

POROS® Strong Cation Exchange Resins: XS and HS 50

Pub. No. 100031321 Rev. A

Product description

POROS® Strong Cation Exchange Resins (POROS® CEX resin) are 50-µm, rigid, polymeric, ion-exchange chromatography resins that can be used for the chromatography of biomolecules including recombinant proteins, monoclonal antibodies, DNA, viruses, and peptides. The resin backbone consists of crosslinked poly[styrene divinylbenzene].

A polyhydroxyl surface coating provides low non-specific binding and surface functionalization with sulphopropyl yields a strong cation-exchanger ionizable with pH 1–14.

POROS® CEX resins are 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. In addition, the 50-µm particle size provides superior resolution for unprecedented impurity clearance independent of scale and flow rate. Each of the resins offers unique selectivity that can impact both capacity and separation in a process.

Specifications

Table 1 POROS® CEX resin product characteristics

Characteristic	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Surface functionality	Sulfopropyl (-CH ₂ CH ₂ CH ₂ SO ₃ -)
Dynamic binding capacity	<ul style="list-style-type: none"> XS^[1]: ≥100 mg/mL HS 50^[2]: 58–80 mg/mL
Shipping solvent	18% ethanol
Average particle size	50 µm
Shrinkage/swelling	<1% from 1–100% solvent
Mechanical resistance	100 bar (1450 psi, 10 MPa)

^[1] 5% breakthrough of Polyclonal Human IgG in 20 mM Tris, 40 mM NaCl, pH 5.0 at 300 cm/hour in 0.46 cmD × 20 cmL column

^[2] 50% breakthrough of Lysozyme in 20 mM MES, pH 6.2 at 100 cm/hour in 0.46 cmD × 5 cmL column

Table 2 POROS® CEX resin chemical and thermal resistance

Characteristic	Description
pH Range	1–14
Ionic strength range	0 to 5 M, all common salts
Buffer additives	All common agents, including 1 M sodium hydroxide, 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents
Solvents	Water, 0 to 100% alcohol, acetonitrile, 2 M acetic acid, 1 M HCl, other common organic solvents Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.
Operating temperature	2 to 30°C Do not freeze

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

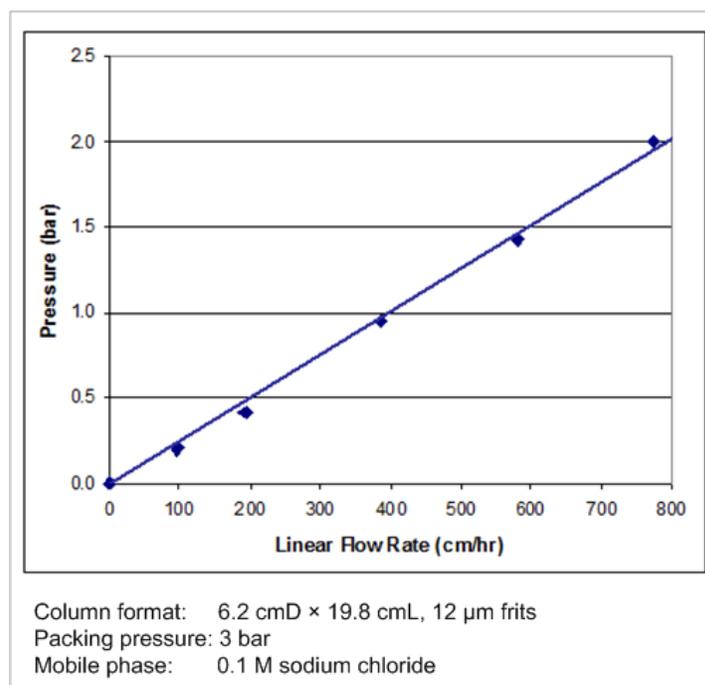


Figure 1 Pressure-flow properties of POROS® XS resin

Packing considerations

- POROS® resins are mechanically rigid and incompressible and can be packed effectively in low-pressure glass columns and in high-pressure stainless steel columns. The lack of wall support with increasing column diameter has minimal impact on chromatography performance because the beads support themselves, allowing for flexible column packing approaches and consistent and robust results. Columns can be packed with traditional flow pack, axial compression, or pack-in-place/stall pack packing methods.
- The 1.06 packing factor is recommended to account for the difference in bed volume between a gravity-settled bed in 0.1 M sodium chloride and a 1- to 3-bar pressure-packed bed. This factor, along with the slurry ratio, is used to determine the volume of slurry required to yield the intended final column volume (CV).
- Standard 10–23 µm screens (frits) can be used.
- 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. Funnel-like column packing devices do not work well for packing POROS® resins.

