- Wash the column with 50 mM Tris-HCl, pH 8, until UV absorbance returns to baseline (1-4 cv). Other buffers may be used.
- Elute bound antibody using a dilute buffer at pH 4. Buffers typically used include 50 mM sodium acetate or sodium citrate (2-4 cv).
- Regenerate the sorbent with 0.5-1 M sodium hydroxide for 30 to 60 min.

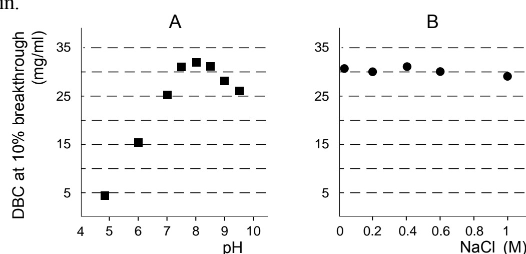

Figure 2. Influence of pH and ionic strength on the binding capacity of MEP HyperCel. IgG capacities obtained at 10% breakthrough on MEP HyperCel vs. pH (A) and ionic strength (B) of the binding buffer. Experimental conditions : Column : 1.1 cm ID x 9 cm. Sample : hu IgG (2 mg/ml). Flow rate : 90 cm/h.

During preliminary studies, it is recommended that loading be conducted at a flow rate not to exceed 70 - 100 cm/h. Maximum capture efficiency is achieved when the residence time is around 6-8 min.

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

Protein-free cell culture supernatant

- The sample is loaded directly, without preliminary diafiltration or concentration.
- Capacities of 30 mg/ml or more are generally obtained.

Note The synthetic iron carriers present in protein-free cell culture media may cause the sorbent to take on significant brown color during loading. If this occurs or if IgG recovery is low, 10 mM Na_4 EDTA may be added to the feedstock to reduce iron complex formation; Use an EDTA stock solution (~ 200 mM) adjusted to pH 8.0 - 8.5. See “Cleaning” section for additional information.

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, the column is washed first with pure water, followed by 25 mM sodium caprylate in 50 mM Tris-HCl, pH 8. Continue all washes until UV absorbance returns to baseline (~ 2 cv).

Ascites fluid, colostrum or other crude, viscous feedstocks

Employ the protocol described above for albumin-containing feedstocks. 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 5-10 volumes of buffer.

Cleaning and maintenance

In order to avoid frequent regeneration, it is advisable to introduce into the column only samples and buffers that are clear and previously filtered (0.22) µm. Make sure that changes in pH and ionic strength during elution do not cause precipitation of sample components. In most cases, 30 to 60 min wash procedures using 0.5-1 M NaOH are sufficient for cleaning and column regeneration. If some impurities are still adsorbed on MEP HyperCel, additional washes (2-3 cv) with 6 M guanidine or 2 M urea are recommended.

If you are working with protein-free cell culture media and the addition of EDTA to the feedstock is not feasible, sorbent performance can be preserved by use of an EDTA solution during cleaning. Following antibody elution, and before the column is washed with NaOH, wash the column with 100 mM Na_4 EDTA (adjusted to pH 7.2). Alternatively, 200 mM sodium citrate, pH 3.0, may be used.

CAUTION ! When using NaOH during the clean-in-place procedure, MEP HyperCel may become colored. This does not affect the chromatographic performance of the sorbent and is due to the cyclic group on the 4-Mercapto-Ethyl-Pyridine ligand.

Storage

Once opened, the MEP HyperCel containers or drums must be stored at +4°C. They must never be frozen. Between runs, store MEP HyperCel at +4°C in a neutral buffer containing 1 M NaCl and 20% (v/v) ethanol.

Ordering information

Product	Cat. No.	Size
MEP HyperCel	12035-010	25 ml
	12035-028	100 ml
	12035-036	1 L
	12035-044	10 L
	12035-051	50 L

CONTACT INFORMATION

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BioSepra® PRODUCT INSERT
Process Chromatography

MEP HyperCel™

Hydrophobic Charge Induction Chromatography (HCIC) Sorbent

Product Description

MEP (Mercapto-Ethyl-Pyridine) HyperCel is a high capacity, high selectivity sorbent specially designed for the capture and purification of monoclonal and polyclonal antibodies.

