

MEP HYPERCEL[®]

Hydrophobic Charge Induction Chromatography (HCIC) Sorbent

- Specially designed for purification of antibodies.
- Direct sample loading without any adjustment of pH or ionic strength.
- High purity achieved in a single step.
- High IgG capacity, independent of subclass or species.
- Easy cleaning with sodium hydroxide.

MEP 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 fluid and cell culture supernatant. 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 IgG₁) 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. Binding occurs at neutral pH, and is independent of ionic strength. No need to add lyotropic or other salts.
 - Concentration of dilute samples is not necessary. Efficient capture is achieved even with feedstocks as dilute 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 in one step. Product purities of 70-90%, or greater, are typically achieved.

TABLE 1 : MEP HYPERCEL Main Properties.

Particle size	80-100 µm
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.

- Gentle elution reduces the risk for antibody aggregation and eliminates the need for desalting or diafiltration. A simple lowering of the pH to 4, at low ionic strength, will elute the antibody.
- **Chemically stable to base.**
 - Easy long term cleaning with 1 M sodium hydroxide gives this material a significant advantage over Protein A-based sorbents.

MEP HYPERCEL is supplied in 1 M NaCl containing 20% ethanol and is available in a range of package sizes. Custom packaging to meet specific manufacturing requirements is available on request.

Hydrophobic Charge Induction Chromatography Mechanism

Hydrophobic Charge Induction Chromatography (HCIC) is based on the pH-dependent behavior of ionizable, dual-mode ligands. MEP HYPERCEL carries an antibody-selective ligand, 4-Mercapto-Ethyl-Pyridine (4-MEP). As shown in figure 1, adsorption is based on mild hydrophobic interaction, and is achieved without addition of lyotropic or other salts. Desorption is based on charge repulsion. It is performed by reducing the pH.

In contrast to traditional hydrophobic interaction chromatography, HCIC is controlled on the basis of pH rather than salt concentration. Antibody elution is conducted at low ionic strength, eliminating the need for extensive diafiltration in applications where ion exchange chromatography will follow capture. Compared to chromatography on Protein A sorbents, elution from MEP HYPERCEL is achieved under relatively mild conditions (pH 4.0). Thus, aggregate formation is reduced and antibody activity is preserved.

BIOSEPRA MEP HYPERCEL is composed of cellulose matrix to which 4-Mercapto-Ethyl-Pyridine (4-MEP) is linked. The cellulose bead confers high porosity, chemical stability and low non-specific interaction. Bead size is 80-100 μm to allow a good compromise between capacity and flow property. 4-MEP was chosen for its high selectivity and capacity for antibodies, and for its pKa which is 4.8. It contains a hydrophobic tail and an ionizable headgroup. At physiological pH, the aromatic pyridine ring (figure 2) is uncharged and hydrophobic. Additional contributions to binding are provided by the aliphatic spacer arm. Antibody binding is further enhanced by interaction with the thioether group. Both ligand structure and ligand density are designed to provide effective binding in the absence of lyotropic or other salts.

When pH of the mobile phase is adjusted to values below 4.8 (typically pH 4.0), the ligand takes on a distinct positive charge. Under such conditions, antibody molecules also carry a positive charge. Electrostatic repulsion is induced and antibody is desorbed.

Figure 1. Adsorption elution mechanism.

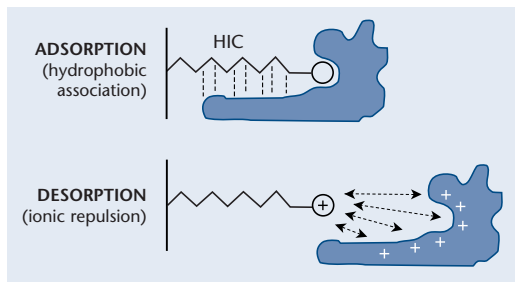
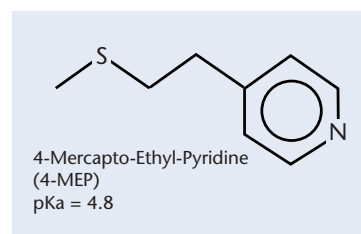


Figure 2. MEP HYPERCEL head group.



Capacity

The binding capacity specification for MEP HYPERCEL is ≥ 20 mg human IgG/ml sorbent. As shown below, however, dynamic binding capacity values ≥ 30 mg/ml are more typical in practical application.

Compared to Protein A sorbents, MEP HYPERCEL is remarkably independent of feedstock composition. Likewise, binding capacity for IgG variants is largely independent of subclass or species.

