Instructions for Use

HyperCel STAR AX

Salt Tolerant Advanced Recovery Anion Exchange Chromatography Resin

1 Product Description

HyperCel STAR AX resin is an industry-scalable ion exchanger designed for high productivity protein purification in broad feedstock conductivity operating conditions, avoiding or limiting unit operations like dilution or ultrafiltration | diafiltration (UF | (DF). The resin can be used for direct protein capture from undiluted feedstocks (e.g., mammalian cell culture supernatants, plasma) or for early contaminant removal in multi-step purifications (i.e., for host cell protein clearance before an affinity capture).

HyperCel STAR AX resin provides:

- High dynamic binding capacity at short residence time (2 minutes or lower)
- Salt "tolerance": dynamic binding capacity is less affected by conductivity changes in a broad feedstock conductivity range (2 to 15 mS/cm), in contrast to conventional anion exchangers
- Excellent flow rate properties
- Fast re-equilibration allowing buffer and time savings

The resin is also available in other configurations: in PRC prepacked columns for rapid selectivity screening under reliable and reproducible conditions and in miniaturized RoboColumn®* for fully automated and parallel chromatographic separations.

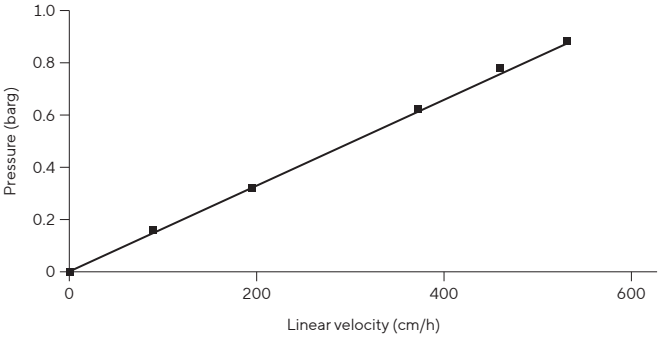
2 Properties

Average particle size (µm)	60 – 90
Capacity for BSA (mg/mL)[1]	> 100 mg/mL within pH 7.5 – 8.0 (conductivity 2 – 15 mS/cm)
Working pressure at 1,000 cm/h[2]	< 1.5 bar
Working pH	Binding: pH 7.0 – 8.5; Elution: pH 3.0 – 8.5
Cleaning pH	1 – 4
Cleaning in place	0.5 – 1 M NaOH, 1 hour contact time

[1] Dynamic binding capacity at 2 minutes residence time, using 5 mg/mL BSA in Tris-HCl, pH 8.5, supplemented with NaCl to vary conductivity.

[2] Determined using 50 mM Tris-HCl, pH 8.5 on an I.D. 15 lab scale column.

Figure 1: Pressure vs. Flow Rate



Column: 200 mm I.D. x 150 mm bed height. Packing Buffer: 10 mM NaCl.

3 Column Packing

3.1 General Considerations

HyperCel STAR AX resin is supplied as a slurry | suspension in 1 M NaCl containing 20% (v/v) ethanol or as a moist cake for process-scale applications (the moist cake resin facilitates the resin transfer, avoiding the agitation and suspension of large material volumes). For process-scale column packing support, contact Technical Service.

3.2 Packing a Small Column (≤ 5 cm I.D.)

HyperCel STAR AX resin is compatible with traditional low or medium pressure chromatography columns and equipment. For preliminary laboratory studies, a column equipped with an adjustable piston facilitates optimal packing. Direct scale-up is accomplished by maintaining bed height constant while diameter is increased. We recommend a chromatography system able to deliver a linear flow rate of 1,000 cm/h (600 cm/h minimum for packing operations).

3.2.1 Preparing the Resin Slurry

⚠ **Do not use stainless steel paddles or magnetic stirrers as they may damage the beads and create fine particles.**

1. Gently agitate the container to fully suspend the resin.
2. Depending on the desired bed volume, transfer a suitable volume of slurry to a graduated beaker including a "practical" excess of resin (20 to 25% of the desired bed volume are recommended).
3. Allow the resin to settle and remove the supernatant. Add 3 to 5 volumes of packing buffer (10 mM NaCl).
4. Gently agitate the slurry and let the resin settle. Remove the supernatant and add 3 to 5 volumes of fresh packing buffer.
5. Repeat step 4 a total of 3 times minimum.
6. Gently agitate the slurry, pour it into a measuring cylinder and allow the resin to settle.
7. Remove the supernatant and add a volume of packing buffer equal to one-half the volume of settled resin. A slurry of 50% (v/v) — the concentration recommended for packing — is obtained.