MEP HyperCel supports efficient capture and purification of 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 sorbents, IgG binding capacity on MEP HyperCel is essentially independent of subclass or species. "Weakly-binding" variants (e.g., murine IgG_1) are well retained.

MEP HyperCel provides significant benefits at both laboratory and process-scale :

Sample preparation is reduced to clarification :

- Feedstock may be applied without adjustment of pH or ionic strength. Binding is conducted at near-neutral pH and is accomplished without addition of lyotropic or other salts. Binding capacity is independent of ionic strength over a broad pH range (~ 6.5 - 9.5).

- Concentration of dilute samples is not required. Efficient capture is achieved from feedstock containing as little as ~50-100 µg IgG/ml.

Rapid and efficient sample processing :

- Large volumes of sample can be processed rapidly and efficiently. Dynamic binding capacities ≥ 30 mg IgG per ml of sorbent (at 10% breakthrough) are routinely achieved.

- High purity is achieved in a single chromatography step. Purity values of 70-90%, or greater, are typical.

- Gentle elution at pH 4 reduces the risk of antibody aggregation.

- No need for extensive desalting/diafiltration following HCIC. In contrast to traditional hydrophobic interaction chromatography, the IgG fraction isolated using MEP HyperCel is recovered in low ionic strength buffer, and can be applied to an ion exchange column without extensive desalting.

Chemically stable to base :

- MEP HyperCel is completely stable to repeated cleaning with 1 M sodium hydroxide, providing a significant advantage as compared to Protein A-based sorbents.

Table 1 : MEP HYPERCEL Main Properties.

Property	Value
Particle size	80-100 µm (av.)
Dynamic binding capacity for hu IgG (10% breakthrough)*	≥ 20 mg/ml
Ligand	4-Mercapto-Ethyl-Pyridine
Ligand density	70-125 µmol/ml
Working pH	3 - 12
Cleaning pH	3 - 14
Pressure resistance	< 3 bar (44 psi)
Typical working pressure	< 1 bar (14 psi)

** Determined using 5 mg/ml human IgG in PBS, flow rate : 60 cm/h.*

Column packing

General considerations

Depending on package size, MEP HYPERCEL is supplied as either a traditional slurry/suspension or as a moist cake. In process-scale applications, the latter form facilitates convenient transfer of sorbent without the need to agitate and suspend large volumes of material. The slurry form is supplied in 1 M NaCl containing 20% (v/v) ethanol. The moist cake contains the same solution. MEP HYPERCEL is available in 25 ml, 100 ml, 1 L, 10 L and 50 L packages. Each contains sufficient sorbent to pack a column of the specified volume.

Preparing sorbent for packing

When working with sorbent supplied in slurry form, gently agitate the container to fully suspend the sorbent. Do not use magnetic stir bars at any point in the process. As supplied, the slurry concentration is approximately 70-75% (v/v). Based on the desired bed volume, transfer a suitable volume of slurry to a graduated cylinder. Include a practical excess of sorbent (20-25% of the desired bed volumes is recommended). Remove the supernatant storage solution (1 M NaCl in ethanol 20% (v/v)) and add a volume of packing buffer equal to one-half the volume of settled resin. This procedure yields a 67% (v/v) slurry – the concentration recommended for packing.

When working with sorbent supplied in moist-cake form, the packing slurry is prepared by addition of packing buffer to a weighed quantity of moist sorbent. As supplied, 1 g of moist sorbent yields 0.8 - 1.0 ml of packed bed. For enhanced accuracy in preparing pilot or process-scale beds of a specific volume, it is useful to pack a laboratory-scale column and calculate the ratio of weighed sorbent to column volume. This determination should be conducted using a column no less than 1.6 cm ID. A bed of at least 5 cm depth should be prepared. As with all non-rigid sorbents, some variation of weight-to-volume ratio is expected as scale is increased.

Following preparation of packing slurry by either of the methods above, mix to obtain a homogeneous suspension and carefully degas the mixture under vacuum. In laboratory-scale applications, the suspension may be swirled manually to minimize "bumping".