• Capacity and pH

The influence of pH on binding capacity is illustrated in figure 3. The relationship is consistent with the chromatographic mechanism described above. At pH values from 7 to 9, the binding capacity for human polyclonal IgG ranges from 25 to 33 mg/ml. At pH 6.5, binding capacity is ~ 20 mg/ml. As pH is reduced further toward the pKa of 4-MEP, there is a distinct decline in binding capacity as the ligand – and antibody – take on increasing positive charge. At pH 4.0, antibody is entirely unretained in most applications.

The data demonstrate that high binding capacity is achieved at pH values representative of typical feedstocks. Precipitation, sometimes observed during adjustment of feedstock pH, is thus avoided.

During chromatography on Protein A sorbents, elution of antibody at pH 3 frequently leads to aggregation and loss of activity. Elution from MEP HYPERCEL is achieved under milder conditions.

• **Capacity and ionic strength**

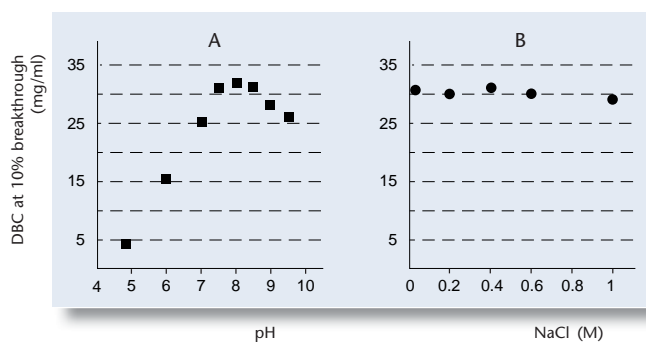
A plot demonstrating the influence of ionic strength on binding capacity is shown in figure 3. Dynamic binding capacity was determined in the presence of sodium chloride, at concentrations ranging from 50 mM to 1 M. Binding capacity is constant over this range. Such behavior is typical at pH values where the ligand carries little or no charge (e.g., pH 6.5-9.0). The data demonstrate that typical feedstock may be loaded without adjustment of ionic strength. Neither diafiltration nor addition of lyotropic salt is required. Viscous feedstocks such as animal sera or ascites fluids may be diluted.

Antibodies are eluted using dilute buffer (e.g., 50 mM sodium acetate or sodium citrate, pH 4).

• **Capacity and antibody concentration**

The influence of IgG concentration on dynamic binding capacity is illustrated in figure 4. Concentration ranges from 50 µg/ml to 5 mg/ml. No significant variation in capacity is observed. MEP HYPERCEL supports efficient capture of antibody from highly dilute feedstock.

Figure 3. 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 : IgG (2 mg/ml) ; Flow rate: 90 cm/h.

Preliminary concentration is not required. Indeed, harvest and concentration of antibody on MEP HYPERCEL can be used to replace traditional concentration procedures. Concentration and initial purification can be achieved in a single step.

• **Capacity for IgG variants**

The data in table 2 illustrate that binding capacity for IgG is little influenced by subclass or species. In contrast with Protein A sorbents, there is no significant difference between capacity for murine IgG_{2a} and the IgG₁, the later being "weakly bound" by Protein A.

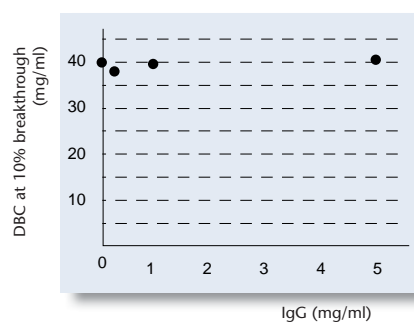
MEP HYPERCEL is "antibody selective". Nevertheless, its selectivity is sufficiently broad to provide high binding capacity for a broad range of IgG variants.

Purity

When antibody is isolated from protein-free cell culture supernatant using MEP HYPERCEL, product of >90% purity (SDS-PAGE) is typically obtained. In the example shown in table 3, product of 99% purity was recovered

	Binding capacity
Human polyclonal IgG	32 mg/ml
Murine monoclonal IgG ₁ (from ascites fluid)	37 mg/ml
Murine monoclonal IgG _{2a} (from cell culture)	34 mg/ml

Figure 4. Influence of human IgG concentration on the binding capacity of MEP HYPERCEL.



Working buffer : PBS, pH 7 ; Flow rate : 70 cm/h.

