

CMM HyperCel Mixed-Mode Resin



Product Information

CMM HyperCel resin is composed of a rigid cellulose matrix that has flow properties compatible with the needs of manufacturing scale protein production. It is a high-performance, industry-scalable cation exchange Mixed-Mode Resin designed for efficient capture and impurity removal at moderate conductivity.

Features and Benefits

- Superior separation performance, typical of a mixed-mode resin, without the traditional limitations commonly associated with this resin class:
 - Ability to separate proteins with similar isoelectric point and | or hydrophobicity at low or high conductivity
 - High dynamic binding capacity over repeated purification cycles
 - High recovery, low elution volume
 - Easy regeneration
- Designed for capturing monoclonal antibodies (MAbs), Fab fragments and recombinant proteins from challenging samples

Introduction

The proprietary ligand (Figure 1), containing both a primary amine and a carboxyl group, confers cation exchange and hydrophobicity properties to the chromatography resin. At working pH (4 to 9), the amine group is never charged ($pK_a < 4$). The carboxyl group is weakly charged at adsorption pH (4 to 6) to allow protein adsorption based on hydrophobicity.

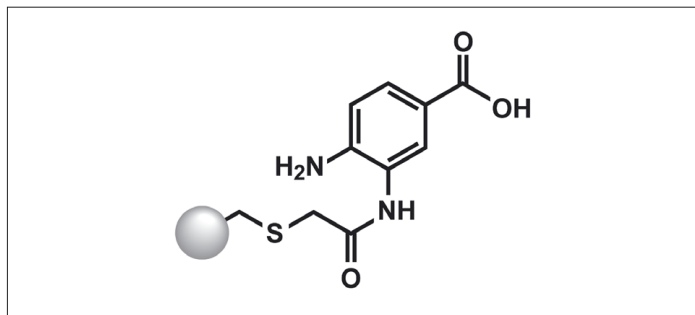
At elution pH (7 to 9), the carboxyl group is fully deprotonated and the elution will be based on negative charge repulsion. The flexibility of the ligand enables the separation of proteins with a large variety of isoelectric points and hydrophobicity levels, and multiple conditions can be employed to separate targeted molecules from contaminants.

The resin is available in a variety of configurations: 200 and 600 μL RoboColumns^{®*} for initial resin screening, and convenient 1 mL and 5 mL PRC prepacked columns for rapid method optimization, selectivity screening or small preparative work.

CMM HyperCel resin is also 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.

CMM HyperCel resin has a chemical stability that ensures simple clean-in-place (CIP) and storage. For standard CIP, 0.5 to 1 M NaOH treatment is recommended, while long-term storage in 10 to 100 mM NaOH is possible.

Figure 1: Chemical Structure of CMM HyperCel Ligand



Technical Specifications

Table 1: Main Properties of CMM HyperCel

Particle size range	50 – 80 μm
Ligand description	Aminobenzoic acid
Ligand density	Av. 70 $\mu\text{eq/mL}$
Dynamic binding capacity	<ul style="list-style-type: none">▪ BSA¹▪ IgG² <ul style="list-style-type: none">▪ > 50 mg/mL at pH 4.5, 15 mS/cm▪ > 60 to 100 mg/mL at pH 4.0 to 5.0, 4 to 12 mS/cm
Working conditions	<ul style="list-style-type: none">▪ Binding▪ Elution <ul style="list-style-type: none">▪ pH ~4 to 6; conductivity up to 50 mS/cm³▪ pH ~4 to 9; conductivity up to 50 mS/cm³
Working pressure at 1,000 cm/hr ⁴	at ~ 1.0 bar g
Working pH	2 to 13
Cleaning pH	1 to 14
Cleaning in place	1 M NaOH – 1 hour contact time – 5 CV

¹ 4 g/L BSA in 50 mM Na acetate complemented with NaCl, 7 minute residence time

² 5 g/L IgG in 50 mM Na acetate complemented with NaCl, 2 minute residence time

³ Conductivity adjustment with NaCl (~0 to 0.5 M)

⁴ Determined using 50 mM Na acetate, pH 5.0 on laboratory scale column of 15 mm I.D. x 200 mm length.

