POROS™ HIC Resins: Ethyl, Benzyl, and Benzyl Ultra

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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 information

Product description

POROS™ Hydrophobic Interaction Chromatography (HIC) resins are rigid, 50-µL polymeric resins with a range of hydrophobic functionalities for the purification of antibody fragments, antibody drug conjugates (ADCs), recombinant proteins, viruses, and other biomolecules. The resin backbone consists of crosslinked poly(styrene-divinylbenzene) with a unique pore structure that provides rapid mass transport and enables enhanced productivity. The particle surface is coated with a novel polymer coating, which is then further derivatized with a range of hydrophobic ligands for flexible purification process design.

POROS™ HIC resins are suitable for bind/elute and flow-through applications at lower salt concentrations. These resins have superior resolution capability, high capacity, and differentiating selectivity for a range of biomolecules, and this performance is independent of flow rate.

Storage

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

Specifications

Table 1 Ligands, hydrophobicity, and applications

Resin	Ligand	Relative hydrophobicity [1]	Application
Ethyl	Novel ethyl	Low	Bind/elute mode to bind moderately to strongly hydrophobic molecules.
Benzyl	Low-density benzyl/ aromatic	Moderate	Bind/elute or flow-through mode depending on the hydrophobicity of the molecule.
Benzyl Ultra	High-density benzyl/ aromatic	High	Flow-through mode in lower salt concentration to bind impurities such as aggregates.

[1] Hydrophobicity results are based on lysozyme gradient elution. Column size: 0.66 cmD × 20 cmL; 1.7-M ammonium sulfate, 50-mM sodium phosphate pH 7.0; Gradient elution: 1.7-M ammonium sulfate/50 mM sodium phosphate pH 7.0 to 50-mM sodium phosphate pH 7.0 over 10 column volumes; Flow rate: 100 cm/hr.

Table 2 Characteristics and stability

Characteristic	Description
Support matrix	Crosslinked poly(styrene-divinylbenzene)
Shipping solution	18% ethanol
Average particle size	50 µm
Mechanical resistance	100 bar (1450 psi, 10 MPa)
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
Salts	Ammonium sulfate, sodium sulfate, sodium chloride, sodium acetate, sodium citrate and other common salts **IMPORTANT!** POROS™ Benzyl and POROS™ Benzyl Ultra are designed for use with lower salt concentration than traditional HIC resins. With some molecules, high salt concentration can cause poor recovery due to a strong interaction between the target and the ligand.
Solvents	Water, 0–100% alcohol, acetonitrile, 1 to 2 M acids (for example, acetic, hydrochloric, phosphoric), 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.
Shrinkage/swelling	<1% from 1–100% solvent
Operating temperature	2– 30°C Do not freeze

POROS™ HIC resins can be operated at high linear flow rates with a pressure drop that allows use with conventional low-pressure chromatography columns and systems. POROS™ HIC resins have linear and predictable pressure flow responses as column diameter increases (Figure 1 and Figure 2).

Product usage: See "Ordering information" on page 5.