Feedstock	Total proteins (mg)	IgG content (mg)	IgG initial purity (%)	Final IgG purity (%)	Recovered IgG (mg)	Overall IgG yield (%)	Purification factor
CCS* with FBS	1740	30	1.7	69	23	76	40
Protein-free CCS*	330**	17	5**	99	17	99	19**
Bovine serum	440	121	28	75	105	87	3
Ascites fluid	55	9	16	83	7	79	5

* CCS = Cell culture supernatant

** BCA methodology

in a single chromatographic step. Isolation from crude feedstock – bovine serum and ascites fluid or CCS supplemented with FBS – is also illustrated, with purity values ranging from 69 to 83%. When antibody is isolated from albumin-containing feedstock, albumin is the principal remaining impurity. In such applications, product of 70 to >90% purity is generally obtained in including an additional washing step with water or sodium caprylate to selectively desorb the albumin. If a simple anion exchange procedure is used to bind residual albumin, product of 99% purity can be obtained. Since anion exchange chromatography is frequently

included in schemes for antibody purification, this approach is convenient and effective.

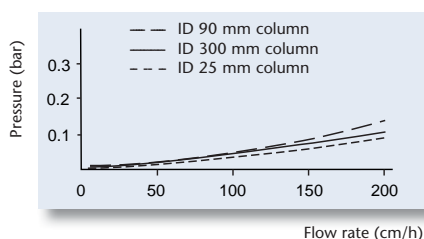
Stability and Cleaning

The physical and chemical properties of MEP HYPERCEL are well suited to both laboratory and process scale use. MEP HYPERCEL is compatible with systems routinely used for low or medium-pressure process chromatography. Sorbent-related backpressure is less than 0.5 bar in practical application at linear velocities up to 200 cm/h. Pressure/flow curves for columns up to 300 mm I.D. are shown in figure 5. To assure best capture efficiency, it is recommended that initial loading studies be conducted at 70 cm/h.

MEP HYPERCEL is chemically stable from pH 4 to 14. Sodium hydroxide, 0.5-1.0 M, is recommended for cleaning. Submitted to a series of 200 clean-in-place cycles with 1 M sodium hydroxide (1 hour contact/cycle), the sorbent maintained its initial properties. Ligand density was unchanged.

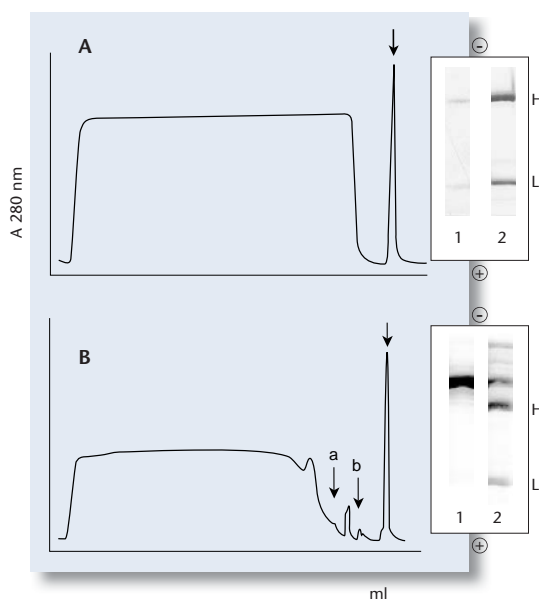
Other useful cleaning agents include 4-6 M guanidine, 8 M urea and 50/50 ethylene glycol/ water.

Figure 5. Backpressure as a function of flow rate.



Column height 150 mm ; PBS, pH 7.4.

Figure 6. Monoclonal antibodies purification on MEP HYPERCEL from cell culture supernatants.



Sample A = 300 ml protein-free cell culture supernatant.
 Sample B = 300 ml cell culture supernatant containing 5% fetal bovine serum ; Equilibration : 50 mM Tris-HCl, pH 8 ; Elution: 50 mM acetate, pH 4 ; Flow rate : 70 cm/h. In curve B, (a) and (b) are respectively water and 25 mM sodium caprylate washings ; SDS-PAGE (reduced conditions) : (1) = crude sample, (2) = purified IgG. H = Heavy chain ; L = Light chain.

Applications

MEP HYPERCEL provides excellent selectivity for a broad range of antibody variants. Representative applications are summarized below.

• Direct capture from cell culture supernatant.