Prepare slurry: lab-scale columns (≤ 100 mL)

Resins are supplied as approximately 56% slurry in 18% ethanol. For column packing, exchange the shipping solution with 0.1 M sodium chloride.

Buffer-exchange using a 0.2–0.45 µm bottle-top 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 resin cake to the starting resin slurry volume with the desired packing solution. Mix with a plastic or rubber spatula. Do not grind the resin bed or tear the filter membrane.
4. Repeat step step 2 and step step 3 for a total of three exchanges.
5. Resuspend the exchanged resin to the original slurry concentration and proceed with column packing.
6. Verify that the slurry concentration is 50–70% by sampling 10–100 mL of slurry in a 10–100 mL graduated cylinder (respectively) and gravity settling for > 4 hours.
7. If needed, adjust the slurry concentration to 50–70%.

Prepare slurry: lab scale and larger scale columns (> 100 mL)

Resins are supplied as approximately 56% slurry in 18% ethanol. For column packing, exchange the shipping solution with 0.1 M sodium chloride.

Buffer-exchange using repeated gravity settling:

1. Allow the resin to settle in the shipping container. Settling requires > 4 hours because the density of the resin is approximately that of water.
As vessel diameter and depth increases, settling can require more time. Large vessels may need to settle overnight to ensure good separation. As vessel size increases, the supernatant can be pumped off.
2. Carefully decant the supernatant. Do not disturb the bed.
Some particles/turbidity may be present in the decant as beads slough off the settled bed or come loose from the carboy side walls. This is not problematic.
3. Replace the supernatant with the same volume of the desired packing solution.
4. Resuspend the resin by gentle agitation by hand, resin wand, air sparging, paddle, flat bed shaker, top-mounted impellor mixer, or rotary mixer, then allow the resin to settle by gravity.
As with any resin, do not use a magnetic stirrer. It may abrade the particles and cause fines to form.
5. Repeat step step 1 through step step 4 two to three times to thoroughly exchange into the 0.1 M sodium chloride packing solution.
6. Verify that the slurry concentration is 50–70% by sampling 10–100 mL of slurry in a 10–100 mL graduated cylinder (respectively) and gravity settling for > 4 hours.
7. If needed, adjust the slurry concentration to 50–70%.

Pack the column

Use a 3- or 4-way valve on the top and bottom of the column (if possible) to allow bypass of the column and avoid introducing air during packing and column use. Place a calibrated pressure gauge at the inlet of the column.

While adjusting the flow rate and forming the bed, you may observe some turbidity in the eluent as packing begins. Turbidity will clear as packing proceeds and 1–2 bed volumes of packing buffer pass through the column.

1. Determine the required slurry volume:
Required slurry volume = target CV / slurry ratio x packing factor
Example for a 40 cmD × 20 cmL 25 L column using slurry with a 56% slurry ratio:
 $25\text{L} / 0.56 \times 1.06 = 47.3 \text{ L slurry required}$
2. Ensure that the column outlet is closed and plumbed directly to waste. Do not connect the column outlet to the chromatography system. Plumbing into the system creates backpressure that fights against the inlet pressure trying to settle the bed and pack the column.
3. Ensure that the column is level and locked in place before beginning the pack.

- 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. Use a squirt bottle containing packing solution to remove any residual resin from the column wall.

POROS® 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 before packing.

