POROS™ 50 HE Heparin Affinity Resin

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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™ 50 HE Heparin Affinity Resin is on a 50-µm, rigid, polymeric bead. The resin backbone consists of crosslinked poly[styrene divinylbenzene] and is coated with a polyhydroxylated polymer. This coating is further derivatized by covalent immobilization of heparin functional groups.

POROS™ 50 HE Heparin Affinity Resin is optimized for very rapid mass transport. This resin supports chromatographic separations at considerably faster flow rates than conventional liquid chromatography separations (up to 1,000 cm/hr) while maintaining high dynamic binding capacity. In addition, the 50-µm particle size provides superior resolution for unprecedented impurity clearance independent of scale and flow rate.

Storage

Store resins at 2–30°C. Do not freeze.

Specifications

Table 1 POROS™ 50 HE Heparin Affinity Resin product characteristics

Characteristic	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Immobilized ligand	Heparin
Dynamic binding capacity	≥25 mg/mL[1]
Shipping solvent	0.1 M sodium phosphate, pH 7, 18% ethanol
Average particle size	50 µm
Shrinkage/swelling	<1% from 1–100% solvent
Mechanical resistance	103 bar (1,500 psi, 10 MPa)
Recommended maximum flow rate	1,000 cm/hr

[1] Lysozyme, pH 6 to 7 at 3,600 cm/hour in 4.6 cmD × 20 cmL column

Table 2 POROS™ 50 HE Heparin Affinity Resin chemical and thermal resistance

Characteristic	Description
pH Range	5–10
Ionic strength range	0 to 5 M, all common salts
Buffer additives	All common agents, including 8 M urea, 6 M guanidine/HCl, ethylene glycol, and detergents. Agents that may degrade the ligand are not recommended.
Solvents	Water, 0 to 100% alcohol, acetonitrile, other common organic solvents **Note:** Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), or acetone.
Operating temperature	5 to 40°C Do not freeze

POROS™ 50 HE Heparin Affinity 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. The pressure-flow curve of POROS™ 50 HE Heparin Affinity Resin is shown in Figure 1.

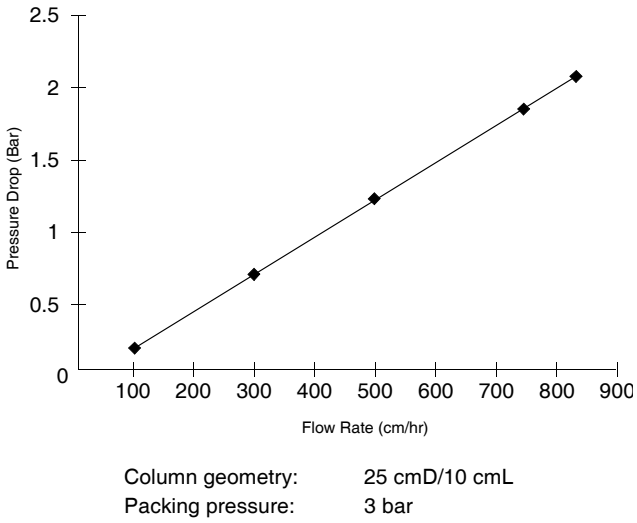

Figure 1 Pressure-flow properties of POROS™ 50 HE Heparin Affinity Resin

See "Product use" on page 6

Packing considerations

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

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 water. Mix with a plastic or rubber spatula. Do not grind the resin bed or tear the filter membrane.
4. Repeat the vacuum and resuspension steps for a total of three exchanges.
5. Resuspend the exchanged resin to the original slurry concentration, then 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)

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 impeller mixer, or rotary mixer, then allow the resin to settle by gravity.

   As with any resin, do not use a magnetic stirrer. It can abrade the particles and cause fines to form.
5. Repeat steps 1 to 4 two to three times to thoroughly exchange into the 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

For larger columns, 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.

When you adjust the flow rate to form the bed, you may observe some turbidity in the eluent as packing starts. 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 L / 0.56 × 1.06 = 47.3 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 starting the pack.

4. 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.

5. 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, then 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.

6. Change the valve back to flow through the system on the top, then open the column bottom.

7. 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 all of the required resin has been transferred 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.

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8. **Flow packing only**: 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.

9. After the bed is formed, bring the adapter into contact with the top of the bed without pushing the resin over the O-ring by closing the column outlet and displacing liquid through the top of the adapter to waste through the bypass line.

   POROS™ resin does not shrink or swell, so an open headspace is not recommended.

10. Flow at the packing flow rate again for 1–2 CVs, taking note of the bed height at the desired pressure. Adjust the adapter again to the noted bed height by displacing the liquid through the top of the adapter and to waste.

11. 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.

12. 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 that is 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 several 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.