Features and Benefits

High Selectivity to Separate Proteins with Similar Isoelectric Point and/or Hydrophobicity

The ability to separate acidic (e.g., ovalbumin) from basic proteins (e.g., MAbs), along with the power to separate hydrophobic proteins (e.g., MAbs) from more hydrophilic proteins (e.g., β -lactoglobulin), illustrates the powerful selectivity of CMM HyperCel resin for a broad range of molecules.

Figure 2: Protein Classification as a Function of Their Isoelectric Point and Hydrophobicity

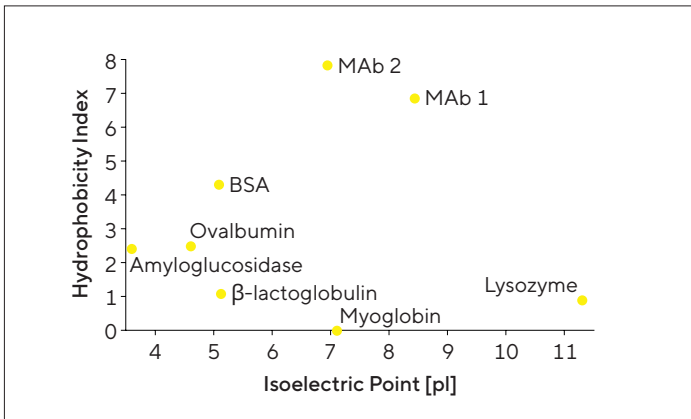


Figure 3: Separation of a Mixture of Proteins on CMM HyperCel Resin

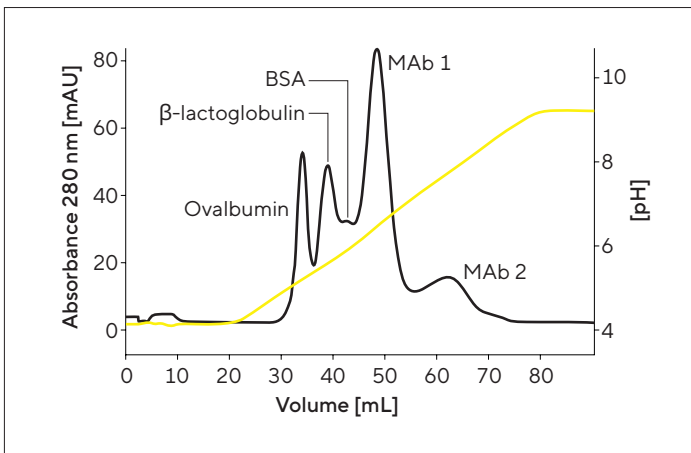
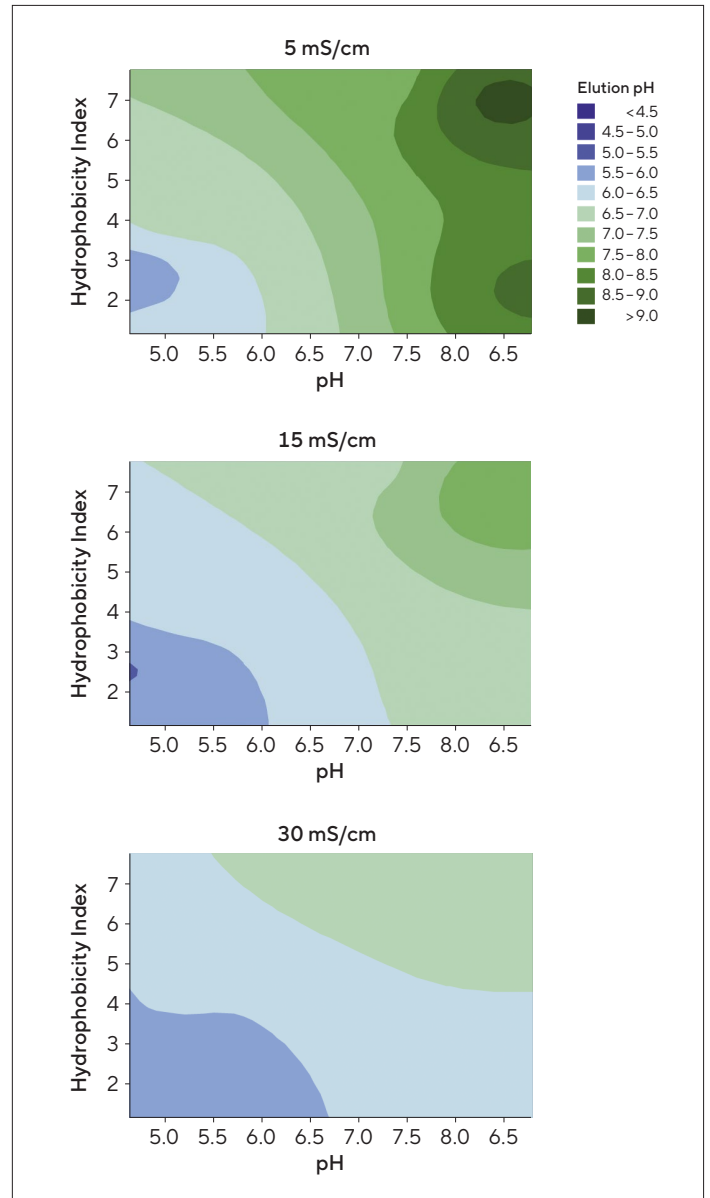


