



Life Sciences

Product Note

USD 2629

MEP HyperCel™ Mixed-Mode Chromatography Sorbent

Hydrophobic Charge Induction Chromatography/HCIC

Product Description and Application Overview



Introduction

Pall MEP HyperCel mixed-mode chromatography sorbent is a flexible chromatography sorbent designed for capture and purification of antibodies and various recombinant proteins from laboratory to manufacturing scale. It offers:

- A unique separation mechanism and selectivity for protein separations
- A no-salt/low-salt alternative to Hydrophobic Interaction Chromatography (HIC)
- Monoclonal and polyclonal IgG capture and intermediate purification (aggregate, DNA and HCP removal)
- Enhanced process economics

MEP HyperCel sorbent brings a specific mixed-mode or multi-modal separation mechanism, different from conventional ion exchange or affinity mechanisms, and is particularly effective to replace conventional HIC. MEP HyperCel sorbent provides significant benefits when used at process scale; particularly, in contrast to conventional HIC, MEP HyperCel sorbent does not require massive addition of salt to promote protein binding, resulting in simplification of process operations, saving of unit operations (e.g., diafiltration or ultrafiltration), and better process economics. Due to its ligand structure, MEP HyperCel sorbent is immunoglobulin-selective. It can be used for direct capture or intermediate purification of IgG from various feedstocks in combination with other conventional methods such as cation exchange, HIC, or even following Protein A affinity capture for enhanced DNA clearance, HCP (Host Cell Proteins), and aggregate removal.

For “non-antibody” proteins (e.g., recombinant proteins, enzymes, etc.), MEP HyperCel sorbent also can be used for capture or intermediate steps in a purification sequence. When used at the capture step, feedstock is typically loaded directly without pH or ionic strength adjustments.

MEP HyperCel sorbent contributes to a simplification of process development, saving of unit operations such as diafiltration or ultrafiltration, and lower waste disposal; anticipated long service life is expected due to MEP HyperCel sorbent’s resistance to harsh cleaning-in-place methods (0.5 to 1 M NaOH, 30 to 60 minutes contact time) – all factors contributing to lower purification cost.

Note: For recent related publications, see References on back page.

Features and Benefits

Unique Separation Mechanism and Differentiated Selectivity

The mixed-mode mechanism allows purification of antibody or other proteins that cannot be easily achieved by conventional techniques such as ion exchange or conventional HIC; for example, playing on differences in hydrophobicity, and separation of proteins with very close isoelectric points.

Direct Immunoglobulin Capture from a Variety of Feedstocks

Due to its unique ligand structure, MEP HyperCel sorbent is immunoglobulin-selective. Antibody binding occurs at neutral pH and is largely independent of ionic strength. Concentration of dilute samples is not necessary (e.g., efficient capture is achieved even with feedstocks as dilute as 50 to 100 µg IgG/mL). Immunoglobulin purification from protein-free and protein-supplemented cell culture supernatants, transgenic milk, ascites fluids and serum has been reported. In contrast to Protein A affinity sorbents, IgG binding capacity on MEP HyperCel sorbent is essentially independent of subclass or species. “Weakly-binding” variants (e.g., murine IgG1 or Rat IgG) are well retained. MEP HyperCel sorbent contributes to HCP removal and virus clearance, and provides a very efficient one-step DNA clearance from cell culture supernatants. Note that the addition of Tween[®] and Triton[®] in feedstock or buffers is not recommended, because such surfactants may interfere with the binding of protein to MEP HyperCel sorbent.

A basic protocol for antibody capture is described in Pall’s Product Information Insert USD 2518.

IgG Elution in Mild Conditions and Separation of Contaminants

IgG is typically eluted in the pH 5.5 to 4.0 range, depending on isoelectric points and contaminants profiles. This milder elution compared to Protein A affinity may contribute to reduced aggregate formation and better preservation of the antibody biological activity. Moreover, MEP HyperCel sorbent’s pH-dependent elution mechanism allows a separation of HCPs, DNA, antibody aggregates and misfolds from the monomeric, IgG based on differences of hydrophobicity. In some cases, the addition of arginine in MEP HyperCel elution buffers (0.1 to 1.0 M) reduces the risk of antibody aggregation and prevents the loss of solubility encountered at acidic pH with many antibodies and allows even milder pH elution (around pH 7.0). (See Reference 14 in Bibliography.)

Protein Binding in No-salt or Low-salt Conditions

Several families of “non-antibody” recombinant proteins have been purified using MEP HyperCel sorbent. In contrast to conventional HIC (e.g., Phenyl or Butyl ligands), protein binding to the sorbent does not require the massive addition of salt such as ammonium sulphate or other lyotrope. This results in lower process costs and waste disposal benefits. Product can be recovered in dilute buffer, and unit operation steps such as ultrafiltration or diafiltration are minimized, contributing to better process flow and enhanced process economics.

