POROS™ CaptureSelect™ AAV Resins: AAV8, AAV9, AAVX

Pub. No. 100038399 **Rev.** E

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™ CaptureSelect™ AAV Resins are 50-µm, rigid, polymeric affinity chromatography resins designed for the purification of native and recombinant adeno-associated virus subtypes with high purity. The resin backbone consists of crosslinked poly[styrene divinylbenzene] with a unique pore structure optimized for rapid mass transport and chromatography. The particle surface is coated with a cross-linked polyhydroxylated polymer. This coating is further derivatized with an affinity ligand which is a single-domain monospecific antibody fragment. These affinity resins enable single-step purification with high purity and yield at higher flow rates and improved throughput.

Storage

Store resins at 2–8°C. Do not freeze.

Specifications

Characteristic	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Immobilized ligand	Single--domain [V_HH] antibody fragment
Serotype affinity	• **AAV8**—AAV8 • **AAV9**—AAV9 • **AAVX**—AAV1 through AAV9, numerous recombinant and chimeric serotypes
Binding capacity	$>10^{13–14}$[1]
Shipping solvent	18% ethanol
Average particle size	50 µm
Recommended flow rate	≥3-minute residence time
Shrinkage/swelling	<1% from 1–100% solvent
Mechanical resistance	100 bar (1,450 psi, 10 MPa)
pH Range (all ligands)	1–10
Ionic strength range	0 to 5 M, all common salts
Buffer additives	All common agents, including 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents. Agents that may degrade the protein ligand are not recommended (for example, pH > 10).
Solvents	Water, 0–20% alcohol , acetonitrile, 1–2 M acetic acid (for example, acetic, hydrochloric, phosphoric) other common organic solvents. Do not expose the resin to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.
Operating temperature	2°C to 25°C Do not freeze

[1] Binding capacity varies based on serotype, feedstream, additives, and mutations to parent serotypes.

Pack and qualify the column

Packing guidelines

- Resins are supplied as approximately 56% slurry in 18% 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.

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 the desired packing solution. 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.

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 1 shows a typical column qualification peak. The peak void volume of a POROS™ column is typically 0.7–0.8 CV.

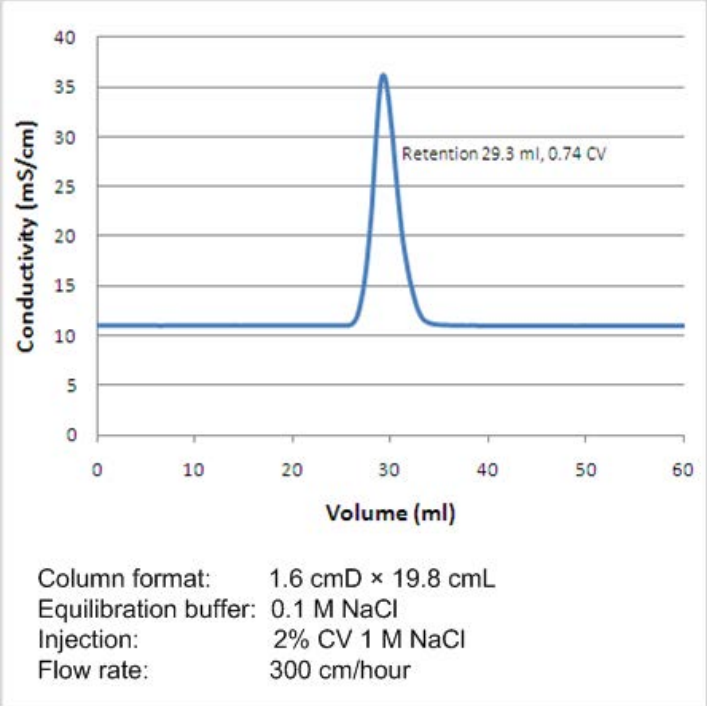

Figure 1 POROS™ column qualification

Chromatography condition optimization

Equilibration/binding conditions guidelines

PBS pH 7.0 to 7.5 is a good starting buffer. However, other standard neutral pH buffers such as 10–50 mM sodium phosphate or Tris can be used. pH must be in the range of 6–8. Adding 0.1–0.2 M NaCl or KCl may prevent nonspecific adsorption due to protein/protein interactions.