- With the column inlet line connected to the system and the bottom outlet closed, bring the primed top flow adapter to 1–2 cm from the slurry level and tighten the O-ring. Do not push the resin up and over the o-ring. Change the top valve to force the air and liquid out the top of the adapter and to waste using the bypass line. Continue to lower the adapter slowly to remove the bubbles from the top of the column. Do not allow large air bubbles between the top adaptor and the top of the resin slurry.
- Change the valve back to flow through the system on the top and open the column bottom.
- Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used:

- Flow packing** – Pack at a flow rate at least 50% greater than the maximum operating flow rate for your chromatography operation, with an approximate final packing pressure of 3 bar at the inlet of the column (not the inlet of the system). This flow should yield a pressure higher than the desired operating pressure for all column steps. For smaller diameter columns (≤ 1 cm), we recommend higher packing flow rates of 1000–2000 cm/hour.
- Flow packing with axial compression** – Place the top flow adaptor at a height that will accommodate all of the slurry. Pump the slurry into the column using the slurry nozzle and follow with 0.1 M sodium chloride to chase the remaining resin or use extra slurry to avoid introducing air into the line.

Pack at flow rates/pressures up to the limits of the column. Pack at a flow rate at least 50% greater than the maximum operating flow rate for your chromatography operation. This flow should yield a pressure higher than the desired operating pressure for all column steps.

After about 2 CVs, lower the top adapter until the pressure limit of the hydraulics. Pack the column to at least 2.5 bar. The top flow adaptor will stop when the resin bed is fully packed. The column inlet pressure drops to zero when the pack is complete.

- Axial compression** – Pack at flow rates/pressures up to the limits of the hydraulics of the column (at least 2.5 bar). Add the slurry to the column as you would for flow packing, but proceed directly with axial compression by lowering the adapter using the hydraulics at the flow/pressure limit of the column. The top flow adaptor will stop when the resin bed is fully packed. The column inlet pressure drops to zero when the pack is complete.
- Pack-in-place/Stall pack** – Pack at flow rates/pressures up to the limits of the column. Lock the top adapter into place at the desired bed height and pump resin into the column until the column is full or the pump stalls.

Characterize the flow versus pressure output for the slurry transfer skid. A final packing pressure of at least 2.5 bar should be attained.

If a pressurizable slurry tank is available, pressurize to 3 bar and execute a constant pressure pack.



CAUTION! If the column is not packed at a high enough flow/ pressure, flowing a more viscous solution (like a cleaning solution) over the column at the same flow rate will further compact the bed and create a head space.

- Continue flow until a clear space forms between the column top adjuster and the slurry (~2 CVs). Monitor the pressure; it will gradually rise as the column packs.
- After the bed is formed, bring the adapter into contact with the top of the bed without pushing the resin over the oring. This is most easily accomplished by closing the column outlet and displacing liquid through the top of the adapter to waste using the bypass line. POROS® resin does not shrink or swell, so an open headspace is not recommended.
- Flow at the packing flow rate again for 1–2 CVs, taking note of the bed height at the desired pressure. Adjust the adapter as described in step 9 to the noted bed height by displacing the liquid through the top of the adapter and to waste.
- After the column is packed, flow 2–3 CVs of packing solution through the packed bed at the operating flow rate to stabilize the bed.
The flow rate used should generate no more than 80% of the final packing pressure.
- If you will reverse the flow of the column during operation, condition the column in upflow:
 - Flow 2–3 CVs in upflow at the operating flow rate.
 - Flow 2–3 CVs in downflow at the operating flow rate, then adjust the adapter if needed.
 - Flow 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”).

Recommended column qualification conditions

Condition	Recommendation
Flow rate	Target operating flow rate (cm/hour)
Equilibration buffer	0.1 M sodium chloride
Plug solution	1 M sodium chloride
Plug volume	2% of column volume

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 to the column inlet and the column outlet to the detector(s).
- Execute at the flow rate defined for the intended unit operation, typically 100–300 cm/hour.
- Equilibrate with at least 4 CVs of equilibration buffer before injection.

Setting specifications

Qualification results depend on a number of factors, including the:

- Solutions and method used
- Scale
- Column hardware
- Chromatography system

After you define a column qualification procedure for a specific system (column plus chromatography system), base the qualification acceptance criteria on historical values and ranges instead of theoretical qualification results. Performing the column qualification method consistently and reproducibly is critical to obtaining meaningful results.

Qualification example

Figure 2 shows a typical column qualification peak. The peak void volume of a POROS® column is typically 0.7–0.8 CV.

