

# SARTORIUS

Instructions for Use

## MEP HyperCel

### Mixed-Mode Chromatography Resin

\* RoboColumn is a trademark of Repligen GmbH.

\*\* Tween is a trademark of CRODA AMERICAS LLC.

\*\*\* Triton is a trademark of Union Carbide Corporation.

## 1 Product Description

MEP (mercapto-ethyl-pyridine) HyperCel resin is a high capacity, high selectivity resin designed for the capture and purification of various recombinant proteins as well as monoclonal and polyclonal antibodies.

MEP HyperCel ligand shows selectivity for antibodies from a broad range of sources, such as animal sera, ascites fluids and cell culture supernatants.

A variety of cell culture formulations are readily accommodated, including protein-free, albumin-supplemented and serum-supplemented media. In contrast to Protein A resins, IgG binding capacity on MEP HyperCel resin is essentially independent of subclass or species. "Weakly binding" variants (e.g., murine IgG1, rat IgGs) are well retained. Therefore, MEP HyperCel resin can be used for the capture of antibodies, but also for intermediate step purification or polishing (aggregate removal), in combination with other conventional techniques (e.g., ion exchange or following protein A affinity...)

MEP HyperCel resin can also be an excellent alternative to traditional hydrophobic interaction chromatography (HIC) resins for the capture of hydrophobic molecules which are non antibody proteins, or for impurity removal.

MEP HyperCel resin provides significant benefits at both laboratory and process-scale. 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	90 $\mu\text{m}$
Bead composition	High porosity cross-linked cellulose
DBC for hu IgG <sup>(1)</sup>	$\geq 20$ mg/mL
Ligand	4-Mercapto-Ethyl-Pyridine (4-MEP)
Ligand density	80 – 125 $\mu\text{mol/mL}$
Adsorption pH	7.0 – 9.0
Elution pH	4.0 – 5.8
Cleaning pH, short term <sup>(2)</sup>	2 – 14
Working pH, long term	2 – 12
Pressure resistance	< 3 barg (44 psig)
Typical working pressure	< 1 barg (14 psig)

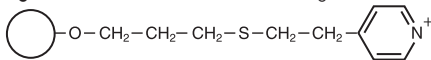
<sup>(1)</sup> Dynamic binding capacity determined using 5 mg/mL human polyclonal IgG in PBS, flow rate: 70 cm/h (6 min residence time), 10% breakthrough

<sup>(2)</sup> Less than 8 hours.

## 3 Technical Overview

MEP HyperCel resin operates by a mixed-mode mechanism also described as hydrophobic charge induction chromatography (HCIC). HCIC is based on the pH-dependent behavior of ionizable, dual-mode ligands. The structure of the 4-MEP ligand is shown in Figure 1.

Figure 1: Chemical Structure of MEP Ligand



## 4 Column Packing

### 4.1 General Considerations

MEP HyperCel 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.

### 4.2 Packing a Small Column ( $\leq 5$ cm I.D.)

MEP HyperCel resin is compatible with traditional low or medium pressure chromatography columns and equipment. For preliminary laboratory studies, a column equipped with an adjustable piston, of dimensions such as 1 cm I.D. x 20 cm length 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).

#### 4.2.1 Preparing the Resin Slurry

1. Gently agitate the container to fully suspend the resin. Do not use magnetic stirrers at any point in the procedure.
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 is recommended).
3. Allow the resin to settle, and remove the supernatant. Add 3 to 5 volumes of packing buffer (equilibration buffer).
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 three 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 67% (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

#### 4.2.2 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 on 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 4.2.3.

#### 4.2.3 Packing the Column

1. Gently resuspend the slurry and pour it into the column in one continuous 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 (i.e., equilibration buffer such as PBS, pH 7.4) up to the top of the glass tube. Allow the suspension to settle so that a layer of clear supernatant  $\leq 1$  cm is visible at the top of the column.
3. Connect the upper piston to the system and prime with packing buffer to ensure that no air is trapped under the frit. Stop the pump, 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, leaving no visible space between the frit and the packed resin at any point around the circumference.
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.

#### 4.2.4 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. This is an example for measurements:

1. Equilibrate a column of I.D. 1 cm x 12 cm length (10 mL) with packing buffer (equilibration buffer such as PBS, pH 7.4).
2. Inject 1% column volume (CV) of 5% acetone solution (i.e., 100  $\mu$ L acetone for a 10 mL column). Apply a flow rate of 100 cm/h. Record UV absorbance at 280 nm.

Alternatively to acetone, a sample of 1 to 2 M NaCl in packing buffer can be injected (corresponding to 1% (v/v) of the column volume). A flow rate of 100 cm/h is applied and conductivity (mS/cm) is recorded instead of absorbance.