Optional: The final slurry concentration can be estimated by centrifuging between 20-g and 200-g, about 10 mL of homogenized slurry in a graduated vial tube for 5 minutes. The concentration of slurry expressed as: **[Resin Volume | Total Volume* x 100]** can be directly estimated in the tube. The 10 mL slurry can be poured back into the total suspension before packing.

* Total Volume = Resin + Supernatant

3.2.2 Preparing Column and System for Packing

1. Prime the solvent delivery system, lines and valves to assure that all air is displaced. Assure that top and bottom frits or nets are fully wetted and free of air. Fill the column with packing buffer and operate the system over a range of flow rates representative of values that will be used during packing and anticipated chromatographic procedures. Record pressure associated with the empty column and system (including detectors, etc.).
2. Determine pressure | flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatographic operation.
3. Stop the pump, close the column outlet, and remove the upper flow adaptor or piston.
4. Open the outlet and drain buffer from the column, leaving 1 to 4 cm of buffer above the bottom frit.

Following completion of blank pressure | flow measurements, proceed with column packing as described in Section 3.2.3.

3.2.3 Packing the Column

1. Gently resuspend the slurry and pour it into the column in one contin uous motion against the wall of the glass tube to minimize introduction of air bubbles (if the pouring process is done in several motions, gently homogenize the slurry in the column using a plastic rod).
2. Fill with packing buffer up to the top of the glass tube. Allow the suspension to settle so that a layer of clear supernatant ≤ 1 cm is visible at the top of the column.

3. Connect the upper adjustable piston to the system and prime with packing buffer to ensure that no air is trapped under the frit. Stop the pump and insert the adjustable piston into the column. Tighten it.
4. Open the column outlet and operate the pump at a selected linear velocity of 1,000 cm/h (~13.1 mL/min for a 1 cm I.D. column) for efficient packing. In any case, flow rate must not be lower than 600 cm/h for guaranteeing high packing performance.
5. When the top of the bed stabilizes, stop the pump and untighten the piston. Position the adjustable piston at the top of the packed resin by turning the screw-lock mechanism, leaving no visible space between the frit and packed resin at any point around the circumference. Re-tighten the O-ring seal.
6. Operate the pump again and repeat the adjustment of the piston until no visible space appears under flow (1,000 cm/h) between the frit and the top bed.

3.2.4 Evaluating Column Performance

Column performance is evaluated by determining column efficiency, expressed as either plates/meter (N/m), or HETP (height equivalent to one theoretical plate). Additionally, the asymmetry factor (AF) is calculated. Required formulas are shown below. Measurements are made as follows:

1. Equilibrate a column of 15 cm I.D.x 10 cm length with equilibration buffer (i.e., 50 mM Tris-HCl pH 8.5).
2. Inject 1% column volume (CV) of 5% acetone solution. Apply a flow rate of 100 cm/h. Record UV absorbance (280 nm).

To determine the packing performance, use the following formulas:

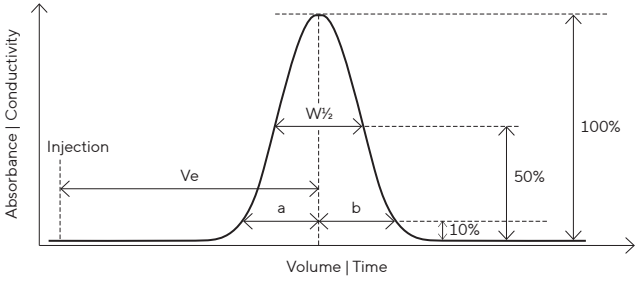
$$N/m = \frac{5.54 \times 100 \times (Ve / W½)^2}{BH}$$