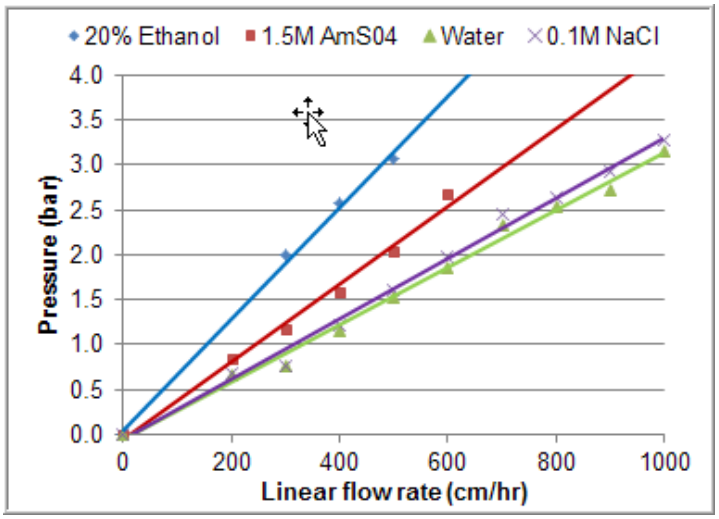

Fig. 1 Pressure-flow properties of POROS™ Ethyl resin—20-cm bed height

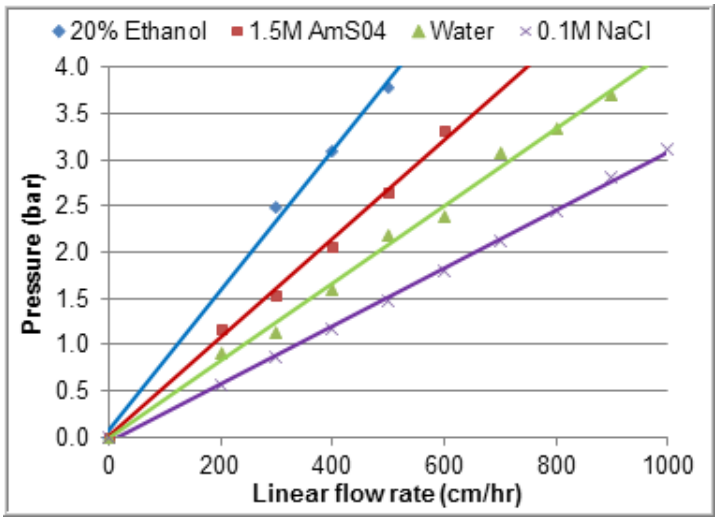

Fig. 2 Pressure-flow properties of POROS™ Benzyl Ultra resin—20-cm bed height

Pack and qualify the column

Packing guidelines

- Resins are supplied in 18% ethanol. For column packing, exchange the shipping solution with water to remove the ethanol.
- 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.
- Standard 10–23 µm screens (frits) can be used.

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% (see "Determine the slurry concentration" on page 2).
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 > 8 hours because the density of the resin is approximately that of water.
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. Replace the supernatant with the same volume of water.
5. Resuspend the resin by gentle agitation, then allow the resin to settle by gravity.
6. Repeat steps 1 to 4 two to three times to thoroughly exchange into water.
7. Verify that the slurry concentration is 50–70% (see "Determine the slurry concentration" on page 2).
8. If needed, adjust the slurry concentration to 50–70%.

Determine the slurry concentration

1. Separate the slurry using either of the following methods.
   - **Gravity settling**—Add 100 mL of slurry in water to a 100-mL graduated cylinder, then allow to settle for >72 hours.

     Note: The time that POROS™ HIC resins take to gravity settle can be inconsistent due to hydrophobicity. We recommend using the centrifugation method for faster, more consistent results.
   - **Centrifugation**—Add 10 mL of slurry in water to three (3) 15-mL conical tubes. Centrifuge at 3,000 rpm for 10 minutes at 20°C with the brake off. Remove the tubes from the centrifuge and allow the tubes to sit for 5 minutes before determining the concentration.
2. Calculate the concentration: Volume of resin/total volume in the graduated cylinder or conical tube.

Pack 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:

   Example for a POROS™ Ethyl 40 cmD × 20 cmL 25-L column using slurry with a 50% slurry ratio:

   25 L / 0.56 × 1.06 = 47.3 L slurry required

   The 1.06 packing factor above accounts for the difference in bed volume between a centrifuged bed in water and a 3-bar pressure-packed bed. Use a 1.12 packing factor for POROS™ Benzyl and POROS™ Benzyl Ultra.
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 up the resin 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.
8. 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.
9. 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.
10. 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.
11. 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	50 cm/hour
Equilibration buffer	Water
Plug solution	0.5 M sodium chloride
Plug volume	1% of column volume

Guidelines for qualification

- 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).
- 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.

Chromatography condition optimization

General guidelines

Standardized conditions or platform-type evaluations are not recommended. Different HIC resins that are operated with the same process conditions can yield variable results.

When optimizing conditions:

- Test different loading and elution conditions to evaluate static binding capacity and yield based on the target molecule characteristics and process challenges.
- Limit static binding load incubation time to 15 minutes.
- Optimize the chromatography step for peak separation: Use conditions that remove some of the bound impurities during the flow-through/wash phase and that retain other bound impurities until elution during the strip and cleaning-in-place (CIP) steps.
- Use buffer salts and reagents of the highest purity.
- Filter (0.22 or 0.45 µm) all buffers, solutions, and load before use.