Figure 4: Contour Plots of Elution pH as a Function of pI and Hydrophobicity of the Model Proteins



A detailed study was performed to understand the selectivity of CMM HyperCel resin as a function of the binding conductivity. The six chosen model proteins were loaded as a mixture on a 1 mL column. The elution was performed with a mix of buffers to provide a near linear pH gradient from 4.5 to 10, at three conductivities: 5, 15 and 30 mS/cm.

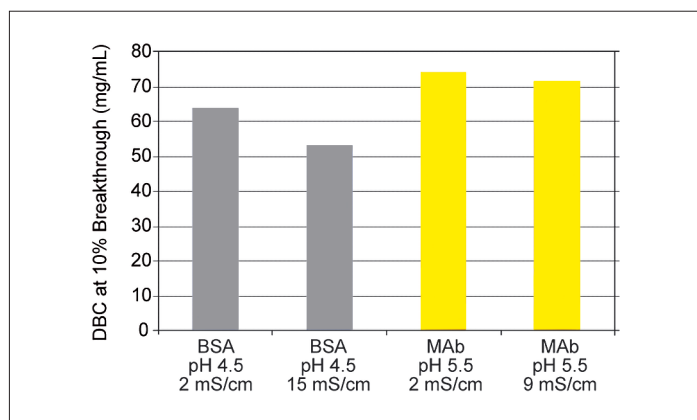
Figure 4 shows the contour plots of pH elution as a function of the isoelectric point and hydrophobicity of the proteins. The data shows the resin is still able to separate proteins at high conductivity, expanding the operating range of the resin.

High Binding Capacity for Protein Capture

Mixed-mode resins are well-known to be able to resolve purification challenges which cannot be solved by ion exchangers, this is normally at the expense of reduced capacity. However, CMM HyperCel resin demonstrates capacity performance competitive with other chromatography technologies.

Figure 5 shows dynamic binding capacities (DBC) for two pure molecules (BSA, MAb) at two different conductivities. The DBC is higher than 60 mg/mL for both cases and remains high, even at 15 mS/cm for MAb, which facilitates process integration without the need for buffer exchange or dilution before loading. The pH for binding and elution is compatible for maintaining the integrity of MAb.

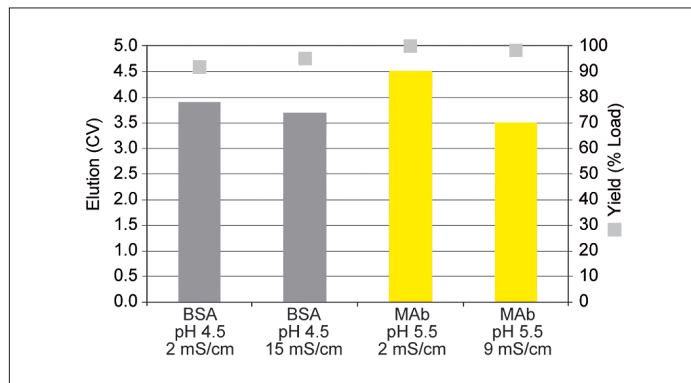
Figure 5: Dynamic Binding Capacity for Two Pure BSA and MAb at Two Conductivities



