 NaCl also reduces pH to the desired range and minimizes refractive index effects (see below).
• For antibody subclass fractionation on POROS A columns, use eluents of pH 5.0 or lower.

Because antibodies differ by both species and subclass in their binding/elution behavior, the best elution conditions can be determined only by trial and error.
• In general, more severe pH conditions are required for elution from POROS G columns than from POROS A columns.
• To elute all antibodies from Protein A and Protein G, use a mixture of 20% (v/v) acetic acid, 0.3 M MgCl₂. The magnesium chloride is particularly effective as an elution agent.

The high speed of Perfusion Chromatography allows you to screen a variety of conditions quickly.

5.1 Running RI Blank Peaks
In general, the eluents used in Protein A and G affinity chromatography have a refractive index (RI) substantially different from the starting/wash buffer. This accounts for the artifactual (RI) peak that appears when you switch to the elution buffer.

The shape and magnitude of this RI peak, and whether it is positive or negative, depend strongly on the buffer/eluent combination and the detector. This RI peak can affect quantitation, especially at low concentrations where high sensitivity is required. Always make blank runs and examine peak integration results carefully for artifacts or an improperly drawn baseline.

In applications that require maximum sensitivity, minimize the RI blank peak by using:
• 50 mM phosphate pH 7, 0.15 M NaCl as the starting/wash buffer
• 0.1% (12 mM) HCl, 0.15 M NaCl as the eluent

Danger CHEMICAL HAZARD. Hydrochloric acid (HCl) causes severe eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This is the most effective wash/eluent system with the minimum RI shift. HCl can denature antibodies, so it is not recommended when biological activity is required.
5.2 Eluting the Sample

To elute the sample, follow these steps:

1. Wash the column with the starting/wash buffer.
   Generally a 5 to 15 column-volume wash is sufficient to remove all unbound proteins from the column. Samples with high contaminant concentrations, however, may require a longer wash to return to a stable baseline.
   All unbound proteins must be removed, because some proteins may precipitate in the acidic eluents.

2. Use 5 to 15 column-volume steps to elute with the chosen eluent and to reequilibrate with the starting/wash buffer.

6 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 you see:

- Increased bandspreading
- Loss of binding capacity
- Loss of recovery
- Increased pressure drop
- Trace or “ghost” peaks different from the usual RI shift peak during blank 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 bed. Also, slow the flow rate to expose the column to the regeneration solution for several minutes at each step of the cleaning protocol.

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.

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.

To regenerate the column, follow these guidelines:

1. Wash with 3 to 5 ml of 20% (v/v) acetic acid, 0.3 M MgCl₂ to remove any bound antibody that may remain.
2. Remove precipitated protein, bound lipids, and so on by other means.
   Brief washes with alcohols, acetonitrile, or mixtures of these solvents with 20% acetic acid may help in these cases.
   2 M guanidine hydrochloride is also very effective.

Multiple Injections

You can use multiple injections of regeneration solutions instead of pumping the solutions directly. The multiple injection 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 to expose the column to the regeneration solution for several minutes.

Important: Backpressure increase is sometimes caused by a plugged inlet frit. If backflushing the column does not solve the problem, replace the inlet frit. Refer to Section 10, Accessories, Spare Parts, and Ordering Information, for details.

7 Storing the Column

When you store your column, always:

- Store columns in the refrigerator, but DO NOT FREEZE THEM!
- Store columns with the endcaps in place, carefully sealed to prevent drying. Drying results in decreased chromatographic efficiency.

Short-Term Storage

Store columns in any appropriate starting/wash buffer.

Long-Term Storage

Store the column in 0.02% sodium azide as a preservative.

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

8 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

8.1 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:
   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 in step 1 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 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 for POROS columns is provided in Table 2.

Typical pressure drops of POROS columns (at 2,000 cm/hr with water as the mobile phase) are shown in Table 5. Refer to this table to verify column performance or to help you decide if column regeneration is required.

### Table 5 Typical Column Pressure Drops

<table>
<thead>
<tr>
<th>Pressure: Bar (psi)</th>
<th>30 mmL</th>
<th>50 mmL</th>
<th>100 mmL</th>
</tr>
</thead>
<tbody>
<tr>
<td>POROS 10</td>
<td>40 (600)</td>
<td>60 (900)</td>
<td>80 (1,200)</td>
</tr>
<tr>
<td>POROS 20</td>
<td>20 (300)</td>
<td>30 (450)</td>
<td>40 (600)</td>
</tr>
</tbody>
</table>

### 8.2 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 VISION or BioCAD Workstation, gradient performance is tracked on each run with the internal conductivity detector. If you have any question about gradient performance on other systems, visualize the gradient 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.