Resin selection guidelines

- If the hydrophobicity of the target molecule is unknown:
  - Run a small-scale bind/elute gradient separation on POROS™ Benzyl resin to determine the elution conductivity of the target molecule, contaminants, and impurities.
  - Optimize conditions on POROS™ Benzyl Ultra resin (higher hydrophobicity) or POROS™ Ethyl resin (lower hydrophobicity).
  - If needed, continue to optimize conditions on POROS™ Benzyl resin (mid-range hydrophobicity).
- If the target molecule is hydrophobic, optimize conditions on POROS™ Ethyl resin or on POROS™ Benzyl with lower salt concentration.
- For flow-through applications where the target molecule is less hydrophobic, optimize conditions on POROS™ Benzyl or POROS™ Benzyl Ultra with lower salt concentration.

After you select the resin, continue to optimize other process conditions.

Binding capacity and loading condition screening guidelines

Perform high-throughput static binding capacity testing in spin columns or in a 96-well plate to screen POROS™ HIC resin. Optimize loading conditions as needed.

Note: A 96-well high-throughput protocol is available on request. This protocol can be used to evaluate static binding capacity and to screen several resins and loading conditions per resin in a single 96-well plate.

Bind/elute chromatography optimization guidelines

Binding conditions guidelines

- **Salt and salt concentration** — POROS™ HIC resins are designed to use less lyotropic salts and to bind at lower concentrations than traditional HIC resins with similar functional groups (Table 3).

  Table 3 Typical salts used in hydrophobic interaction chromatography in order of decreasing lyotropic ("salting out") effect and increasing chaotropic ("salting in") effect

Effect	Anionic salts	Cationic salts
Most lyotropic	$C_6H_5O_7^{3-}$	NH_4^+
	PO_4^{3-}	Rb^+
	SO_4^{2-}	K^+
	$CH3COO^-$	Na^+
	Cl^-	Cs^+
	Br^-	Li^+
	NO_3^-	Mg^{2+}
	ClO_4^-	Ca^{2+}
Most chaotropic	I^-	Ba^{2+}

  Note the following:
  - You can perform initial binding experiments with lower salt concentrations than are typically used for HIC chromatography. For example, you can start with 25%, 50% and 75% of the salting out concentration, instead of the typical 10–15% lower than the salting out concentration.
  - The optimized ionic salt concentration for the salts listed above can differ from ammonium sulfate, which has been traditionally used for HIC chromatography.
  - Different salts can provide different selectivity.

  Optimize the salt concentration for the target molecule by using a "salting out" experiment: increase the ionic strength of the loading buffer until the sample precipitates. Alternatively, you can measure increasing optical density of the loading buffer to detect aggregation (~350 nm, but this value may differ with each salt). Salts that are commonly used to perform salting out experiments are ammonium sulfate, sodium chloride, sodium citrate, sodium sulfate, and sodium acetate.
- **Buffer system**—Buffer systems are not as critical for HIC processes as they are for ion-exchange chromatography steps. Citrate, acetate, Bis-Tris propane, HEPES, MES, sodium phosphate, succinate, and Tris are commonly used.

  The buffer system is typically dictated by the upstream purification step. When selecting the upstream buffer system, consider molecule stability in the buffer, binding optimization, and buffering capacity.
- **pH**—pH is not typically critical for HIC processes, but it can affect the binding strength and selectivity. Because pH effects are unpredictable on HIC, test a few pH values over the stability range of the target molecule.
- **Flow rate**—The target operating flow rate is flexible. Start optimization at 4-minute residence time (300 cm/hr in a 20-cmL column).
- **Temperature**—Temperature can significantly impact HIC performance. Perform all optimization at the final intended process temperature.

Elution conditions guidelines

Start elution optimization with a gradient elution. Most often, after elution performance is determined, you can implement a step elution.

- **Salt gradient**—To determine where the target molecule and contaminants/ impurities elute, start with a 20-CV gradient from high salt to buffer only. To do so, assay fractions across the peaks (~1/10 CV). Based on this information, the process can be further optimized.
- **Dynamic binding capacity (DBC)**—Assess separation as a function of DBC. The maximum DBC depends on several factors, including sample solubility, column selectivity, buffer pH, and loading buffer conductivity.
- **Bed height**—Initial screening can be run with shorter bed heights and a constant residence time. Use the final desired bed height for scale up development (typically 15 cm to 30 cm).