Preparing the column and system for packing

MEP HYPERCEL is compatible with traditional low or medium pressure chromatography columns and equipment. A column equipped with an adjustable flow adapter facilitates optimal packing and is recommended. For preliminary laboratory studies, a column of 1 cm ID x 15 cm is recommended. Scale-up is best accomplished according to well-known principles : bed height is maintained constant while diameter is increased.

- 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...).
- Determine pressure/flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatographic operation.
- Stop the pump, close the column outlet, and remove the upper flow-adaptor or piston.
- Open the outlet and drain buffer from the column, leaving 1-4 cm of buffer above the bottom frit (e.g., 1 cm for a 1 cm ID column ; 4 cm for a 30 cm ID column).
- Adjust the pump to provide a volumetric flow rate (i.e., ml/min) equivalent to 600 cm/h linear velocity.

Following completion of blank pressure/flow measurements, proceed with column packing as described below. The following recommendations apply to packing of columns for laboratory, pilot and production-scale use.

Packing the column

1. Gently stir the packing slurry to obtain a homogeneous suspension.

2. Pour the slurry into the column in one continuous motion. In order to minimize introduction of air bubbles, pour the slurry along a glass rod held against the column.

3. Gently stir the slurry in the column using a plastic rod or paddle.

4. Allow the suspension to settle for 4-5 min, so that a layer of clear supernatant, ≥ 1 cm, is visible at the top of the column.

5. Insert the upper plunger into the column and adjust carefully to ensure that no air is trapped under the net or frit. Avoid turbulence or other disturbance of the sorbent as it settles.

6. Open the column outlet and operate the pump at the packing flow rate (equivalent to 600 cm/h). When the bed height is stabilized and no further compaction is observed, stop the pump and close the column outlet.

7. Position the plunger so that 1 to 4 mm of buffer is visible between the plunger and the top of the bed.

8. Start the pump again and repeat step 7. If further compression of the bed occurs, re-position the plunger.

9. Make a final adjustment of the flow adaptor so that the frit is just in contact with the bed, leaving no visible space between the frit and bed at any point around the circumference. If possible, this final step should be conducted under flow at 600 cm/h.

The measured pressure/flow characteristics of the bed should reflect the typical values shown in Table 1.

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 is calculated. Required formulas are shown below. Measurements are made as follows :

- Equilibrate the column with packing buffer.
- Inject a sample of 5% (v/v) acetone solution and/or 0.5 M sodium chloride corresponding to 0.5% (v/v) of the total column volume. Record UV absorbance (280 nm) and conductivity.

HETP (as cm/plate) is calculated as follows :

$$HETP = d^2 \times L / 5.54 \times t^2$$

The number of plates/m (N/m) is calculated as follows :

$$N/m = \frac{5.54 \times t^2}{d^2 \times L}$$

t = Retention value of acetone or sodium chloride peak expressed in units of time, volume or measured distance on the chromatogram.

d = Peak width at half height expressed in same units as "t".

L = Column length (in units of cm for HETP or m for N/m).

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

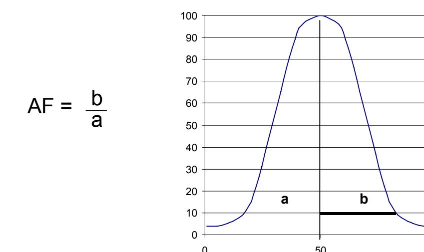

Figure 1. Peak trace in a typical test evaluation of column performance. "a" and "b" are respectively first and second half peak width at 10% of peak height. Typical values for N/m are 1,000-3,000 plates/meter. Typical values for asymmetry factor (AF) range from 0.8 and 2 at 10% of peak height. These values are given as the average of experimental values.

Working conditions

General considerations

The procedure described below is appropriate for antibody-containing feedstocks that do not contain albumin. Variants of this procedure suitable for isolation of product from albumin-containing feedstocks, and other crude or viscous samples, are described following the basic protocol.

The basic protocol

- Equilibrate the MEP HYPERCEL column with PBS, pH 7.4 (3-8 cv). In most cases, this buffer is also the packing buffer. A useful alternative buffer is 50 mM Tris-HCI, pH 8. Maximum capacity is obtained when pH ranges between 6.5 and 9.5. Binding capacity is independent of ionic strength in this pH domain (Figure 2).
- 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.

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