INSTRUCTIONS

POROS XS

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82071 82072

Number	Description
82071	**POROS XS,** 10mL settled resin
82072	**POROS XS,** 50mL settled resin

Resin: Crosslinked poly(styrene-divinylbenzene)

Supplied: 50% slurry in 20% ethanol

Storage: Upon receipt store at 2-30°C. DO NOT FREEZE. Product shipped on wet ice.

Table of Contents

Introduction

Thermo Scientific™ POROS™ XS Strong Cation Exchange Resin is designed for charge-based chromatographic separation of biomolecules including recombinant proteins, monoclonal antibodies, DNA, viruses, and peptides. The POROS XS resin is functionalized with a high density of sulphopropyl groups that ionize over a broad range of pH from 1–14. This produces a resin with high binding capacities over a range of pH and conductivity conditions, increasing flexibility in method development.

The resin backbone of POROS XS consists of a rigid, highly porous, crosslinked poly[styrene divinylbenzene] with an additional polyhydroxyl surface coating to ensure low nonspecific binding. The intrinsic stability and open pore structure of POROS beads allow rapid binding of large molecules, consistently producing high dynamic binding capacities over a wide range of flow conditions. POROS resins improve the speed, flexibility, and optimization of purifications when compared to soft supports (e.g., agarose), thus increasing performance of purifications over a wide range laboratory and process-scale applications.

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Table 1. Properties of Thermo Scientific POROS XS resin.

Support matrix	Crosslinked poly(styrene-divinylbenzene)
Surface functionality	Sulfopropyl
Dynamic binding capacity	≥100mg/mL [1]
Shipping solvent	20% ethanol
Average particle size	50µm
Shrinking/swelling	<1% from 1-100% solvent
Mechanical resistance	100bar/1450psi/10MPa
Compatibility	
pH range	1-14
Ionic strength range	0-5M, all common sales
Buffer additives	All common reagents including 1M sodium hydroxide, 8M urea, 6M guanidine hydrochloride, ethylene glycol, and detergents
Solvents	Water, 0-100% alcohol, acetonitrile, 1-2M acids (e.g., acetic, hydrochloric, phosphoric), and other common organic solvents Do not expose to strong oxidizers (e.g., hypochlorite), oxidizing acids (e.g., nitric), strong reducing agents (such as sulfite), acetone, or benzyl alcohol
Operating temperature	2-30° C Do not freeze

[1] 5% breakthrough of polyclonal human IgG in 20mM MES, 40mM NaCl, pH 5.0 at 300 cm/hr in 0.46cm D x 20cm length column

Important product information

- To aid in resin handling, resin should be adjusted into a slurry containing ≥0.1M NaCl.
- For all column chromatography applications, we recommend that highly pure buffer components be used and that all buffers and samples are degassed and filtered through a 0.45µm filter before use.
- Final protein yield and purity during a chromatographic separation are dependent upon the sample complexity and concentration in load sample, as well as the buffer conditions and flow rates used. Therefore, it is important to optimize these parameters before attempting a large-scale purification.
- If required to stir the resin slurry over an extended period of time, use a free-standing paddle stirrer. Do not use magnetic stir bars or paddles that mate with the bottom of the container.

Resin handling (<100mL)

Below are general recommendations for handling resin, adjusting slurry percentage, and exchanging resin into new buffers.

1. Transfer the required volume of resin slurry to a bottle-top filter or sintered-glass frit.
2. Apply vacuum to remove the liquid from the top of the resin bed.
3. Resuspend the resin cake to the desired final resin slurry volume with 0.1M NaCl. Mix with a plastic or rubber spatula. Do not grind or press resin against the sintered glass material. If using a paper filter membrane, take care to not damage or rip the filter material.
4. Repeat steps 2 and 3 for a total of three exchanges. After the third exchange, recover the resin slurry into an appropriate container and store appropriately.
5. Verify the slurry concentration by transferring 10-100mL of slurry into a graduated cylinder and allow the resin to settle for ≥4 hours. If needed, adjust the slurry concentration to 50–70%.
6. Proceed with column packing (below).

Column packing for lab-scale columns (<100mL)

Note: POROS resins are mechanically rigid and incompressible and can be packed effectively in low-pressure glass columns and high-pressure stainless steel columns. We do not recommend packing in pure water, ethanol, or low-conductivity solutions because of the high ionic surface charge of POROS anion exchange resin. For volumes greater than 100mL, please refer to the POROS column packing instructions at https://tools.thermofisher.com/content/sfs/manuals/4468731.pdf.