To determine the packing performance, use the following formulas:

$$N/m = \frac{5.54 \times 100 \times (V_e / W_{1/2})^2}{BH}$$

With:

N = Number of theoretical plates

$V_e$  = Elution volume on the chromatogram (cm)

$W_{1/2}$  = Width of the acetone peak at half of the height (cm)

BH = Bed height (cm)

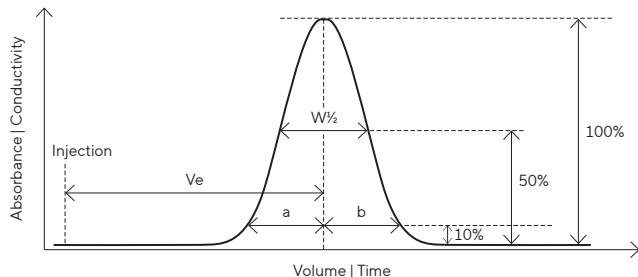
$$AF = \frac{b}{a}$$

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 at 100 cm/h for N/m are 1,500 – 3,000 plates/m after packing in sodium phosphate, pH 7.0 and for asymmetry factor (AF), are from 0.8 and 1.8. 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.

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

Please contact Technical Service.

## 5 Working Conditions and Basic Protocol

Use only samples and buffers that are previously filtered on a 0.2 µm membrane. Make sure that changes in pH and ionic strength to be used during the chromatography do not cause precipitation of sample components.

### 5.1 Equilibration

Equilibrate the column with equilibration buffer (e.g., PBS, pH 7.4 or 50 mM Tris-HCl, pH 8) for 5 to 8 CVs. In most cases, the equilibration buffer is also the packing buffer.



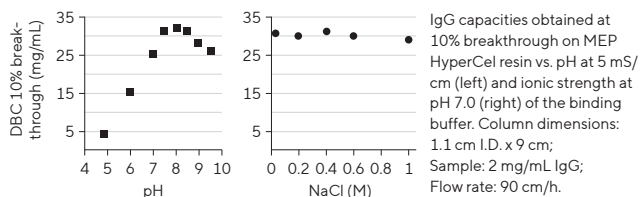
**Recommendation:** After packing or after a storage period, and before use, we recommend a blank run including the following sequence, to ensure the robustness and the stability of the packed bed:

1. Cleaning with 5 CVs of 1 M NaOH, 60 min contact time.
2. Re-equilibration with equilibration buffer until pH and conductivity are stable (e.g., 8 to 12 CVs of PBS, pH 7.4) or alternatively with 1.5 CVs of elution buffer (e.g., 100 mM sodium acetate pH 4.0) followed by 4 to 5 CVs of equilibration buffer (e.g., PBS, pH 7.4).
3. Check that the resin is well equilibrated (UV, conductivity, pH). If further settling is observed after this step, readjust the column piston.

### 5.2 Sample Application

Load pre-filtered sample at typical residence time of 5 to 8 minutes to reach optimal capacity. Antibody-containing feedstock can be loaded directly onto columns of MEP HyperCel resin, without preliminary diafiltration or concentration. Maximum capacity is obtained when pH ranges between 6.5 and 9.5. Binding capacity of IgG is independent of ionic strength in this pH domain (Figure 3). Capacities ≥ 20 mg/mL are generally obtained.

**Figure 3:** Influence of pH and Ionic Strength on the Binding Capacity of MEP HyperCel Resin



### 5.2.1 The Basic Protocol

The following procedure is appropriate for antibody-containing feedstocks that do not contain albumin.

1. Check the pH and the conductivity of the feedstock. If it is between 6.5 and 9.5, no adjustment is necessary. Diafiltration, concentration or dilution are not typically required. Load feedstock onto column.
2. Wash the column with equilibration buffer until UV absorbance returns to baseline (1 to 4 CVs).

### 5.2.2 Specific Recommendations for an Optimal Use

#### Protein-free Cell Culture Supernatant

- The synthetic iron carriers present in protein-free cell culture media may cause the resin to take on significant brown color during loading. If this occurs or if IgG recovery is low, 10 mM Na<sub>4</sub> EDTA (final concentration) may be added to the feedstock to reduce iron complex formation. Use a Na<sub>4</sub> EDTA stock solution (~200 mM) buffered at pH 8.0.
- If feedstocks are concentrated (e.g., UF | TFF) prior to column loading, high molecular weight iron carrier, often present in protein-free cell culture media, may aggregate and accumulate on the retentate side of the UF | TFF membrane. Such aggregates can bind strongly to MEP HyperCel resin and lead to column fouling. The addition of 10 mM Na<sub>4</sub> EDTA to the feedstock prior to concentration will limit the accumulation of aggregates. Use a Na<sub>4</sub> EDTA stock solution (~200 mM, pH 8.0).
- If the addition of EDTA to the feedstock is not feasible, resin performance can be preserved by using an EDTA solution during cleaning. Following antibody elution, and before the column washing with NaOH, wash the column with 100 mM Na<sub>4</sub> EDTA (adjusted to pH 7.2).
- Tween™\*\* and Triton™ X-100\*\*\* should not be included in feedstock or buffers. Such surfactant may interfere with the binding of protein to MEP HyperCel resin.
- Lipid supplements added to the culture medium may compete for the MEP ligand, fouling the resin and reducing binding capacity. Contact Technical Service for assistance.