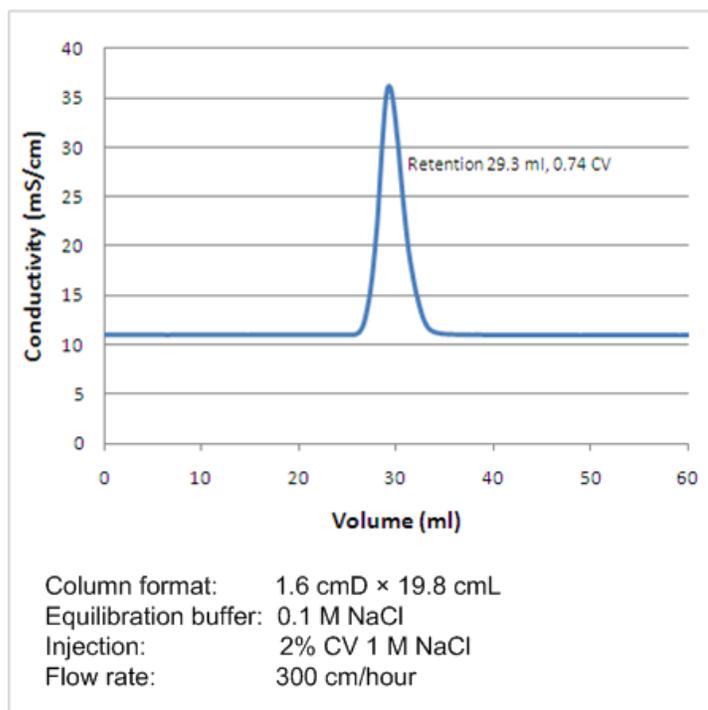


Figure 2 POROS® column qualification

Optimize chromatography conditions

- Use buffers of the highest purity practical.
- Filter (0.22 or 0.45 µm) all buffers and load materials before use.

General considerations

Although similar sulphopropyl functional groups are used on most strong 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. 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.

Binding conditions considerations

- **pH** – Use the same pH for load solution and equilibration buffer. Use a binding pH 1 to 3 units below the isoelectric point (pI) of the target molecule – pH 4.5 to 6.5 for most monoclonal antibodies. The dynamic binding capacity (DBC) decreases as pH increases (away from the pI).
- **Buffer system** – Acetate, citrate, citrate-acetate, MES, and phosphate are commonly 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® resin is salt-tolerant, so that high DBC can be obtained under higher conductivity conditions. High protein 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. Most proteins require some salt for stability. These conditions are determined by the physical characteristics and stability of the protein.
- **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).

Elution conditions considerations

Begin elution optimization with a gradient elution. Most often, after elution performance is determined, you can implement a step elution. Because of the increased salt tolerance of the 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 elute, start with a 20 CV gradient from low salt, typically matched to the wash buffer, to approximately 500 mM–1 M NaCl. This can be accomplished by assaying fractions across the peaks (~1/10 CV). Based on this information, the process can be further optimized.
- **pH** – Use the same starting pH for load solution and equilibration buffer, then optimize the pH of the elution buffer. Optimum binding and elution pH can differ.

- **DBC** – Assess separation as a function of DBC. The maximum DBC 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 final development should be done with the final desired bed height, typically 15 to 25 cm.

Clean the column

Clean the resin with 3 to 5 CVs of 1–2 M NaCl followed by 3 to 5 CVs of 0.5–1 M NaOH.

Different solutions may be required for column cleaning if the resin is used for capture chromatography.

Degas more viscous solutions such as 1 M acetic acid or 20% ethanol before use on the column to avoid gassing out during operation.

Store the resin

Store the resin in 20% ethanol or 0.1 M NaOH at 2–30°C.

