POROS™ Caprylate Mixed-Mode Chromatography Resin

Catalog Number Multiple, see below

Pub. No. MAN0029949 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

POROS™ Caprylate Mixed-Mode Chromatography Resin is a rigid, 50-µm polymeric resin for use in the purification of antibodies and antibody derivatives. The resin backbone consists of crosslinked poly(styrene-divinylbenzene) with a superior pore structure that provides rapid mass transport and facilitates increased productivity. The particle surface is coated with a proprietary hydrophilic polymer, which is further derivatized with caprylic acid.

The POROS™ Caprylate resin is designed for use in flow-through mode for the removal of antibody aggregates with high selectivity, as well as for the removal of residual process-related impurities, including host cell proteins and leached protein A resin ligand.

Contents and storage

Cat. No.	Volume	Storage
POROS™ Caprylate Mixed-Mode Chromatography Resin		
A51049[1]	50 mL	Store at 2–30°C. Do not freeze.
A51050[1]	250 mL	
A51051[2]	1,000 mL	
A51052[2]	5,000 mL	
A51053[2]	10,000 mL	
POROS™ Caprylate Mixed-Mode Prepacked Column		
A51054[1]	5 mmD x 50 mmL (GoPure™) V = 1 ml	Store at 2–30°C. Do not freeze.
A51055[1]	8 mmD x 100 mmL (GoPure™) V = 1 ml	
A51056[1]	200 µL Robocolumn	
A51057[1]	600 µL Robocolumn	

[1] For Research Use Only. Not for use in diagnostic procedures.
[2] Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.

Specifications

Characteristic	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Immobilized ligands	Caprylic (octanoic) acid
Shipping solvent	0.1 M sodium hydroxide
Average particle size	50 µm
Mechanical resistance	100 bar (1,450 psi, 10 MPa)
pH Range	1–14
Ionic strength range	0 to 5 M, all common salts
Buffers and additives	All common agents including 1 M sodium hydroxide, sodium chloride, sodium phosphate, acetic acid, citric acid, and other common buffer salts.
Storage conditions	2–30°C Do not freeze

General considerations

POROS™ Caprylate resin incorporates hydrophobic interaction characteristics with a weak cation exchange functionality. These properties allow the resin to function across a wide range of pH and ionic strength. Binding strength for some molecules varies with pH or ionic strength concentration. Strong monomer binding conditions may reduce selectivity and product recovery. Chemical modifiers, such as arginine, may be helpful to modulate selectivity and product recovery.

Recommended operating conditions

POROS™ Caprylate resin is designed as the intermediate or final polishing step in antibody purification processes after capture using Protein A.

Recommended operating conditions are a pH between 4 and 6 and a conductivity in the range of 8–30 mS/cm (for example, 50 mM acetate + 0.250 M NaCl, pH 5.0). It may be possible to operate in a broader range of pH and conductivity. Binding may be less sensitive to conductivity than for cation exchange resins lacking hydrophobic character.

For a given set of buffer conditions, achieving balance between aggregate reduction and monomer recovery requires optimal load-density determinations. In most cases, higher loading increases monomer recovery (yield), while lower loading results in greater aggregate reduction (purity). To determine the optimal

loading range, generate a breakthrough curve of aggregates at load densities in the range of 100–200 mg mAb/mL resin.

After loading, wash the column with 5 CV of equilibration buffer to displace the remaining product in the column and return the UV signal to baseline.

Resin cleaning and storage

Cleaning

- Clean the resin with 3 to 5 CVs of 25 mM acetate pH 3.0, followed by 3 to 5 CVs of 0.5 to 1 M NaOH.
- Other solutions may be needed for column cleaning.

Storage—Store the resin in 0.1 M NaOH at 2–30°C.

Pressure-flow characteristics

POROS™ resins are rigid. Therefore, columns packed with these resins exhibit a linear relationship between pressure and flow.

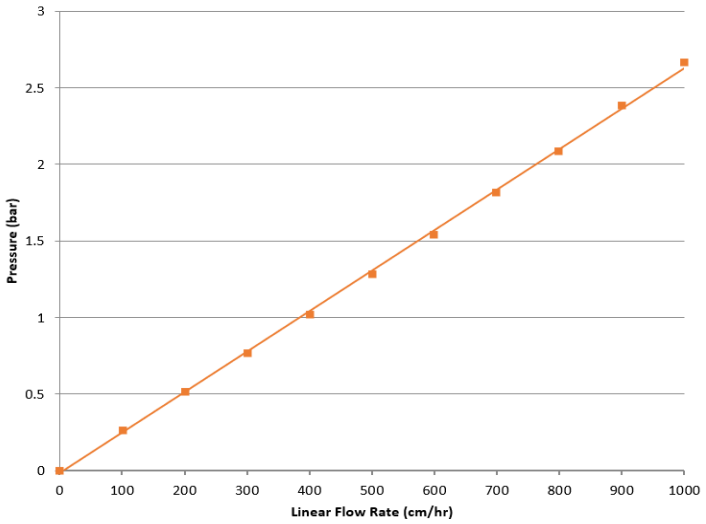

Figure 1 Pressure-flow characteristics of POROS™ Caprylate Mixed-Mode Chromatography Resin

Column format—1.6 cmD x 20 cmL

Packing pressure—3 bar

Mobile phase—0.1 M sodium chloride

Column packing instructions

Because POROS™ Caprylate resins are mechanically rigid, they can be packed effectively both in low-pressure glass columns and in high-pressure stainless-steel columns.

