POROS® XS Strong Cation Exchange Resin

POROS® XS Strong Cation Exchange Resin (POROS® XS resin) is a 50 µm, rigid, polymeric cation-exchange chromatography resin that can be used for the chromatography of biomolecules including monoclonal antibodies, recombinant proteins, and peptides. The resin backbone consists of cross-linked poly[styrene-divinylbenzene]. A polyhydroxyl surface coating provides low non-specific binding, and surface functionalization with sulphopropyl yields a strong cation exchanger ionizable over the pH range of 1 to 14.

POROS® XS resin is designed for high dynamic binding capacity over a range of pH and conductivity conditions. This allows target-molecule binding and impurity removal over a range of process conditions, thereby increasing process development flexibility and manufacturing throughput. (See “Optimize chromatography conditions” on page 3 for recommended starting conditions). In addition, the 50 µm particle size provides superior resolution for unprecedented impurity clearance independent of scale and flow rate. The combined features of high dynamic binding capacity, salt tolerance, and high resolution make POROS® XS resin a unique strong cation exchange resin.

Specifications

Table 1 POROS® XS resin product characteristics

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support matrix</td>
<td>Cross-linked poly(styrene-divinylbenzene)</td>
</tr>
<tr>
<td>Surface functionality</td>
<td>Sulphopropyl (-CH2CH2CH2SO3–)</td>
</tr>
<tr>
<td>Dynamic binding capacity</td>
<td>≥100 mg/mL</td>
</tr>
<tr>
<td></td>
<td>5% breakthrough of Polyclonal Human IgG</td>
</tr>
<tr>
<td></td>
<td>in 20 mM MES, 40 mM NaCl, pH 5.0 at</td>
</tr>
<tr>
<td></td>
<td>300 cm/hr in 0.46 cmD x 20 cmL column</td>
</tr>
<tr>
<td>Shipping solvent</td>
<td>20% ethanol</td>
</tr>
<tr>
<td>Ionic capacity</td>
<td>88 to 120 µmol/mL</td>
</tr>
<tr>
<td>Average particle size</td>
<td>50 µm</td>
</tr>
<tr>
<td>Mechanical resistance</td>
<td>100 bar (1,450 psi, 10 MPa)</td>
</tr>
</tbody>
</table>

POROS® XS resin chemical stability characteristics make it compatible with a variety of solvents and buffer salts listed in Table 2.

Table 2 POROS® XS resin chemical and thermal resistance

<table>
<thead>
<tr>
<th>Property</th>
<th>Range/Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH Range</td>
<td>1 to 14</td>
</tr>
<tr>
<td>Ionic strength range</td>
<td>0 to 5 M, all common salts</td>
</tr>
<tr>
<td>Buffer additives</td>
<td>All common agents, including 5 M sodium hydroxide,</td>
</tr>
<tr>
<td></td>
<td>8 M urea, 6 M guanidine hydrochloride, ethylene glycol,</td>
</tr>
<tr>
<td></td>
<td>and detergents.</td>
</tr>
<tr>
<td>Solvents</td>
<td>Water, 0 to 100% alcohol, acetonitrile, 2M acetic acid,</td>
</tr>
<tr>
<td></td>
<td>1M HCl, other common organic solvents</td>
</tr>
<tr>
<td>Note</td>
<td>Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>2 to 40 °C</td>
</tr>
<tr>
<td><strong>DO NOT FREEZE</strong></td>
<td></td>
</tr>
</tbody>
</table>

The pressure-flow curve of POROS® XS resin is shown in Figure 1. POROS XS resin can be operated at high linear flow rates with a pressure drop that allows for use with conventional low pressure chromatography columns and systems.

Figure 1 Pressure-flow properties of POROS® XS resin

![Pressure-flow properties of POROS® XS resin](image)

Column format: 6.2 cmD x 19.8 cmL, 12 µm frits
Packing pressure: 3 bar
Mobile phase: 0.1M sodium chloride
Pack the column

- POROS® XS resin is mechanically rigid and can be packed effectively both in low-pressure glass columns and in high-pressure stainless steel columns.
- Columns can be packed with traditional flow pack, pack-in-place, or axial compression packing methods.
- A 1.06 packing factor is recommended to account for the difference in bed volume between a gravity-settled bed and a 3 bar pressure-packed bed. You use this factor, along with the slurry ratio, to determine the volume of slurry required to yield the intended final column volume.
- 10 to 23 µm screens (frits) are recommended.
- For best results, use a column tube or column fitted with an extender large enough to contain the entire slurry so that the bed can be packed all at once.

Prepare the slurry

POROS® XS resin is supplied as a 56% slurry in 20% ethanol. A 1.8 L volume of resin slurry yields a 1 L packed-bed volume when packed at 3 bar.

Exchange-out the 20% ethanol shipping solution with 0.1 M sodium chloride for column packing.