Capture of antibody from both protein-free and serum-supplemented (5% fetal bovine serum) cell culture supernatant (CCS) is described. In both cases, clarified samples were loaded without concentration or adjustment of composition. The IgG concentration in the protein-free CCS was 114 µg/ml. Chromatograms are shown in figure 6 and results are summarized in table 3.

In both applications, the column was equilibrated with 50 mM Tris-buffer, pH 8.0. After loading, the column was washed with the same buffer. PBS, pH 7.4 may also be used.

During chromatography of serum-supplemented cell culture supernatant, two

additional wash steps (figure 6) were added to promote desorption of albumin.

In both cases, the IgG fraction was eluted under the influence of 50 mM sodium acetate, pH 4.0. Product isolated from protein-free CCS was 99% pure (SDS-PAGE). Despite the presence of abundant albumin in the feedstock, product isolated from serum-supplemented CCS was 69% pure. The latter could be brought to 99% purity by application of a simple anion exchange procedure.

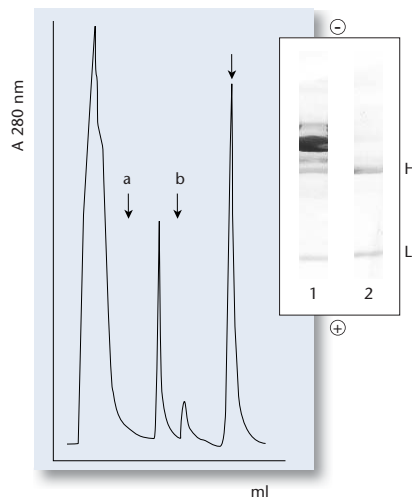
• **Purification of monoclonal IgG from ascites fluid.**

IgG was isolated from ascites fluid using the same procedure as described above for use with serum-supplemented CCS. In order to reduce viscosity, the sample was diluted with an equal volume of equilibration buffer prior to loading. The chromatogram appears in figure 7. Results are summarized in table 3. Isolated product was 83% pure. Purity of the IgG fraction could be brought to 98% using the anion exchange procedure summarized below.

• **MEP HYPERCEL followed by Anion Exchange Chromatography.**

Efficient removal of residual albumin present in the IgG fraction can be accomplished using DEAE Ceramic HYPERD. The column is

Figure 7. Immunoglobulin capture from ascites fluid on MEP HYPERCEL.



(a), (b) = Contaminant elution peaks after the 2 washing steps ; Same conditions as those of figure 6. SDS-PAGE (reduced conditions) analysis : (1) = crude sample ; (2) = purified IgG.

equilibrated with 25 mM Tris buffer, pH 8.8, containing sodium chloride sufficient to provide a conductivity of 10 mS. The IgG fraction obtained following HCIC is diluted with two volumes of the same buffer and applied to the column.

After loading, elution is continued with the above buffer. Antibody appears as an unrestrained peak, while albumin is retained. The latter is desorbed using 25 mM Tris buffer containing 0.5-1.0 M sodium chloride, pH 8.8.

• **Additional Applications :**

MEP HYPERCEL has been studied in a broad range of applications, including :

- Isolation of antibodies from sweet-whey and colostrum.
- Isolation of antibodies from transgenic plant and animal sources.
- Isolation of IgA and selected fusion proteins.

For industrial applications, a validation package is available and provides necessary information to assist users in development of validation procedures.

References

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Ordering Information

Product	Cat. No.	Size
MEP HYPERCEL	12035-069	5 ml
	12035-010	25 ml
	12035-028	100 ml
	12035-036	1 L
	12035-040	5 L
	12035-044	10 L

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About BIOSEPRA® Products & Collaborative Services

CIPHERGEN Biosystems develops, manufactures and markets BIOSEPRA process chromatography sorbents that greatly simplify protein purification development and significantly improve biopharmaceutical manufacturing productivity.

Over the past 25 years, BIOSEPRA chromatography products and services have earned an outstanding reputation for product innovation and technical support. Our expanded R&D sorbent program, new ISO 9001 manufacturing plant and recently launched MEP HYPERCEL represent our latest commitment to the biopharmaceutical industry.

With the acquisition of BIOSEPRA products and services, CIPHERGEN has been able to combine chromatography development expertise with SELDI-based PROTEINCHIP® technology to set in motion an entirely new approach to protein purification called Process Proteomics. With the addition of PROTEINCHIP technology, sample analysis and purification development are combined. This single-step, on-chip approach dramatically accelerates and simplifies purification development and analysis. The future of Process Proteomics begins with CIPHERGEN.

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