CaptureSelect™ Affinity Matrices – Column packing guidelines

Pub. No. MAN0009645 **Rev.** B.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

CaptureSelect™ Affinity Matrices (resins) are based on highly cross-linked agarose beads with an average particle size of 65 µm.

Pressure-flow curves are shown below.

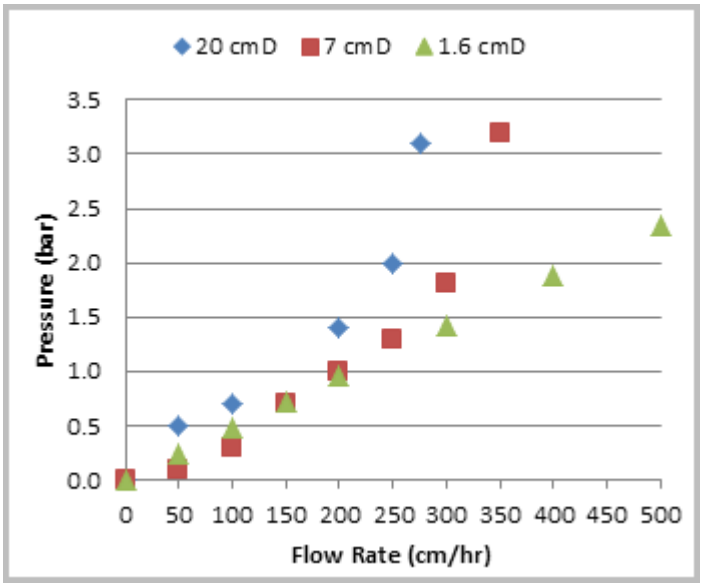

Figure 1 Typical pressure-flow curve of CaptureSelect™ resin at different column diameters

Column formats:	20 cmD x 22 cmL, 7 cmD x 21 cmL, 1.6 cmD x 22.5 cmL
Packing pressure:	3 bar
Mobile phase:	Water

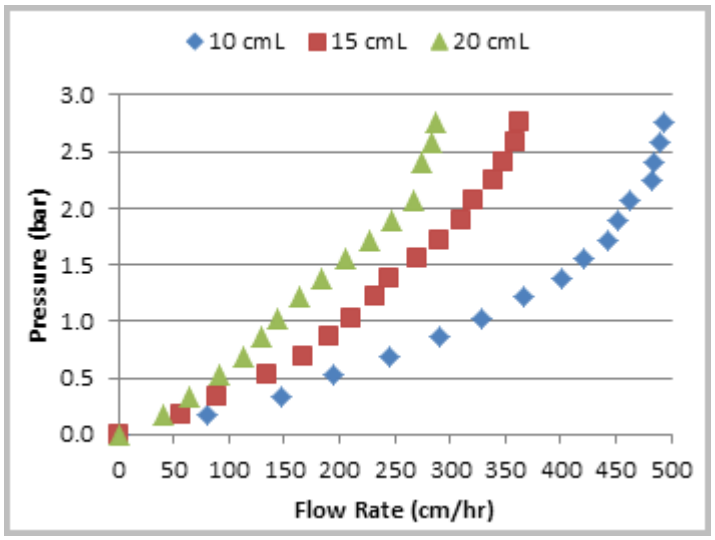

Figure 2 Typical pressure-flow curve of CaptureSelect™ resin with increased bed height

Column diameter:	10 cm
Packing pressure:	3 bar
Mobile phase:	0.1 M NaCl

Packing considerations

- Resins are supplied as approximately 50% slurry in 20% ethanol.
- For column packing, exchange the 20% ethanol shipping solution with packing solution. Recommended packing solutions are water or 0.1 M NaCl.
- Packing factors are typically in the range of 1.1–1.3 at a 15-20% compression with packing pressures up to 2.5–3 bar. Packing factors for smaller diameter columns may be lower. The packing factor accounts for the difference in bed volume between a gravity-settled bed and a 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).
- Packing flow rates of at least 50% greater than the maximum process flow are recommended, if achievable within system pressure limitations.
- Standard 10–23 µm screens (frits) can be used.

Prep the slurry

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

   As with any resin, do not use a magnetic stirrer. It may 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 40–60% 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 40–60%.

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.

1. Determine the required slurry volume:

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

   25 L / 0.50 × 1.17 = 58.5 L slurry required

   Optimize the packing factor as needed.

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.

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

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.

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

Recommended column qualification conditions

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").

Condition	Recommendation
Flow rate	Target operating flow rate (cm/hour)
Equilibration buffer	Water or 0.1 M NaCl
Plug solution	2–5% acetone in equilibration buffer or 1.0 M in 0.1M NaCl
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 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 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.

Qualification example

Figure 3 shows a typical column qualification peak.

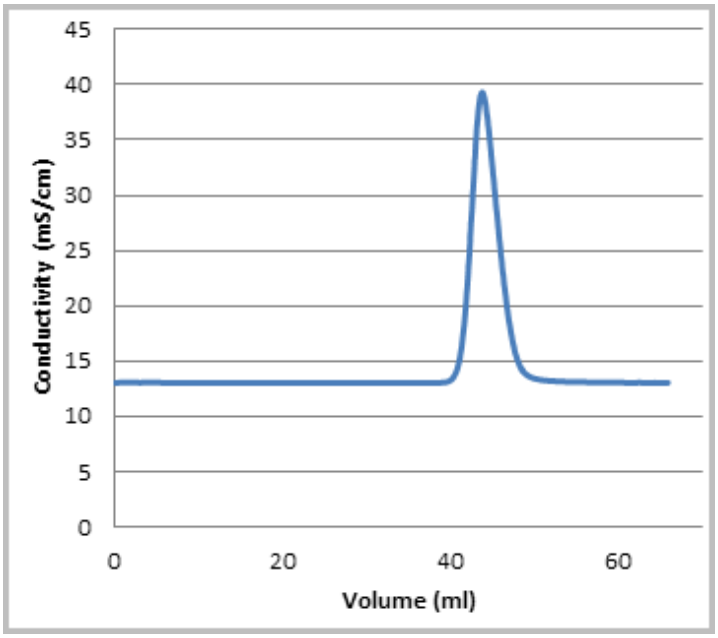

Figure 3 CaptureSelect™ column qualification

Column format:	1.6 cmD x 22 cmL
Equilibration buffer:	0.1 M NaCl
Injection:	2% CV 1M NaCl
Flow rate:	100 cm/hr

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.

Observation	Possible cause	Recommended action
High backpressure *(continued)*	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 HETP pulse solution	• Characterize an acetone 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.
	Pulse injection method is not optimized	Verify that the desired plug volume 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	Column is overpacked or packed inconsistently	Repack the column following the recommended procedure.
Column qualification – low plates or high HETP	The system and plumbing allow for dilution of the HETP pulse solution	• Characterize an acetone 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.

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.

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