applied biosystems
by life technologies

POROS® 50 µm Resin – General Column Packing

INSTRUCTIONS

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Product description

POROS® Chromatography Resins are 50 µm, polymeric beads that can be used for the chromatography of biomolecules, including monoclonal antibodies, recombinant proteins, DNA, viruses, and peptides. The plastic backbone yields incompressible beads that are chemically stable and inert, features that facilitate handling during packing and maintenance of packed bed heights. POROS® Chromatography Resins are mechanically rigid and can be packed effectively both in low-pressure glass columns and in high-pressure stainless steel columns. The lack of wall support with increasing column diameter has minimal impact 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 using a range of packing solutions (for example, water, sodium chloride, hydroxide). This document outlines Life Technologies best practice recommendations for obtaining fast and consistent results.

Notes:

- A 1.06 packing factor is recommended to account for the difference in bed volume between a gravity-settled bed and a 1–3 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 the slurry

POROS® resins (except POROS® R 150 and R 250 resins) are supplied as approximately 56% slurry in 20% ethanol or buffered ethanol. When packed at 3 bar, a 1.8-L slurry volume yields a 1-L packed bed volume.

Note: POROS® R 150 and R 250 resins are supplied as a dry powder. The amount of dry powder needed to form the final bed volume is calculated using the formula: 1 g of powder = 4 mL of resin. The packed bed volume specified on the column label is based on a packing pressure of 7 bar.

For column packing POROS® ion exchange and affinity resins, exchange the 20% ethanol shipping solution with 0.1 M sodium chloride. For POROS® R 150 and R 250 resins, add a volume of organic solvent equivalent to 2–3 times the final bed volume; we recommend 13% isopropanol.

Note: POROS® 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 (≤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 dry resin bed 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 2 and 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%.

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.

   Note: 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.

   Note: 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.

   Note: As with any resin, 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 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.

Note: 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 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 1.06

   Example for a 40 cmD x 20 cmL, 25 L, column using slurry with a 56% slurry ratio:

   25 L / 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. 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.

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.

   Note: 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 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.

6. Change the valve back to flow through the system on the top and 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 POROS® 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 POROS® 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. Slurry should be transferred at a flow rate equal to 500–1000 cm/hour and final packing pressure should attain 2.5–3 bar.

  If a pressurizable slurry tank is available, the slurry tank can simply be pressurized to 3 bar and a constant pressure pack can be executed.

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

8. 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. 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.

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 as described in the previous step to the noted bed height by displacing the liquid through the top of the adapter and to waste.

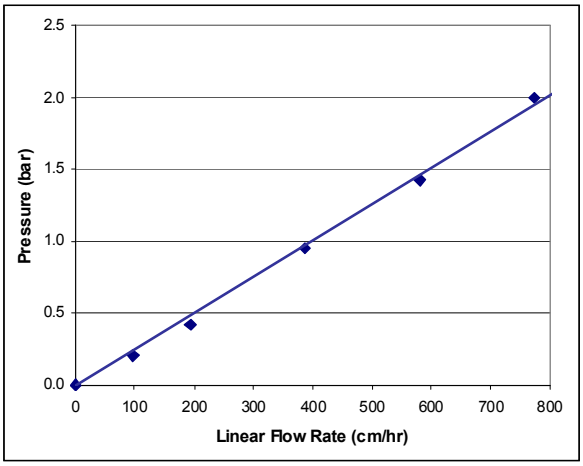
11. After the column is packed, flow 2–3 CVs of packing solution through the packed bed at the operating flow rate to stabilize the bed.

    Note: 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.

Figure 1 shows a typical pressure-flow curve of a POROS® 50 µm resin. POROS® 50 µm resins can be operated at high linear flow rates with a pressure drop that allows use with conventional low-pressure chromatography columns and systems. The column and system backpressures have been subtracted, so this curve represents the packed bed backpressure only.

Figure 1 Pressure-flow properties of a POROS® 50 µm resin



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

Qualify the column

Recommended column qualification conditions

For ion exchange and affinity resins:

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

For R 150 and R 250 resins:

- Flow rate: operating flow rate (cm/hour)
- Equilibration buffer: 80% acetonitrile
- Plug solution: 1% acetone in 80% acetonitrile
- Plug volume: 2% of column volume

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"). Common plug solutions are listed below.

Solution	Concentration for pulse
Sodium chloride†	0.5–1.0 M
Sodium hydroxide	0.5–1.0 M
Sodium nitrate‡	50–200 mg/mL
Acetone (POROS® R 150 and R 250 only)§	1–50%

† Sodium chloride concentrations ≥ 2 M NaCl are not recommended for column qualification because a shoulder will be detected on the backside of the peak and will yield erroneous results.

‡ Add 1.0 M NaCl to the nitrate solutions if running on anion exchange resins.

§ Do not use acetone for POROS® ion exchange resins or Protein A resins. Acetone binds to POROS® resins in the absence of high organics, therefore add acetone only to an acetonitrile solution (for example, 80–90%).

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).
- Use:
  - Plug volume: 1–3% of the total column volume.
  - Plug concentration: 5–10 times the mobile phase concentration (for example 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. If the packing buffer is different from the mobile phase, equilibrate the column for at least 4 CVs with the mobile phase.
- Execute at the flow rate defined for the intended unit operation, typically 100–300 cm/hour.
- Equilibrate with at least 2 CVs of equilibration buffer before injection.
- Monitor:
  - Conductivity for sodium chloride and sodium hydroxide.
  - Absorbance for sodium nitrate and acetone.

Setting specifications

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

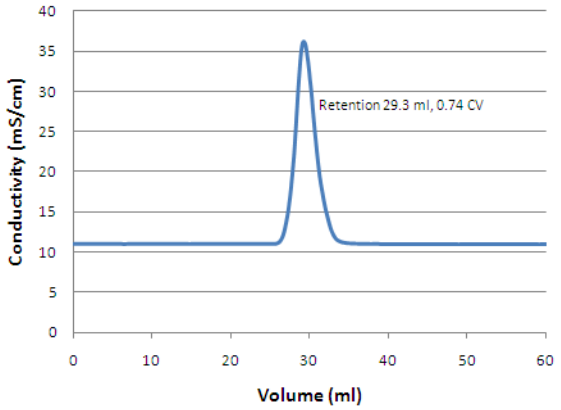
- Solutions and method used
- Scale
- Column hardware
- Chromatography system

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.

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® HS column qualification



Column format: 1.6 cmD x 19.8 cmL
Running buffer: 0.1 M NaCl
Injection: 2% CV 1 M NaCl
Flow rate: 300 cm/hour

Troubleshooting

The table below provides troubleshooting information for common column packing and qualification observations.

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: • 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 POROS® resin in the column before packing.
	Resin allowed to freeze	Store resin at 2–40°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.
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.
	Column not equilibrated long enough with 0.1 M sodium chloride before salt injection	Equilibrate ≥ 4 CVs if the packing solution is different from the qualification mobile phase.
	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.
	2 M sodium chloride or higher salt is used for the salt plug or an analyte that interacts with the resin is used	Use recommended column qualification conditions.
	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.
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.

Support

For service and technical support, go to **www.lifetechnologies.com/poros**, or call Toll-Free in US: 1.800.831.6844.

For the latest services and support information for all locations, go to **www.lifetechnologies.com**, then click the link for **Support**, or contact your local Life Technologies representative.

Safety information

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

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 found on Life Technologies website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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