Product Description

MEP HyperCel sorbent is composed of a proprietary rigid 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. An 80 to 100 μm average bead diameter allows excellent flow properties at low column backpressures, compatible with large-scale production. MEP HyperCel sorbent can be used from laboratory to hundred-liter production-scale columns. The sorbent is available in a variety of packaging configurations as well as convenient Pall PRC prepacked columns of 1 mL and 5 mL designed for sorbent screening, fast methods optimization and scale up. MEP HyperCel sorbent is supplied in 1 M NaCl containing 20% ethanol, and custom packaging is available on request.

Table 1

Main Properties of MEP HyperCel Sorbent

Particle Size (average)	80 – 100 μm
Dynamic Binding Capacity for Human IgG* (10% breakthrough)	≥ 20 mg/mL
Ligand	4-Mercapto-Ethyl-Pyridine
Ligand Density	80 – 125 $\mu\text{mol/mL}$
Working pH (long-term)	2 – 12
Cleaning pH (less than 6 hours)	2 – 14
Pressure Resistance	< 3 barg (44 psig)
Typical Working Pressure	< 1 barg (14 psig)

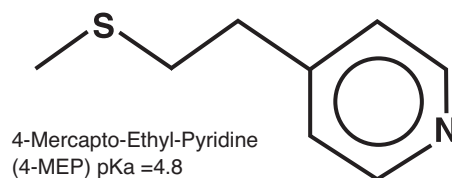
* Determined using 5 mg/mL human IgG in PBS, 6 minute residence time (flow rate 70 cm/h).

Separation Mechanism

MEP HyperCel sorbent operates by a mixed-mode or multi-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

Structure of 4-MEP Ligand



Protein binding: Neutral pH, No Feedstock Adjustment

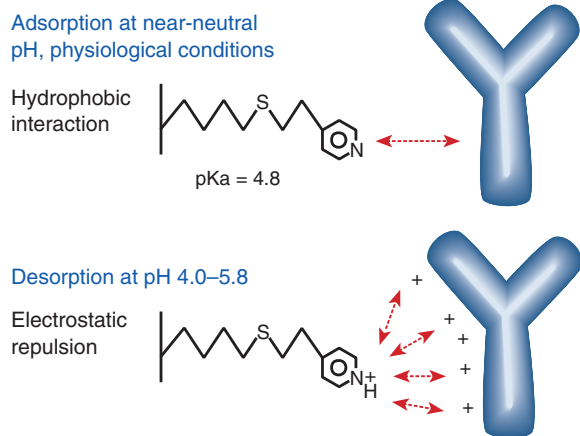
Binding of proteins is based on mild hydrophobic interaction and conducted at near-neutral pH, conditions where the pyridine group of the ligand is uncharged.

4-MEP ligand has a pKa of 4.8, and contains a hydrophobic tail and an ionizable headgroup. At physiological pH, the aromatic pyridine ring is uncharged and hydrophobic. 4-MEP is immunoglobulin-selective; additional contributions to IgG binding are provided by the aliphatic spacer arm and interactions with the thioether group. Both ligand structure and ligand density are designed to provide effective protein binding in the absence of salt or at low salt concentrations.

Protein Elution: Electrostatic Charge Repulsion by Decreasing pH Steps

Protein desorption is prompted by electrostatic charge repulsion. By reducing the pH of the mobile phase, like charges are established on both the ligand and protein. When pH of the mobile phase is reduced, the magnitude of the opposing charges depends on the pI of the target protein and the pKa of the ligand. Figure 2 illustrates the binding mechanism of IgG; with a pKa of 4.8, the ligand will carry 50% positive charge at pH 4.8 or 10% positive charge at pH 5.8. Even with only 10% positive charge present on the ligand, desorption will occur if the protein carries a net positive charge of sufficient magnitude. This electrostatic charge repulsion mechanism is not specific to IgG and can also be exploited to purify a broad variety of “non-antibody” proteins or to remove specific contaminants from a complex feedstock.

Figure 2
Antibody Binding and Elution on MEP HyperCel Sorbent



Binding of IgG is supported by a combination of hydrophobic interaction and molecular recognition. Desorption is prompted by electrostatic charge repulsion by reducing the pH of the mobile phase.

Capacity

As for any other chromatography sorbent, the capacity of MEP HyperCel sorbent depends on many parameters including the nature of the target protein, its isoelectric point, and the feedstock composition. Typically, capacities reported for “non-antibody” recombinant proteins vary from 10 to 100 mg/mL, and capacities for IgG are in the 20 to 30 mg/mL range.