#### Albumin-containing Cell Culture Supernatant

- The sample is loaded directly, without preliminary diafiltration or concentration.
- To desorb bound albumin, additional wash steps are recommended prior to elution. Following the load and the wash with 50 mM Tris-HCl, pH 8, wash first the column with pure water, followed by 25 mM sodium caprylate in 50 mM Tris-HCl, pH 8.0. Continue all washes until UV absorbance returns to baseline (~2 CVs).

#### Ascites Fluid, Colostrum or Other Crude, Viscous Feedstocks

- Employ the protocol for albumin-containing feedstocks described above. Dilute viscous feedstocks with equilibration buffer. Ascites fluid is typically diluted with an equal volume of buffer. More viscous feedstocks may need to be diluted with 5 to 10 volumes of buffer.

As with all chromatographic procedures, varying degrees of optimization will be required to account for variation in feedstock composition.

### 5.3 Working Flow Rate

Typical residence times of 5 to 8 minutes correspond to linear flow rates between 75 and 120 cm/h for a column of 10 cm height (0.98 to 1.57 mL/min volumetric flow rates for 1 cm I.D. column).

### 5.4 Elution

A step-elution sequence using buffers of decreasing pH (e.g., 5.5, 5.2, 4.9, 4.6, 4.3, 4.0, 3.0) is recommended. Buffers typically used include 50 to 100 mM sodium acetate. Elution at each step should be continued for 2 to 4 CVs, or

until complete elution of an observed peak is accomplished. Based on analysis of collected fractions, a second step-elution experiment at narrower pH increments or at alternative values may be useful. Ultimately, the elution procedure would be simplified to a three-step sequence. At the highest pH, elution of basic impurities would be accomplished. At the optimized, intermediate pH, the target protein would be recovered. At the lowest pH in the sequence, acidic impurities, aggregates and misfolds would emerge. Depending on the characteristics of the acidic and hydrophobic impurities, this final step may be conducted at pH 3.0.

## 6 Regeneration and Cleaning

We recommend to regenerate the column with a wash at low pH (e.g., 50 mM sodium acetate, pH 3.0 or 4.0) followed by a general cleaning-in-place (CIP) with 5 CVs of 1 M sodium hydroxide, 60 min contact time. After CIP, re-equilibration of the column in 1.5 CVs of an acidic solution (sodium acetate pH 3.0 or 4.0) hastens the neutralization of the column before equilibration in the desired buffer for the next cycle (e.g., PBS, pH 7.4). Refer to "Protein-free cell culture supernatant" in Section 5.2.2 in the case of presence of iron and iron carrier, lipids, or surfactants. For specific CIP challenges (strongly adsorbed contaminants), 3 to 5 CVs of denaturing agents (6 M guanidine-HCl or 8 M urea) or 3 to 5 CVs of 40% isopropanol are recommended prior to the step in 1 M NaOH.

If CIP sequence requires optimization, please contact Technical Service.

**⚠** MEP HyperCel resin may become coloured when using NaOH during the cleaning-in-place procedure. This is due to the cyclic group on the 4-mercapto-ethyl-pyridine ligand and does not affect the chromatographic performance of the resin.

## 7 Thermal Stability and Storage

Temperature of use	2–30 °C
Storage temperature	2–8 °C
Storage solution between runs	Neutral buffer containing 1 M NaCl and 20% (v/v) ethanol.
<b>⚠</b>	Product must never be frozen. Avoid long exposure to light.
<b>i</b>	Product is shipped at ambient temperature

## 8 Ordering Information

### MEP HyperCel Bottled Resin

Pack Size	Part Number	Pack Size	Part Number
25 mL	12035-010	5 L	12035-040
100 mL	12035-028	10 L	12035-044
1 L	12035-036		

### Prepacked Columns

Description	Part Number
<b>PRC Columns</b>	
PRC Prepacked Column 5 x 50 MEP HyperCel, 1 mL, 1/pkg	PRC05X050MEPHCEL
PRC Prepacked Column 8 x 100 MEP HyperCel, 5 mL, 1/pkg	PRC08X100MEPHCEL
<b>RoboColumn**</b>	
RoboColumn** MEP HyperCel 200 µL, row of 8	SR2MEP
RoboColumn** MEP HyperCel 600 µL, row of 8	SR6MEP

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