Note: POROS® XS resin beads are rigid and incompressible and do not desiccate. Therefore, non-traditional methods can be used for exchanging the shipping solution.

Lab scale columns (≤ 100mL)
Buffer-exchange using a 0.2 µm bottle filter or sintered-glass filter:

1. Transfer the required volume of resin slurry to the top of a bottle-top filter.
2. Apply vacuum to remove the shipping solution.
3. Resuspend the dry resin bed to the starting resin slurry volume with the desired packing solution.
4. Resuspend the resin with a plastic or rubber spatula. Do not grind the resin bed or tear the filter membrane.
5. Repeat step 2 through step 4 for a total of three exchanges.
6. Resuspend the exchanged resin to the original slurry concentration and proceed with column packing.
7. Check the slurry concentration by sampling 10 to 100 mL of slurry in a 10 to 100 mL graduated cylinder and gravity settling for >4 hours.
8. If needed, adjust the slurry concentration to 50 to 70%.

Lab scale and larger scale columns (>100mL)
Buffer-exchange using repeated gravity settling:
1. Allow the resin to settle in a container [will require >4 hours because the density of the resin is about that of water].
2. Carefully decant the clear supernatant. Do not disturb the bed.
3. Replace the supernatant with the same volume of the desired packing solution.
4. Resuspend the resin by gentle agitation by hand, with a flat bed shaker, or on a rotary mixer, then allow the resin to settle by gravity.
   Note: Do not use a magnetic stirrer. It may abrade the particles and cause fines to form.
5. Repeat step 1 through step 4 two to three times to thoroughly exchange the packing solution.
6. Check the slurry concentration by sampling 10 to 100 mL of slurry in a 10 to 100 mL graduated cylinder and gravity settling for >4 hours.
7. If needed, adjust the slurry concentration to 50 to 70%.

Pack the column

1. Determine the required slurry volume:
   Required slurry volume = target CV / slurry ratio × 1.06
   Example for 40cmD x 20cmL:
   25L / 0.56 x 1.06 = 47.3 L slurry required
   The 1.06 packing factor accounts for the difference in bed volume between a gravity-settled bed and a 3 bar pressure-packed bed.
2. Deliver the required slurry volume to the column by hand or with a diaphragm pump, as dictated by your equipment and the intended packing procedure.
   Note: POROS® XS resin beads have a skeletal density similar to the density of water and do not settle rapidly. Do not allow the resin to gravity-settle in the column for more than 1 hour before packing.
3. Bring the primed top flow adapter as close to the resin slurry as possible. Do not push the resin up and over the o-ring, and do not allow large air bubbles between the top adaptor and the top of the resin slurry.
4. Ensure that the column outlet is open and plumbed directly to waste. Do not connect to the chromatography system.
5. Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used:
   - Flow packing – Pack at flow rates approximately 50% greater than the maximum operating flow rate for your chromatography operation with an approximate final packing pressure of 2.5 to
3 bar. This flow should yield a pressure higher than the desired operating pressure for all column steps.

- **Pack-in-place** – Pack at flow rates/pressures up to the limits of the column.
- **Axial compression** – Pack at flow rates/pressures up to the limits of the column. The top flow adaptor will stop when the POROS XS resin bed is fully packed.

**Note:** If the column is not packed at a high enough flow/pressure, then flowing a more viscous solution (like a cleaning solution) at the same flow rate will result in further bed compaction.

6. Continue flow until a clear space forms between the column top adjuster and the slurry.

**IMPORTANT!** 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.

7. After the bed is formed, bring the adapter into contact with the top of the bed without pushing the resin over the o-ring. POROS® XS resin does not shrink or swell, so an open head space is not recommended.

8. Flow at the packing flow rate again for 1 to 2 column volumes (CVs), then adjust the adapter if necessary as described in the previous bullet.

9. After the column is packed, flow 3 to 5 CVs of packing solution through the packed bed at the packing flow rate to stabilize the bed.

**Note:** The flow rate used should generate no more than 80% of the final packing pressure.

10. If you will reverse the flow of the column during operation, condition the column in upflow:
- Flow 2 to 3 CVs in upflow at the operating flow rate.
- Flow 2 to 3 CVs in downflow at the operating flow rate, then adjust the adapter if needed.
- Flow for another 2 CVs after you adjust the adapter.

**Qualify the column**

To qualify the integrity of a packed column, determine HETP (height equivalent to a theoretical plate) and asymmetry using a non-binding analyte (a “plug”) such as sodium chloride (0.5 to 1.0 M), sodium hydroxide (0.5 to 1.0 M), or sodium nitrate (50 to 200 mg/mL). Do not use acetone.