Note: For antibodies, in contrast to Protein A affinity sorbents, there is no significant difference between capacity for different subclasses or species (e.g., for murine IgG2a and IgG1, the latter being “weakly bound” by Protein A).

Table 2
MEP HyperCel Capacities for IgG

	Binding Capacity
Human polyclonal IgG	32 mg/mL
Murine monoclonal IgG1 (from ascites fluid)	37 mg/mL
Murine monoclonal IgG2a (from cell culture)	34 mg/mL

Like many other chromatography sorbents, the key parameters influencing the binding capacity of MEP HyperCel sorbent include:

Residence Time

The residence time (RT) influences the yield/purity ratio and should be optimized on a case-by-case basis (refer to Pall Application Note USD 2409). Usually, the column linear flow rate should be adjusted in order to

keep an average 5 to 8 minutes residence time for optimal capacity. According to purity/yield results, residence time may be decreased to shorten the purification cycle duration.

Binding pH

The relative hydrophobicity of MEP HyperCel sorbent can be modulated by variations of the pH. For separation of weakly hydrophobic protein, MEP would be used at neutral pH, while separation of strongly hydrophobic protein could be conducted at lower pH, where relative hydrophobicity of MEP and binding is weaker.

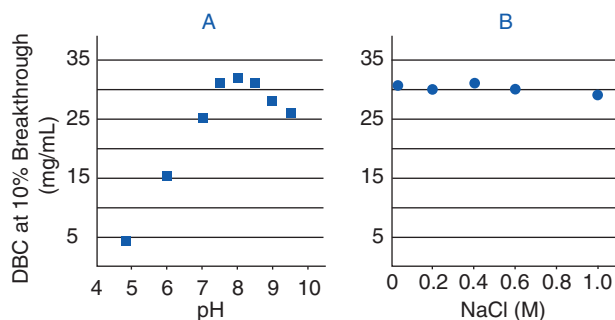
As shown in Figure 3A, at pH values from 7 to 9, the binding capacity for human polyclonal IgG ranges from 25 to 33 mg/mL. At pH 6.5, binding capacity is around 20 mg/mL. As pH is reduced further toward the pKa of the ligand, there is a decline in capacity as the ligand and antibody take on increasing positive charge.

Ionic Strength

As illustrated in Figure 3B, dynamic binding capacity for IgG is largely independent of ionic strength (e.g., in sodium chloride concentrations ranging from 50 mM to 1 M). Typical IgG containing feedstock may be loaded without adjustment of ionic strength.

For “non-antibody” proteins, salt (e.g., in sodium chloride concentrations ranging from 0.5 to 1 M) may be added in certain cases to enhance the hydrophobic component of the interaction and protein binding. This often results in better dynamic binding capacities and recoveries than when using conventional HIC sorbents, still with lower salt concentrations.

Figure 3
Influence of pH and Ionic Strength on the IgG Binding Capacity of MEP HyperCel Sorbent



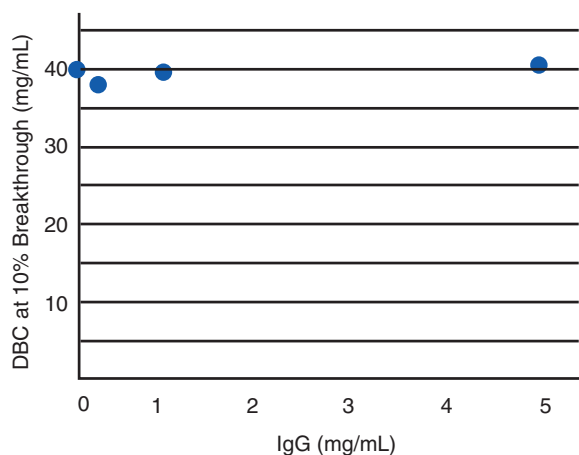
IgG capacities obtained at 10% breakthrough on MEP HyperCel sorbent vs. pH (A) and ionic strength (B) of the binding buffer. Experimental conditions: Column 1.1 cm ID x 9 cm; Sample: IgG (2 mg/mL); Flow rate: 90 cm/h.

Concentration

As shown in Figure 4, no significant variation in capacity is observed with IgG concentrations ranging from 50 µg/mL to 5 mg/mL. MEP HyperCel sorbent therefore supports efficient capture of antibody from highly dilute feedstock, without preliminary concentration.

Figure 4

Influence of Human IgG Concentration on the Binding Capacity of MEP HyperCel Sorbent



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

Temperature

The hydrophobic binding dimension of the mechanism is entropy-driven, and the interaction increases with rise of temperature. For robustness and capacity optimization studies, special attention should be given to keep buffer and operation room temperature consistent.