With:
N = Number of theoretical plates
Ve = Elution volume on the chromatogram (cm)
W½ = Width of the acetone peak at half of the height (cm)
BH = Bed height (cm)

$$AF = \frac{b}{a}$$

With:
b = Right section of the peak at 10% peak height
a = Left section of the peak at 10% peak height

Figure 2: Peak Trace in a Typical Test Evaluation of Column Performance



"a" and **"b"** are respectively first and second half peak width at 10% of peak height. Typical values for HyperCel STAR AX resin N/m range between 1,500 and 3,000 plates/m. Typical values for asymmetry factor range from 0.8 to 1.8 at 10% of peak height. These values are given as the average of experimental values. More important than the values by itself, the reproducibility of the values over the successive packing operations is critical.

3.3 Packing Pilot-scale (> 5 cm I.D.) or Manufacturing Columns

Please contact Sartorius Technical Service.

4 Working Conditions and Basic Protocol

4.1 Equilibration

1. After column packing, wash with 1.5 CV of a buffer of the same composition as that chosen for equilibration (i.e., 25–50 mM Tris-HCl, pH 7.0–8.5).
2. Continue to equilibrate the column until the ionic strength and pH of the buffer at both the outlet and the inlet of the column are identical.

4.2 Sample Application

⚠ **HyperCel STAR AX resin is based on primary amine groups; the use of multivalent buffers such as phosphate containing buffers can impact the dynamic binding capacity of the resin. For optimal results it is therefore recommended not to use such buffers with a molarity higher than 20 mM.**

1. Ensure the absence of bubbles in the sample.
2. Inject the sample into the column through a pump and then connect the pump to a buffer reservoir or to a gradient system.
3. Start the pump, the recorder and the elution gradient maker simultaneously.

4.3 Working Flow Rate

Dynamic binding capacity may slightly vary as a function of residence time. For a 10 cm bed height column, use a flow rate of 300 cm/h, 2 minutes residence time. Higher flow rates can be used (600 cm/h, 1 minute residence time).

4.4 Choice of Elution Gradient and its Slope

For preliminary studies, a 20 CV linear NaCl gradient (0 to 1 M) is often helpful in determining the best separation conditions. Once determined, optimal elution conditions can be achieved through adjustments to the NaCl concentration, gradient slope and sample load.

Elution prompted by pH: in certain cases, elution can be driven without addition of salt, by just decreasing the pH (e.g., pH 4.0).

Note that wash and elution buffers pH and conductivity can impact both yield and purity of the target protein in a complex environment.

5 Regeneration and Cleaning

Regenerate the column with 2 to 4 CV of 1 to 2 M NaCl, followed by a cleaning-inplace (CIP) with 5 CV of 1 M sodium hydroxide, 60 minutes contact time at room temperature. In some cases, 5 CV of 0.1 M HCl can also be used after NaCl regeneration, followed by 1 M NaOH for efficient regeneration.

After CIP, neutralize the column with 2 to 3 CV of a concentrated buffer (e.g., 200 mM Tris-HCl) to prepare for the next cycle, and pump 2 to 3 CV of storage solution onto the column before putting a packed column into storage between campaigns.

6 Thermal Stability and Storage

Temperature of use	2 – 30 °C
Storage temperature	2 – 30 °C (2 – 8 °C once opened)
Storage solution between runs	Neutral buffer containing 1 M NaCl and 20% (v/v) ethanol
⚠	Product must never be frozen
i	Product is shipped at ambient temperature

7 Ordering Information

HyperCel STAR AX Bottled Resin

Pack Size	Part Number
25 mL	20197-026
100 mL	20197-032
1 L	20197-046
5 L	20197-058
10 L	20197-064

Prepacked Columns

Description	Part Number
PRC Columns	
PRC Column 5 x 50 HyperCel STAR AX, 1 mL, 1/pkg	PRCSTARAX1ML
PRC Column 8 x 100 HyperCel STAR AX, 5 mL, 1/pkg	PRCSTARAX5ML
RoboColumn®*	
RoboColumn®* HyperCel STAR AX 200 µL, row of 8	SR2STARAX
RoboColumn®* HyperCel STAR AX 600 µL, row of 8	SR6STARAX

* RoboColumn is a trademark of Repligen GmbH

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