Troubleshooting

Observation	Possible cause	Recommended action
High backpressure	Presence of any amount of ethanol (shipping/storage solution) in the slurry or in the column	Fully exchange the ethanol before packing. Typically, this requires three exchanges.
	Compromised flow path: <ul style="list-style-type: none"> Compressed sanitary gaskets Closed, partially closed, or blocked inlet and outlet valves on the column Improperly functioning valves on the chromatography system Blocked inline filters 	<ul style="list-style-type: none"> Use narrow-bore sanitary gaskets. Characterize the pressure of the entire chromatography system with no column in place, the system and empty column with the column outlet plumbed directly to waste, and the system and empty column with the column outlet plumbed back into the skid. Ensure that the entire flow path is clear. Change the inline filters.
	Clogged or very tiny frits (< 3 µm)	<ul style="list-style-type: none"> Change or clean the frits (screens). Run the column in upflow for 3 CVs, then downflow again. Observe if there is a change in pressure.
	Improperly scaled chromatography systems, including small-diameter tubing anywhere in the system and operating at the high end of the system range	<ul style="list-style-type: none"> Verify that the skid pump and tubing diameters are scaled appropriately for the column operation and replace as needed. Do not operate pumps at over ~70% of their capacity.
	Particle size gradient in the column caused by gravity settling the resin	Do not gravity-settle POROS® resin in the column before packing.
	Resin allowed to freeze	Store and operate the column at 2–30°C. Do not freeze.
Turbid column effluent after >3 CVs during packing	Column frits (screens) are too large for the resin (> 23 µm frit)	Use standard 10–23 µm screens (frits).
	Compromised flow adaptor o-ring, improperly assembled flow adaptor, or defective flow adaptor	Take the adapter apart, inspect all parts, and replace as needed.

Observation	Possible cause	Recommended action
Column qualification — high asymmetry	Column is underpacked; that is, the column is not packed at a high enough flow rate/ pressure	<ul style="list-style-type: none"> Pack at a higher flow rate/pressure. The top adapter position may need to be better seated in the packed resin bed to ensure that a headspace does not form.
	The system and plumbing allow for dilution of the salt plug	<ul style="list-style-type: none"> Characterize a salt plug through the chromatography system at the qualification flow rate to understand how the plug moves through the system with no packed column in line. Verify that the plumbing throughout the system (pre- and post-column) is consistent and that areas for dilution are minimized. Verify that there is no air under the distributor.
	Salt injection method is not optimized	Verify that the desired amount of salt is loaded by checking the peak height and width. Ensure that the injection is consistent and applied as close to the column inlet as possible to minimize dilution from the system. The injection method should be well-described in your operating procedures to maintain reproducibility.
	The column needs more post-pack conditioning to stabilize the packed bed	Equilibrate the column with 2–3 CV of packing solution in downflow at the operating flow rate, 2–3 CV in upflow, and 2–3 CV in downflow again.
	≥ 2 M NaCl salt is used for the salt plug or an analyte interacts with the resin	Use recommended column qualification conditions.
Column qualification – low asymmetry	Water is used as the mobile phase	Add some salt to the mobile phase to reduce the charge interaction between the salt and the bead.
	Column is overpacked or packed inconsistently	Repack the column following the recommended procedure.
	Column not equilibrated long enough with sodium chloride before salt injection	Equilibrate ≥ 4 CVs if the packing solution is different from the qualification mobile phase.
Decreased performance: <ul style="list-style-type: none"> Increased bandspreading Decreased binding capacity Decreased recovery Increased pressure drop Trace or “ghost” peaks during blank runs 	Column fouling can occur due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities	Clean the column (see “Clean the column” on page 5).

Ordering information

POROS® resin	Volume/column size	Part number	POROS® resin	Volume/column size	Part number
XS bulk	10,000 mL	4404334	HS 50 bulk	10,000 mL	1-3359-08
	5000 mL	4404335		5000 mL	1-3359-09
	1000 mL	4404336		1000 mL	1-3359-07
	250 mL	4404337		250 mL	1-3359-11
	50 mL	4404338		50 mL	1-3359-06
	25 mL	4404339		25 mL	1-3359-03

POROS® resin	Volume/column size	Part number	POROS® resin	Volume/column size	Part number
GoPure™ XS	1.2 cmD × 5 cmL	4448884	GoPure™ HS 50	1.2 cmD × 5 cmL	4448875
	1.2 cmD × 10 cmL	4448885		1.2 cmD × 10 cmL	4448876
	1.2 cmD × 15 cmL	4448886		1.2 cmD × 15 cmL	4448877
	1.2 cmD × 20 cmL	4461756	HS 50 pre-packed columns	10 mmD × 50 mmL	1-3352-44
—	—	10 mmD × 100 mmL		1-3352-46	

Support

For service and technical support, go to lifetechnologies.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 www.lifetechnologies.com/support, or contact your local Thermo Fisher Scientific representative.

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