There is a 1.06 packing factor for POROS XS resin to account for the difference in bed volume between a gravity-settled bed in 0.1M 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).

1. Determine the required slurry volume:

$$Required\ slurry\ volume = \frac{Target\ Column\ Volume}{Slurry\ ratio} \times packing\ factor$$

Example: A column of dimensions 2.5cm ID×10.2cm length would have a packed bed of 50mL ($Volume = \pi r^2 H$).

To properly pack this volume using a 50% resin slurry of POROS HQ, 106mL is needed:

$$\frac{50mL}{0.50} \times 1.06 = 106mL\ of\ slurry\ required$$

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. If needed, attach appropriate column packing extender to allow full resin slurry volume to be added.
4. Add 0.1M NaCl to the empty column until the solution is 5cm above the bottom frit.
5. Wet bottom frit by opening the column outlet and allow liquid to enter frit and begin to flow. This will ensure the air trapped in the frit will not enter the resin bed. Close outlet when the solution reaches 3-5mM above the bottom frit.
6. Slowly deliver the required slurry volume to the column and immediately move on to the next steps. Do not allow the resin to fully gravity-settle in the column before packing.

   Note: POROS resin beads typically have a density similar to water and do not settle rapidly.
7. Bring the top flow adaptor to the top of the slurry level and tighten the O-ring until it begins to form a seal with the column. Do not push the resin up and over the O-ring.
8. Continue to lower the adaptor slowly to remove the bubbles from the top of the slurry liquid. Do not allow large air bubbles between the top adaptor and the top of the resin slurry.
9. Tighten the o-ring completely and connect the column to the system.
10. Open the column outlet.
11. Start flow and increase the flow rate to at least 50% above the maximum flow rate used during your planned purifications. For columns with a diameter of <1.0cm, it is recommended that you pass a flow rate of 1000cm/hr.
12. Continue flow until a stable resin bed forms. Monitor the pressure; it will gradually rise as the column packs. Do not exceed maximum pressure recommended for column or instrument hardware.

    Note: While packing the column, 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.
13. After the bed is formed, remove the top adaptor and remove excess solution with a pipette. Leave a few cm of liquid above the resin bed.
14. Re-insert top adaptor and bring the top flow adaptor to 1cm from the resin bed and tighten the O-ring until it begins to make a seal. Lower the adaptor slowly to remove the bubbles from the top of the column.

15. Bring the adaptor into contact with the top of the bed and seal O-ring. POROS resin does not shrink or swell, so an open headspace is not recommended. Do not push the resin over the O-ring or use pressure to compress the resin bed.
16. Flow at the packing flow rate again for 2-5 column volumes, taking note of the bed height and pressure at the desired flow rate.
17. If running the column in a unidirectional flow, then packing is completed. If running the column in both directions, continue on to the next step to further condition the column.
18. If reversing the flow of the column during operation, condition the column in upflow. Switch column inlet and outlet position related to the pump flow for upflow and downflow. Do not invert the column.
    a. Flow 2–3 column volumes in upflow at the operating flow rate.
    b. Flow 2–3 column volumes in downflow at the operating flow rate, then adjust the adaptor if needed.
    c. Flow 2 column volumes after you adjust the adaptor.

Qualify the column

To qualify the integrity of a packed column, determine the HETP (height equivalent to a theoretical plate) and asymmetry using a non-binding analyte (a "plug"). Equation calculators are available in the Thermo Scientific™ ChromaPro™ mobile application available online. Alternatively, use the equations following the protocol.

Setting specifications

Qualification results depend on a number of factors including:

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

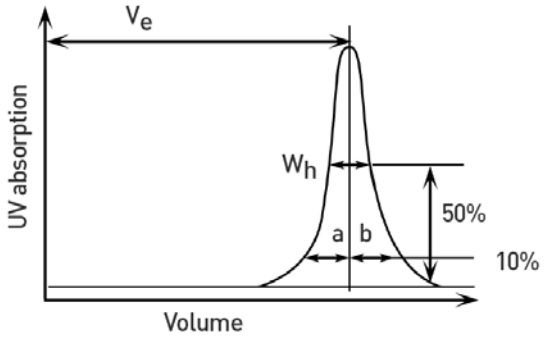
After defining a column qualification procedure for a specific system (column plus chromatography system), base the qualification acceptance criteria on historical values and ranges instead of theoretical qualification results. Performing the column qualification method consistently and reproducibly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

Table 2. Recommended column qualification conditions.

