SARTORIUS

Instructions for Use

CMM HyperCel Cation Exchange

Mixed-Mode Resin

1 Product Description

CMM HyperCel resin is a novel industry-scalable chromatography cation exchange mixed-mode resin designed for high productivity protein capture and process-scale protein separations. It complements current anion exchange mixed-mode resin (MEP, HEA, PPA HyperCel) and offers a different selectivity for a broad range of applications such as the purification of antibodies, antibody fragments, recombinant proteins in bind | elute and flow through modes.

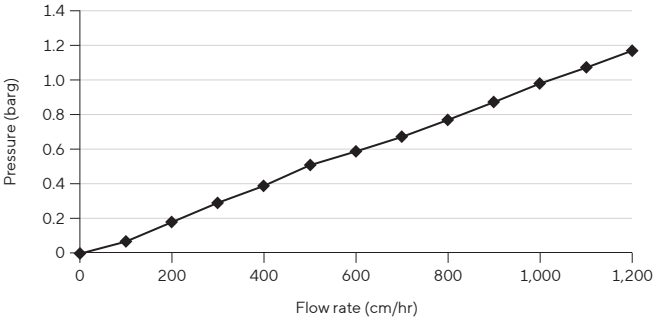
2 Properties

CMM HyperCel resin is designed from HyperCel-based matrix for robust large scale processing of various fermentation or cell culture feedstreams. The resin provides extended selectivity to separate proteins with similar pI and | or hydrophobicity, high dynamic binding capacity for capture of target protein in complex feedstocks, high yield of recovery, and low elution volume (salt or pH-based elution).

Particle size	50 – 80 µm
Ligand	Aminobenzoic acid
Dynamic binding capacity[1]	> 50 mg BSA/mL at pH 4.5, conductivity 15 mS/cm
Working Conditions	
Binding	pH 4.0 to 6.0 — Conductivity up to 50 mS/cm[2]
Elution	pH 7.0 to 9.0 — Conductivity up to 50 mS/cm[2]
Working pressure at 1,000 cm/hr[3]	~1.0 barg (14 psig)
Clean-in-place	Up to 1 M NaOH — 1 hour contact time

[1] 4 g/L BSA in 50 mM Na acetate complemented with NaCl, 7 minutes residence time.
[2] Conductivity adjustment with NaCl (~0 to 0.5 M).
[3] Determined using 50 mM Na acetate, pH 5.0 on laboratory scale column of 15 mm I.D. x 200 mm length

Figure 1: Pressure vs. Flow Rate



Column: 15 mm I.D. x 170 mm bed height.
Packing buffer: 50 mM Na acetate, pH 5.0.

3 Column Packing (for Columns up to 15 mm I.D.)

CMM HyperCel resin is supplied as a slurry | suspension in 1 M NaCl containing 20% (v/v) ethanol. Example for a laboratory column of 10 mm I.D. x 200 mm length.

3.1 Packing Solution

Packing Solution resin is compatible with a broad range of aqueous solutions for packing. The resin can be packed in water, in low concentration (10 mM) NaCl solution or in standard cation exchange equilibration buffer (Na acetate, pH 4.0 to 5.0). This offers many options to prepare the column according to customer's process.

3.2 Slurry Preparation

1. Drain 20 to 40 mL of suspended resin on a frit to remove the storage solution. A moist cake of 15 to 35 mL of drained resin is obtained.
2. Weigh this moist cake resin and add the same weight of packing solution, to obtain a 50/50 (w/w) slurry preparation.

3.3 Packing the Column

1. Homogenize the slurry and pour into the column. Complete with packing solution.
2. Start the pump with the pre-selected 500 cm/hr linear velocity (~6.5 mL/min for a 10 mm I.D. column), then open the column outlet and insert the adjustable piston and tighten it. Wait until the packing solution above the packed resin is clear. Maintain these conditions during 10 minutes.
3. Increase the linear velocity to 1,000 cm/hr (~13 mL/min for a 1 cm I.D. column) and maintain this flow rate during 10 minutes.
4. Untighten the adjustable piston at the top of the packed resin. Apply again a flow rate of 1,000 cm/hr for a few minutes.
5. When the bed height stabilizes, make a final adjustment of the piston until the piston flushes the packed resin, leaving no visible space between the frit and packed resin at any point around the circumference.