Column (0.5 cm ID × 7 cm)

High Recovery and Low Elution Volume

Figure 6: Recovery and Elution Volume Obtained on CMM HyperCel Resin

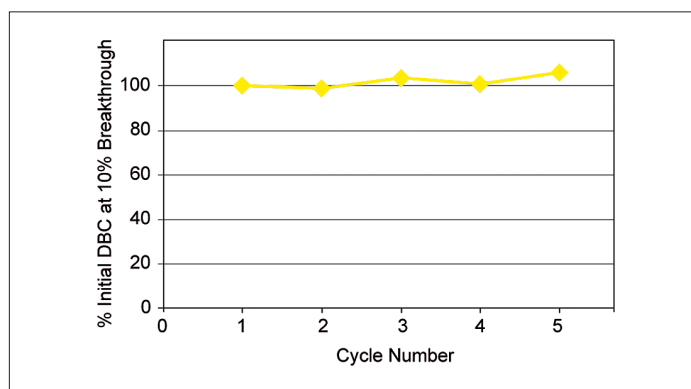


BSA and MAb elution was performed using 50 mM Tris at pH 7.5, 15 mS/cm and pH 8.5, 30 mS/cm respectively. The recovery was higher than 90% (even with elution volumes below 4 CV) for both BSA and MAb when loaded at 15 and 9 mS/cm.

Efficient Regeneration for a Long Service Life

To test the efficiency of regeneration, MAb was purified from a clarified CHO cell culture supernatant. Five full purification cycles were performed. After each elution, the chromatography resin was regenerated with 1 N NaOH (1 hour contact time) and DBC at 10% breakthrough was tested. The DBC remained unchanged, confirming the efficient cleaning of the resin.

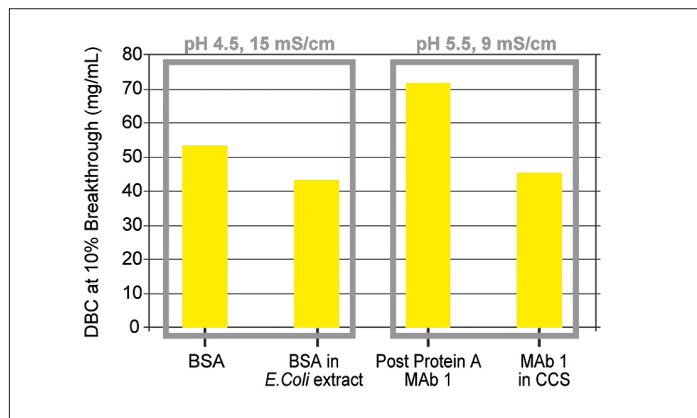
Figure 7: MAb Dynamic Capacity Binding Over Cycles



Application

Capture from a Complex Feedstock

Figure 8: Protein Capture from *E. coli* and CHO Supernatant on CMM HyperCel Resin



Data presented in Figure 8 confirms the high capacity obtained from a pure protein (BSA) or a post Protein A MAb at medium or high conductivities. This reflects the capacity that can be expected when the resin is applied to a polishing application.

High capacities are also maintained under challenging conditions, such as clarified CHO cell culture supernatant and *E. coli* extract.

Ordering Information

Description	Part Number
Bottled Resin	
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
Columns	
PRC Prepacked Column 5 × 50 CMM HyperCel, 1 mL	PRCCMMHCEL1ML
PRC Prepacked Column 8 × 100 CMM HyperCel, 5 mL	PRCCMMHCEL5ML
RoboColumn® CMM HyperCel 200 µL, row of 8	SR2CMM
RoboColumn® CMM HyperCel 600 µL, row of 8	SR6CMM

Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

 **For more information, visit**
[sartorius.com](https://www.sartorius.com)

Specifications subject to change without notice.

©2024 Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany

DIR: 2578758-000-02

Status: 06 | 2024