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

A. Qualification protocol

1. Equilibrate the column and all buffers to working temperature. Ensure that all solutions are degassed.
2. Equilibrate the system.
3. Equilibrate the column with 5-15 column volumes of the equilibration buffer.
4. Inject your plug and continue running the system for 1.5 column volumes.
5. Record the elution volume peak shape of the plug after it passes through the column.
6. Determine HETP and Asymmetry factor.



$N = 5.54\left(\frac{V_e}{W_h}\right)^2$	$HETP = \frac{L}{N}$	$A_s = \frac{b}{a}$
N = Number of theoretical plates	HETP = Height equivalent to a theoretical plate	As = Asymmetry factor
V_e = Elution volume	L = Bed height (cm)	a = Width at 10% height, leading side
W_h = Width at peak's half height (in volume)	N = Number of theoretical plates	b = Width at 10% height, tailing side
		As > 1 = Tailing
		As < 1 = Leading

Purification: Bind and elute

In a bind and elute method, sample components are bound to the resin and then eluted with increasing salt concentration or modification of pH conditions to sequentially elute sample components at different conditions. When performing IEX chromatography, some optimization is always required to get optimal separation. The most common areas to optimize include the buffer conditions used for binding and elution, as well as the number of column volumes over which an elution gradient is applied. The following notes and protocol are provided as a starting point. Adjust as appropriate for your sample and the desired level of separation required. See Troubleshooting section for more tips on optimizing protocols.

Buffer composition

- To ensure resin binding, the binding buffer/sample buffer pH should be 1 to 3 units below the pI of the target molecule. There is a direct correlation between dynamic binding capacity and the difference between the pI and pH of your buffer. Dynamic binding capacity will increase as the difference between the pI and your buffer pH increases.
- In addition to pH, binding is also directly influenced by ionic strength (salt concentration). To ensure proper binding to resin, the starting sample salt concentration should be between 0-150mM for POROS XS.

Flow rate

- We recommend starting with a flow rate that allows for a 1-6 minute resident time (contact time) of a sample across the column. Flow rates can be increased or decreased significantly depending on sample concentration and complexity.

Elution Gradients

- Running a salt gradient starting with your load salt concentration to 1M over 20 column volumes is recommended as a starting point. However, for some purifications >1M salt may be required to remove all bound components. Changes in pH can also be used. As an alternative to a salt gradient and pH gradient, a gradient combining shifts in pH and salt concentration could also be applied.
- The slope at which a salt gradient is applied can have a significant impact on resolution. To optimize a purification protocol, use different gradient slopes throughout the protocol accordingly. Decrease the slope during points of interest to increase the resolution peaks and increase slopes at other times to minimize total purification time.

Fraction collection

- The size of fractions to collect will be dependent on the complexity of your sample and the separation that can be achieved between sample components. This should be optimized for each purification; however, as a starting point, a fraction size of ~1/4–1/10 of the column volume can be used.

Standard purification protocol

1. Equilibrate the column and all buffers to working temperature. Purifications can be performed at 2-30°C. Ensure that all solutions are degassed.
2. Prepare the purification system by washing pumps and filling tubing with buffer.
3. Equilibrate the column with 5-10 column volumes of the equilibration buffer.
4. Apply sample to the column. Under normal bind and elute purifications, total protein load should not exceed 80% of the dynamic binding capacity of the column.
5. Wash the resin with >3 column volumes of wash buffer until the absorbance approaches baseline.
6. Elute bound molecules by applying a gradient from 0-100% elution buffer.
7. Maintain flow at 1M salt for 2-5 column volumes.
8. Wash the column with 3 column volume of equilibration buffer.
9. Proceed to the “Cleaning-in-place and resin storage” section.

Cleaning-in-place and resin storage

Contaminants/impurities can often bind very tightly to POROS CEX resins, so it is important to clean the POROS CEX resin sufficiently to increase column lifetime. The frequency at which this cleaning procedure is performed will be dependent on your sample, column performance, and regulatory needs. At a minimum, perform the following clean-in-place procedure any time an increase in backpressure or decrease in performance is observed.