Flow-through chromatography optimization guidelines

An optional flow-through step can be used remove trace product and process-related impurities such as aggregates from the target molecule. You can add the flow-through step as the second or third chromatography step for polishing in a downstream process. Different target molecules have different degrees of hydrophobicity and other biophysical characteristics. Therefore, it is essential to optimize the process conditions to achieve the desired aggregate clearance and recovery of the target molecule.

Loading conditions guidelines

- **Initial study** —Perform an initial study using a decreasing salt gradient in bind/elute mode:
  - Load approximately 1 mg of protein per 1 ml of resin at 0.5–1.0 M sodium chloride (or other preferred salt), then elute using a gradient over 10 column volumes (CVs) in a buffered solution to determine aggregates, impurities, and target molecule elution profiles.
  - Use the elution conductivity at peak maximum to determine the highest approximate salt concentration that is required to remove impurities, but that allows the target molecule to flow through (Figure 3).

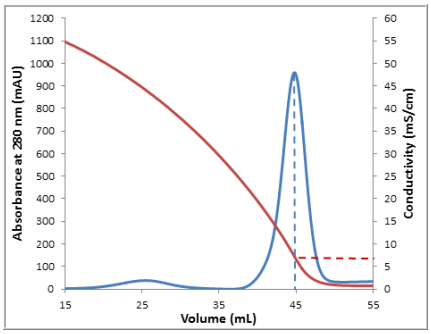

Fig. 3 Example screening chromatogram to obtain the ideal low salt condition for flow-through conditions. Process screening for a monoclonal antibody using POROS™ Benzyl Ultra resin in flow-through mode. Gradient: High conductivity to low conductivity using sodium citrate. Based on this chromatogram, the resin was further optimized in flow-through mode at low salt conditions starting at 7 mS/cm.

- **Buffer system**—Buffer systems are not as critical for HIC processes as they are for ion-exchange chromatography steps. Citrate, acetate, Bis-Tris propane, HEPES, MES, sodium phosphate, succinate, and Tris are commonly used.

  The buffer system is typically dictated by the upstream purification step. When selecting the upstream buffer system, consider molecule stability in the buffer, binding optimization, and buffering capacity.
- **pH**—pH is not typically critical for HIC processes, but it can affect the binding strength and selectivity. Because pH effects are unpredictable on HIC, test a few pH values over the stability range of the target molecule.
- **Flow rate**—The target operating flow rate is flexible. Good impurity binding has been demonstrated at flow rates up to 600 cm/hour on a 20 cmL column (1.6-minute residence time).
- **Temperature**—Temperature can significantly impact HIC performance. Perform all optimization at the final intended process temperature.
- **Dynamic binding capacity (DBC)**—A conservative starting point for DBC determination is 100–250 mg of the target molecule per mL of resin. Determine the DBC for each impurity by using breakthrough analysis under the desired load pH and conductivity conditions.
- **Bed height**—A bed height of 15 cm to 30 cm can be used for this step.

Resin cleaning and storage

Resin cleaning guidelines

- POROS™ resins can tolerate harsh cleaning conditions that allow acceptable column life.
- Clean the resin with 3 to 5 CVs of water followed by 3 to 5 CVs of 1 M NaOH.
- For more stringent cleaning, use 20% ethanol/1 M acetic acid.
- Other 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.

  Note: Low-level gassing out does not impact column performance.

Store the resin

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

Ordering information

Table 4 POROS™ HIC bulk resins

Resin	Cat. No.	Amount	Product usage
Ethyl	A32552	10,000 mL	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	A32553	5,000 mL	
	A32554	1,000 mL	
	A32555	250 mL	For Research Use Only. Not for use in diagnostic procedures.
	A32556	50 mL	
	A32557	25 mL	
Benzyl	A32558	10,000 mL	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	A32559	5,000 mL	
	A32560	1,000 mL	
	A32561	250 mL	For Research Use Only. Not for use in diagnostic procedures.
	A32562	50 mL	
	A32563	25 mL	
Benzyl Ultra	A32564	10,000 mL	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	A32565	5,000 mL	
	A32566	1,000 mL	
	A32567	250 mL	For Research Use Only. Not for use in diagnostic procedures.
	A32568	50 mL	
	A32569	25 mL	

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

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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The information in this guide is subject to change without notice.

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Revision	Date	Description
A	10 July 2017	New document.