### 8.3 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.

### 8.4 Maintain Your Column and System

With Perfusion Chromatography you can perform runs more quickly than other chromatography technologies. For this reason, perform maintenance tasks such as replacing filters or regenerating columns after a certain number of runs rather than after a specified period of time. You can reduce the frequency of such maintenance by always filtering the sample and eluent.

### 8.5 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 those 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. Table 6 provides bed volumes of POROS columns to help you make the necessary calculations.

### Table 6 POROS Prepacked Columns

<table>
<thead>
<tr>
<th>Diameter (mmD)</th>
<th>Length (mmL)</th>
<th>Column Bed Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>30</td>
<td>0.10</td>
</tr>
<tr>
<td>2.1</td>
<td>100</td>
<td>0.35</td>
</tr>
<tr>
<td>4.6</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>4.6</td>
<td>100</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>7.9</td>
</tr>
<tr>
<td>16</td>
<td>100</td>
<td>20.1</td>
</tr>
</tbody>
</table>

When you work routinely with Perfusion Chromatography, always think of gradients in terms of column volumes, because 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 three 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 directly in column volumes.
8.6 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. Refer to Table 6 for POROS column bed volumes to help you make the necessary calculations.

8.7 Measure Recovery Properly

Quantitation (recovery) measurements using peak integration are comparable run to run only if the running 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 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 will cause 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 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.

8.8 Run the Test Standard

Run a standard to verify that your system and column are running properly. Refer to Section 9, Standard Test Protocols, for details.

9 Standard Test Protocols

Applied Biosystems has designed a performance test protocol for POROS A and G columns. Use this test to troubleshoot column performance whenever in doubt.

9.1 Chromatographic Efficiency

The Column Test Certificate provided with the column lists the chromatographic efficiency, asymmetry, and efficiency for 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. Plate count is determined by the half-height method. For detailed test information, contact Applied Biosystems Technical Support.

9.2 Protein Separation

The standard protein sample used is the Protein A/G Test Standards available from Applied Biosystems. Refer to Section 10, Accessories, Spare Parts, and Ordering Information, for more information. The separation is run using simple step elution.

To prepare the protein test standard:

1. Dissolve the test standard in 1 ml of buffer (final concentration is 5 mg/ml bovine serum albumin, 5 mg/ml human gamma globulins).

   *WARNING:* All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

2. Filter the test standard using a .22-µm filter.
3. Store unused reconstituted test mix frozen.

9.3 Conditions

| Buffer | 50 mM phosphate pH 7.0, 0.15 M NaCl |
| Eluent | 0.1% (v/v) (12 mM) HCl, 0.15 M NaCl |
| 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 |

9.4 Results

The standard chromatogram shown in Figure 1 is for a 4.6mmD/100mL POROS 20 micron A column. The retention times and bandspreading may vary with different HPLC systems, but the general profile should be similar.

![Figure 1 POROS 20 Micron A Chromatogram](image-url)
10 Accessories, Spare Parts, and Ordering Information

These accessories are available for your POROS A and G columns:

Table 8 POROS A and G Column Accessories

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A/G Test Standards</td>
<td>Package of 5 vials</td>
<td>1-9006-05</td>
</tr>
<tr>
<td>Frits, PEEK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1mmD</td>
<td>Package of 5</td>
<td>1-9124-05</td>
</tr>
<tr>
<td>4.6mmD</td>
<td>Package of 5</td>
<td>1-9125-05</td>
</tr>
<tr>
<td>10mmD</td>
<td>Package of 1</td>
<td>1-9127-01</td>
</tr>
<tr>
<td>16mmD</td>
<td>Package of 1</td>
<td>1-9128-01</td>
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<tr>
<td>EZ Grip Fittings (SS)</td>
<td>Package of 5</td>
<td>P5-1011-05</td>
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<tr>
<td>Fitting Adaptor Kit</td>
<td></td>
<td>1-9532-00</td>
</tr>
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</table>

The Fitting Adaptor Kit lets you connect POROS columns to M-6 (FPLC) and 1/4-28 low-pressure fitting systems. The kit includes two 10-32 fittings, two low-pressure ferrules, two M-6 nuts, two 1/4-28 nuts, and 1/16-inch OD PEEK tubing.

Column parts are available from Applied Biosystems on special order. Please inquire.

11 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. Please contact Applied Biosystems directly for a publication list.

For further details or for answers to questions on POROS A and G columns, Perfusion Chromatography, or other products, please 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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