1. Wash the resin with 5 column volumes of 1-2M NaCl.
2. Wash the resin with 5 column volumes of ultrapure water.
3. Wash the resin with 5 column volumes of 0.5-1M NaOH.
4. Wash the resin with 5 column volumes of ultrapure water.
5. Store resin in ultrapure water containing 20% ethanol or 0.1M NaOH at 2-30°C.

Troubleshooting

Problem	Cause	Solution
Low protein yield	Poor expression of soluble protein	Optimize expression conditions. Possible conditions to optimize are temperature of induction, length of induction, media, and cell type
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	Protein did not bind to the column	Adjust binding buffer, modify salt concentration, or modify pH to be further from pI of the protein of interest
	Flow rate was too fast	Decrease flow rate during binding to allow for greater residence time and increased binding
Poor protein purity	Co-elution of contaminating molecules	Modify elution conditions or gradient
		Use alternative IEX resin such as Poros 50 HQ or XQ
	Column was overloaded	Apply less protein extract onto the column
	Column was dirty	Follow clean-in-place procedure to remove non-specifically bound impurities
Slow column flow	Extract was too viscous or highly particulate	Dilute lysate with equilibration buffer to decrease viscosity, centrifuge lysate at higher speed to remove particulate, or use Pierce™ Universal Nuclease for Cell Lysis (Product No. 88700, 88701, 88702) to eliminate DNA/RNA
	Column was dirty after multiple uses	Perform a clean-in-place procedure
High backpressure	Rapid switch in buffer type	Avoid rapid switches between organic and aqueous solutions. Switch under reduced flow rates or over a gradient
	Presence of any amount of ethanol in the slurry or column	Fully remove ethanol before packing or sample injection
	Compromised flow path	Characterize the pressure of the entire chromatography system with 1) no column in place, 2) an empty column with the column outlet plumbed to the waste, and 3) an empty column with the column outlet plumbed back to the chromatography system
		Ensure the entire flow path is clear
		Change in-line filters
	Clogged frits	Change or clean frits
		Run the column in reverse for 3 column volumes
	Particle size gradient in the column caused by gravity settling	Do not gravity settle POROS resin in the column before packing
		Repack column
	Resin was frozen	Do not freeze

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Turbid column effluent after >3 column volumes during packing	Column frits were too large for the resin	Use frits with <25 micron pore size
	Defective O-ring or improperly assembled column	Disassemble column and inspect all components
Column qualification – high asymmetry	Column was underpacked	Pack at higher flow rate/pressure
	Salt injection was not optimized	Verify the desired amount of salt is loaded by checking the peak height and width.
	Column needs more post-pack conditioning to stabilize the packed bed	Equilibrate the column with 2-5 column volumes of packing solution in downflow at the operating flow rate
	System plumbing allows for dilution of salt plug	Characterize the 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
	Improper slug solution used	Use the recommended column qualification conditions
Column qualification – low asymmetry	Water was used as the mobile phase	Add some salt to the mobile phase to reduce the charge interaction between the salt and the resin
	Column was overpacked or packed inconsistently	Repack the column following the recommended procedure
	Column not equilibrated long enough with NaCl before salt injection	Equilibrate with >4 column volumes if the packing solution is different from the qualification mobile phase
Decreased performance • Increased band spreading • Dynamic binding capacity decrease • Recovery issues • Increased pressure drop • Ghost peaks during other steps • Protein separation not achieved	Column fouling can occur due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities	Clean the column
	Gradient was not correct	Use a shallower gradient
		Lower flow rate
	Wrong type of gradient used	Perform a pH gradient instead of salt gradient
		Perform a salt gradient instead of a pH gradient

Related Thermo Scientific Products

1255907	**POROS™ 50 HQ**, 1L (see thermofisher.com for larger pack sizes)
4467818	**POROS™ XQ**, 1L
4404336	**POROS XS**, 1L
1335907	**POROS 50 HS**, 1L
90078	**B-PER™ Bacterial Protein Extraction Reagent with Enzymes**
90084	**B-PER™ Bacterial Protein Extraction Reagent**, 250mL
78248	**B-PER Bacterial Protein Extraction Reagent**, 500mL
87786	**Halt™ Protease Inhibitor Cocktail (100X)**
23225	**Pierce™ BCA Protein Assay Kit**, 1L
89934	**Zeba™ Desalting Chromatography Cartridges**, 5 × 5mL
89893	**Zeba™ Spin Desalting Columns**, 5 × 10mL
87717	**Slide-A-Lyzer™ G2 Dialysis Cassettes**

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