Wash conditions guidelines

- 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 may require a longer wash to return to a stable baseline.
- Washing with a secondary or intermediate wash can increase impurity removal and make impurity clearance more predictable especially when there is interaction with the protein of interest and the impurity. Secondary wash options include:
  - High-salt washes of up to 1 M NaCl
  - Varying pH up or down
  - Additives such as Tween™ 20 up to 0.05 (v/v)%
  - Chaotropic salts such as ≤0.2 M $MgCl_2$ (avoid buffers containing phosphates to prevent precipitation which can cause column over-pressurization)
  - ≤20% ethanol, which does not damage the capsid structure of most serotypes

Elution conditions considerations

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

- Start with 50–100 mM citric acid pH 3.0.
- To elute most target molecules, reduce the pH to the range of pH 2 to 3.
- Other elution buffer components that can be used include phosphate, hydrochloric acid, glycine, acetate, or other components that buffer well at low pH. Other additives such as ≤2 M $MgCl_2$(pH 7) or ≤50% propylene glycol may be useful.
  Combinations of these components can be used to optimize elution conditions.
- Use an elution buffer strength greater than the equilibration buffer strength to ensure a good pH transition.
- Use a step elution to obtain a concentrated elution fraction, then a gradient if additional separation for very similar product impurities is needed.
- Do not underload the column. A load significantly below the maximum binding capacity can hamper efficient release due to re-binding events during elution causing poor recovery.
- Immediately neutralize the eluted pool to prevent denaturation of some molecules at low pH. When selecting buffer systems, consider molecule stability, binding optimization for the next step, and the ability of the buffer to control pH in the desired operating range.

Resin cleaning and storage

Column cleaning and lifetime study considerations

To avoid contaminant buildup and to ensure long column lifetime, clean the column in place (CIP) after every run.

In any cleanup method, reversing the flow direction is recommended to help flush out particulates and to prevent contamination of the lower part of the bed. Also, slow the flow rate to give several minutes' exposure to the regeneration solution at each step of the cleaning protocol.

The resin is acid stable and has limited caustic stability. For cleaning, use solutions in the range of pH 2–12. Test cleaning solutions in this order, then optimize cleaning based on results:

- Elution buffer titrated to a lower pH (target pH 1.5–2.0)
- Elution buffer titrated to a lower pH plus 1–2 M NaCl
- 0.1–0.5 M citric acid
- 0.5–1.0 M acetic acid
- 0.5 M phosphoric acid
- 6 M urea
- 2–6 M guanidine hydrochloride
- 20% ethanol (with or without acid)
- 20% isopropanol (with or without acid)
- 10–25 mM NaOH

A typical cleaning procedure is:

1. Strip with 0.1 M phosphoric acid (pH 2.0).
2. Clean with 6 M guanidine hydrochloride.
3. Re-equilibrate with neutral pH buffer such as PBS, pH 7.5.
   or
   Store in buffered ethanol.

Resin storage guidelines

- Store bulk resin at 2 to 8°C. Do not freeze.
- Store packed columns at 2 to 8°C (long-term) or room temperature (short-term) after cleaning (described above) in a neutral-pH solution with a bacteriostatic agent such as 0.1 M sodium phosphate (pH 7.0) with 20% ethanol.

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

Troubleshooting

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 resin in the column before packing.
	Resin allowed to freeze	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 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.
	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 is overpacked or packed inconsistently	Repack the column following the recommended procedure.
	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.

Observation	Possible cause	Recommended action
Column qualification – low asymmetry *(continued)*	Column 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 allow for dilution of the HETP pulse solution	• Characterize an acetone (UV) or salt (conductivity) 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.
Decreased performance: • Increased bandspreading • Decreased binding capacity • Decreased recovery • Increased pressure drop • Trace or "ghost" peaks during blank runs	Column fouling can occur due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities	Clean the column.

Ordering information

POROS™ resins	Cat. No.	Amount
AAV8	A30795	10,000 mL
	A30794	5,000 mL
	A30793	1,000 mL
	A30792	250 mL
	A30791	50 mL
	A30790	25 mL
	A30789	10 mL
AAV9	A27357	10,000 mL
	A27358	5,000 mL
	A27359	1,000 mL
	A27355	250 mL
	A27356	50 mL
	A27353	25 mL
	A27354	10 mL
AAVX	A36745	10,000 mL
	A36744	5,000 mL
	A36743	1,000 mL
	A36742	250 mL
	A36741	50 mL
	A36740	25 mL
	A36739	10 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

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Revision history: Pub. No. 100038399

Revision	Date	Description
E	22 December 2017	Add AAV products.
D	13 August 2017	Add AAV products, change document title, update procedures with latest information.
C	24 February 2017	Change regulatory statement
B	26 April 2016	• Change regulatory statement • Add before first use task to optimize chromatography conditions
A	January 2016	New document.

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