- A 1.10–1.15 compression factor is recommended for laboratory scale columns. Compression factor is the ratio of the gravity-settled volume to the packed-bed volume.
- Bed supports with a porosity of 10–23 µm should be used.

Slurry preparation—Lab scale columns (≤100 mL)

POROS™ Caprylate resin is supplied as approximately 55–60% slurry in 0.1 M sodium hydroxide. For column packing, exchange the shipping solution into 0.1 M NaCl.

Exchange the buffer

Exchange the buffer using a 0.22–0.45 µm bottle-top filter or sintered-glass filter.

1. Transfer the required volume of resin slurry to the top of the filter.
2. Apply vacuum to remove the storage solution.
3. Resuspend the resin cake to the starting resin slurry volume with the recommended packing solution. Swirl the filter top to resuspend the resin.
4. Transfer the resuspended resin into another container (for example, centrifuge tube, or graduated cylinder).
5. Verify that the slurry concentration is 50–70% by gravity settling for more than 4 hours.
6. If needed, adjust the slurry concentration to 50–70%.

Prepare the slurry

Prepare the slurry using repeated gravity settling.

1. Allow resin to settle in the shipping container.

   Settling requires >4 hours because the density of the resin is approximately that of water. The settling time also depends on the geometry of the container and the packing solution. The settling rate is between 17–22 cm/h.
2. Carefully decant the supernatant. Take care not to disturb the settled resin. Replace the supernatant with the same volume of the 0.1 M NaCl solution.
3. Resuspend the resin by gentle agitation (for example by gentle inversion of the container), then allow the resin to settle by gravity. Do not use a magnetic stirrer, which can abrade the particles and cause formation of fines.
4. Repeat step 1 to step 3 two to three times, to exchange the resin fully into the packing solution.
5. Verify that slurry concentration is 50–70% by allowing the slurry to settle by gravity for at least 4 hours, or until there is no change in the gravity-settled volume. If needed, adjust the slurry concentration to 50–70%.

Pack the column

During the initial flow packing stages, some turbidity may be observed in the column effluent. This clears as packing proceeds and 1–2 column volumes of packing buffer pass through the column.

1. Determine the required slurry volume using this equation.

   `required slurry volume = compression factor × target CV / slurry concentration`

   For example, for a 2.5 cmD x 5 cmL 25–mL column using a slurry concentration of 56%, the required slurry volume calculation is: 1.15 × 24.5 mL / 0.56 = 50.3 mL

2. Mix the required slurry volume until it is homogeneous.

3. Pour the slurry into the column.

   If the column is not packed at a high enough flow/pressure, then flowing a more viscous solution (such as a cleaning solution) over the column at the same flow rate results in further bed compression.

4. Apply flow at 200—300 cm/h until clear liquid is visible between the column top flow adapter and the packed bed. Monitor the pressure; it will gradually rise as the bed forms.

5. After the bed is formed, stop the flow and lower the top adapter until it is about 1–2 cm above the packed bed.

6. Reapply flow at 300 cm/h, increase it until the pressure drop across the column is at least 3 bar, then record the bed height.

   For short bed heights or due to system pump limitations, a pressure drop of 3 bar may not be achieved.

7. If a 3-bar pressure drop was not achieved on the previous step, stop the flow and lower the top adapter to the bed height recorded in the previous step or the target bed height based on the target compression factor.

8. *(Optional)* After the column is packed, flow 1–3 CVs of the 0.1 M NaCl solution through the packed bed at the highest operating flow rate in each flow direction to condition the bed.

Qualify the column

To qualify the integrity of a packed column, determine the HETP (height equivalent to a theoretical plate) and asymmetry using a nonbinding analyte (injection). The recommended column qualification conditions are as follows.

- Flow rate—Target operating flow rate (for example, 200–300 cm/hour)
- Equilibration buffer—0.1 M sodium chloride
- Injection solution—1.0 M sodium chloride
- Injection volume—2% of column volume
- Minimize the column tubing lengths between the injection point to the column inlet and the column outlet to the detectors.
- Ensure that the conductivity has equilibrated before the injection.

Troubleshooting

Observation	Possible cause	Recommended action
High backpressure	Compromised flow path: • 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	• 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)	• 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	• 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 resin in the column before packing.
	Resin was frozen	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, if needed.
Column qualification — high asymmetry	Column is underpacked; that is, the column is not packed at a high enough flow rate/ pressure	• 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.

Observation	Possible cause	Recommended action
Column qualification — high asymmetry *(continued)*	The system and plumbing allow for dilution of the salt plug	• 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	Column was overpacked or packed inconsistently	Repack the column following the recommended procedure.
	Water was used as the mobile phase	Add some salt to the mobile phase to reduce the charge interaction between the salt and the bead.
	Column was not equilibrated long enough with sodium chloride before salt injection	Equilibrate ≥ 4 CVs if the packing solution is different from the qualification mobile phase.
Column qualification – low plates or high HETP	The system and plumbing allowed for dilution of the pulse solution.	• Characterize a salt pulse injection through the chromatography system at the qualification flow rate 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 column flow distributor.
Details: Decreased performance: • Increased elution peak volume • Decreased binding capacity • Decreased recovery • Increased pressure drop • Trace or “ghost” peaks during blank runs	Insufficient or unoptimized cleaning leading to precipitation of product or impurities, irreversible binding of lipid material, or other impurities.	Change and improve the cleaning regime.

Support

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 2 Preston Court | Bedford, Massachusetts 01730 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029949 A.0

Revision	Date	Description
A.0	11 September 2023	New document for POROS™ Caprylate Mixed-Mode Chromatography Resin.