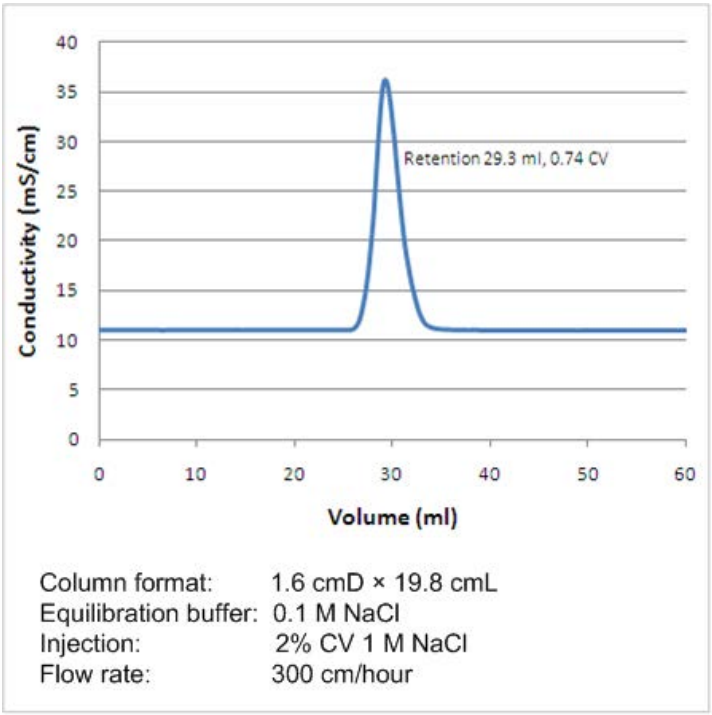

Figure 2 POROS™ column qualification

Optimize chromatography conditions

- Use buffers of the highest purity practical.
- Filter (0.22 or 0.45 µm) all buffers and load materials before use.
- The starting/wash buffer pH can range from 6.0 to 9.0, although binding is strongest near neutral pH.
- Heparin is negatively charged and acts as a cation exchanger. Add salt (0.1-M to 0.2-M NaCl or KCl) to prevent nonspecific binding due to heparin/protein interactions.

Binding conditions considerations

- For equilibration, use standard neutral buffers (pH 6 to 9) such as 10–50 mM sodium phosphate or Tris. As an initial starting condition, try PBS pH 7.0 to 7.5.
- Usually, clarified harvest material can be loaded directly onto the equilibrated column without any further pre-treatment. However, pre-treatments are recommended to:
  - Delipidate samples. Lipids can cause irreversible fouling.
  - Heat-treat serum samples (56°C for 30 minutes). Heat treatment removes remaining fibrinogen that can clog the column on multiple runs.
- Dilute feed capture: POROS™ 50 HE Heparin Affinity Resin can concentrate very dilute samples, such as cell culture supernatants, with high throughput. This can eliminate the need for an ultrafiltration concentration step on scale-up. Use a membrane filtration step to clarify the feed and apply the filtrate directly to the column.

Wash conditions considerations

- After the load, wash unbound material from the column with the equilibration buffer. Generally a 5–10 CV wash is sufficient to remove all unbound proteins from the column. Samples with high impurity levels can require a longer wash to return to a stable baseline.

Elution conditions considerations

Because proteins differ significantly in their binding/elution behavior, the best elution conditions are determined experimentally.

- Elution is usually carried out with an increasing concentration of salt.

  Note: Although most proteins elute with less than 1.5 M of salt, you can use up to 4 M or 5 M if needed.
- You can also elute by adding heparin (1 to 5 mg/mL) in the starting/wash buffer.
- Use a step elution to obtain a concentrated elution fraction, then a gradient if additional separation for similar product impurities is needed.

Resin storage guidelines

- Store bulk resin at 2 to 8°C. Do not freeze.
- Store packed columns at 2 to 8°C or room temperature after cleaning (described above) and equilibration in buffered ethanol such as 100 mM sodium phosphate, 18% ethanol, pH 7.0.

Note: Changing storage temperature from room temperature to refrigerated temperature can affect packed bed stability and buffer outgassing.

Ordering information

POROS™ resin	Volume	Cat. No.
POROS™ 50 Heparin Affinity Chromatography Resin	10 mL	4329435
	50 mL	4333410
	1,000 mL	4329437
	5,000 mL	4329438
	10,000 mL	4329439

Product use

POROS™ resin	Volume	Cat. No.	Product use
POROS™ 50 Heparin Affinity Chromatography Resin	10 mL	4329435	For Research Use Only. Not for use in diagnostic procedures.
	50 mL	4333410	For Research Use Only. Not for use in diagnostic procedures.
	1,000 mL	4329437	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	5,000 mL	4329438	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	10,000 mL	4329439	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.

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 you local Thermo Fisher Scientific representative.

Limited product warranty

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Revision history: Pub. No. 4327859

Revision	Date	Description
C	24 April 2018	Removed outdated sections and updated the content throughout.
B	24 February 2017	Baseline for this revision history.

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