**Guidelines**

- Ensure uniform column plumbing:
  - Avoid using reducers to connect different tubing sizes.
  - Minimize and keep consistent the column tubing lengths between the plug solution and the column inlet and the column outlet to the detector(s).
- Use:
  - Plug volume: 1 to 3% of the total column volume.
  - Plug concentration: 5 to 10 times the mobile phase concentration [for instance 0.1 M sodium chloride mobile phase with a 1 M sodium chloride plug].
  - Use process equilibration buffer or 0.1 M sodium chloride as the mobile phase.
- Execute at the flow rate defined for the intended unit operation, typically 100 to 300 cm/hr.
- Monitor:
  - Conductivity for sodium chloride and sodium hydroxide.
  - Absorbance monitored for sodium nitrate.

After you define a column qualification procedure for a given system (column plus chromatography system), base the qualification acceptance criteria for reproducibility and consistency on historical values and ranges rather than on theoretical qualification results.

**Recommended column qualification conditions**

- Flow rate: operating flow rate (cm/hr)
- Equilibration buffer: 0.1 M sodium chloride
- Plug solution: 1 M sodium chloride
- Plug volume: 2% of column volume

**Optimize chromatography conditions**

**Background**

Although similar sulphopropyl functional groups are used on most strong cation exchange (CEX) resins, the optimal binding and elution conditions can vary significantly due to a number of resin characteristics. Different CEX resins operated with the same process conditions will yield variable results; therefore, standardized conditions or platform-type evaluations are not recommended. For this reason, it is important to test different loading and elution conditions to optimize capacity, separation and yield based on the target molecule characteristics and process challenges.

Always filter the load through a 0.22 or 0.45 µm filter before loading to reduce fouling of the column screens.

**Optimize binding conditions**

- **pH:** Use a binding buffer pH 1 to 3 units below the isoelectric point (pl) of the target molecule, that is, pH 4.5 to 6.5 for most monoclonal antibodies. Dynamic binding capacity (DBC) typically increases as the loading pH decreases.
• **Buffer system**: MES, acetate, phosphate, citrate, and citrate-acetate are often used. When choosing buffer systems, consider molecule stability, binding optimization, and the ability of the buffer to control pH in the desired operating range.

• **Conductivity**: Although DBC typically decreases as load conductivity increases, POROS® XS resin is designed to have increased salt tolerance, so that high DBC can be obtained under higher conductivity conditions. For example, high IgG capacity has been obtained with up to 150 mM sodium chloride (15 mS/cm), reducing the need to dilute or buffer exchange column loads. The load conductivity should be between 2 and 15 mS/cm; however, the optimum buffer condition depends on the target molecule and buffer pH.

• **Flow rate**: The target operating flow rate is flexible, but optimal binding should be obtained with a residence time of ≥3 minutes (that is, ≤ 400 cm/h in a 20 cm length column).

### Optimize elution conditions

Elution optimization should begin with a gradient elution. Most often, once elution performance is understood, a step elution can be implemented. Due to the increased salt tolerance of POROS® XS resin, a slight change in salt or pH may be needed to elute the column and maintain the same elution pool volume and retention time compared to other resins.

• **Salt gradient**: To determine where the target molecule and contaminants/impurities are eluting, start with a 20 column volume gradient from low salt, typically matched to the wash buffer, to approximately 500 mM to 1 M sodium chloride.

• **pH**: Initially, the elution pH should be matched to the binding pH. However, the pH of the elution buffer should be optimized as often the optimum binding and eluting pH can be different.

• **DBC**: Separation as a function of DBC should be assessed. The maximum DBC at which a given separation can be obtained depends on a number of factors, including sample solubility, column selectivity, buffer pH, and buffer conductivity.

• **Bed height**: Initial screening can be run with shorter bed heights, but development should be conducted at the final desired bed height, typically 15-25 cm.

### Clean the column

POROS® XS resin can typically be cleaned with 3 to 5 column volumes of 1 to 2 M sodium chloride followed by 3 to 5 column volumes of 0.5 to 1 N sodium hydroxide. Different solutions may be required for column cleaning if POROS® XS resin is used for capture chromatography.

### Store the resin

Store the resin in 20% ethanol or 0.1 M sodium hydroxide at 2 to 30 °C.

### Technical support

Applied Biosystems is dedicated to supporting your use of POROS® XS resin. Our Application Scientists are available for support ranging from email and telephone consultation, for process development and resin cleaning optimization, to on site support for column packing. We also offer a full line of 50 µm bulk POROS® resins for reversed-phase, ion-exchange, and affinity chromatography. Please contact your Applied Biosystems account representative for technical and ordering information.

### Safety information

#### Obtaining SDSs

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

1. Go to [www.appliedbiosystems.com](http://www.appliedbiosystems.com), click Support, then select SDS.

2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click Search.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

**Note**: For the SDSs of chemicals not distributed by Applied Biosystems contact the chemical manufacturer.