3.4 Packing the Column

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 10 mm I.D. x 10 cm bed height with packing buffer (i.e., 10 mM NaCl).
2. Inject 100 µL of 5% acetone solution. Apply a flow rate of 100 cm/hr. Record UV absorbance (280 nm) and conductivity.

To determine the packing performance, use the following formulas:

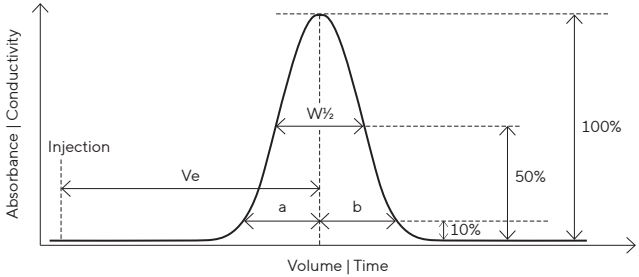
$$N/m = \frac{5.54 \times 100 \times (Ve / W½)^2}{BH}$$

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:
a = First half peak width at 10% peak height
b = Second half peak width 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 CMM HyperCel resin N/m at 100 cm/hr are 4,500 – 5,000 plates/m. Typical values for AF at 100 cm/hr range from 1.0 to 1.5 at 10% of peak height.
These values are given as the average of experimental values.

4 Working Conditions and Basic Protocol

4.1 Equilibration

1. After column packing, wash with 1.5 CV of an equilibration buffer (i.e., 25 to 50 mM Na acetate, pH 4.0 to 6.0). Adjust with NaCl solution according to the conductivity of the sample.
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

CMM HyperCel resin can accommodate significant conductivity (up to 50 mS/cm) for the capture of antibody and non-antibody proteins in complex feedstocks.

However, if increased capacity is required, sample conductivity may be reduced by dilution or buffer exchange before application on the column.

4.3 Working Flow Rate

Dynamic binding capacity will vary as a function of residence time. For a 10 cm bed height column, use a flow rate of 85 to 120 cm/hr, 5 to 7 minutes residence time. Higher flow rates can be used (200 cm/hr, 3 minutes residence time).

4.4 Choice of Elution Gradient and its Slope

For preliminary studies, a 20 CV linear pH gradient (from 4.0 to 9.0) is often helpful in determining the best separation conditions. Once determined, optimal step elution conditions can be achieved through adjustments of pH and |or, conductivity (addition of NaCl).

5 Regeneration and Cleaning

Regenerate the column with 2 to 4 CV of 1 to 2 M NaCl, followed by a cleaning-in-place (CIP) with 5 CV of 1 M sodium hydroxide, 60 minutes contact time at room temperature.
After CIP, neutralize the column with 2 to 3 CV of equilibration buffer (e.g., 50 mM Na acetate, pH 5.0) to prepare for the next cycle, and pump 2 to 3 CV of storage solution (1 M NaCl and 20% (v/v) ethanol) 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 – 8 °C
Storage solution between runs	1 M NaCl with 20% (v/v) ethanol
⚠	Product must never be frozen. Avoid long exposure to light.
i	Product is shipped at ambient temperature

7 Ordering Information

Description	Part Number
CMM HyperCel 25 mL	20270-025
CMM HyperCel 100 mL	20270-031
CMM HyperCel 1 L	20270-041
CMM HyperCel 5 L	20270-055
CMM HyperCel 10 L	20270-066
PRC Column 5 x 50 CMM HyperCel 1 mL	PRCCMMHCEL1ML
PRC Column 8 x 100 CMM HyperCel 5 mL	PRCCMMHCEL5ML
RoboColumn®* CMM HyperCel 200 µL, Row of 8	SR2CMM
RoboColumn®* CMM HyperCel 600 µL, Row of 8	SR6CMM

* RoboColumn is a trademark of Repligen GmbH

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