Purity of IgG Recovered by Chromatography on MEP HyperCel Sorbent

MEP HyperCel sorbent is immunoglobulin-selective; however, other molecules present in the feedstock (cell culture supplements, albumin, iron carriers, surfactants) may interact with 4-MEP ligand. However, data show that even with “protein-rich” feedstreams, it is possible to obtain IgG purity ranging from 95 to 98% in a single capture step after optimization (refer to Application 3 in the Applications section). However, to allow proper discrimination between pure IgG and contaminants from cell culture such as HCP or additives (albumin, transferrin), a careful optimization of elution pH and specific washing sequences is needed (refer to Pall Application Notes USD 2409, USTR 2565 and Product Information Insert USD 2518).

Optimization Guidelines to Achieve Best IgG Purity

Initial evaluation of MEP HyperCel sorbent should include experiments to identify an elution-pH at which the target antibody is desorbed with maximum selectivity and optimum resolution. Identification of optimum elution pH is best achieved by step-elution using a series of buffers with incrementally decreasing pH values. The number of steps will depend on the feedstock, the nature and the antibody pI, but finally a typical step elution sequence will be achieved in a maximum of three pH steps. The first step would be used to prompt elution of basic/hydrophilic impurities (if any), the second to prompt elution of the target antibody, and the third to prompt elution of acidic or hydrophobic impurities. This approach is successfully used to discriminate IgG HCP, IgG aggregates, misfolds or other contaminants. Depending on the characteristics of the acidic and hydrophobic impurities, this final step may be conducted at pH 3.0. It is useful to conduct a final wash at pH 3.0 to prompt desorption of any remaining impurities before the column is cleaned using sodium hydroxide.

Chemical Stability and Cleaning

In regular conditions, the typical working pH for MEP HyperCel sorbent is pH 2 to 12. However, for shorter contact times and cleaning in place, MEP HyperCel sorbent is chemically stable from pH 2 to 14. Therefore, sodium hydroxide, 0.5 to 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. Other adsorbed contaminants may be removed by washing with 6 M guanidine (2 to 3 CVs), 8 M urea, or 40 % isopropanol. (Refer to Pall Product Information Insert USD 2518).

Methods Screening and Scale Up

The physical and chemical properties of MEP HyperCel sorbent are well suited to both laboratory, pilot and process scale use. MEP HyperCel sorbent is compatible with systems routinely used for low or medium-pressure process chromatography. For challenging separations of proteins and impurities, it is recommended to screen MEP HyperCel sorbent along with Pall HEA HyperCel and PPA HyperCel mixed-mode sorbents carrying aliphatic and aromatic synthetic ligands that provide additional chromatographic selectivities (refer to Pall Brochure USD 2443).

At Laboratory Scale or for Methods Development

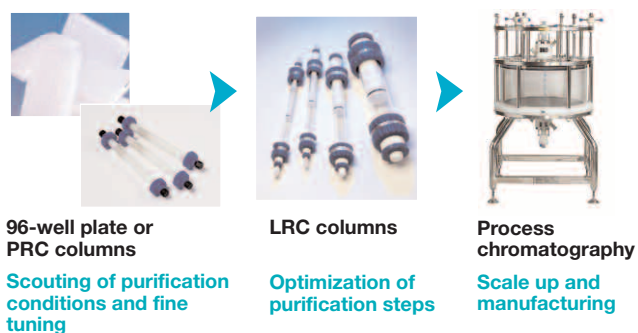
Efficient separations can be achieved using 96-well filter plates or Pall PRC prepacked columns. The 1 mL and 5 mL PRC prepacked columns demonstrate a high packing efficiency (>2500 plates/meter), can be directly connected to commonly used laboratory chromatography systems, and deliver optimal and consistent performance (refer to Pall Brochure USD 2492). Further scale up (up to 900 mL sorbent volume) can be achieved by packing the sorbent in Pall LRC laboratory empty glass tube columns (refer to Pall Brochure USD 2480).

Pilot and Process Scale Applications

MEP HyperCel sorbent has been designed to meet pilot to manufacturing-scale requirements in protein purification, and is currently used in columns of multi-liter up to several hundred liter volumes (see Figure 5). Specific packing protocols and technical support for large-scale column packing are available from Pall. A comprehensive validation package and Regulatory Support File (RSF) are also available to assist users in the development of validation procedures.

Figure 5

Screening and Scale-up Principles



Sorbent performance can be first screened using either Pall AcroWell™ or AcroPrep™ 96 microfilter plates, then on Pall PRC 1 mL or 5 mL prepacked columns, and transferred to Pall LRC empty glass columns. For manufacturing scale, Pall offers a complete range of Resolute® columns from 28 cm to 2 m diameter.

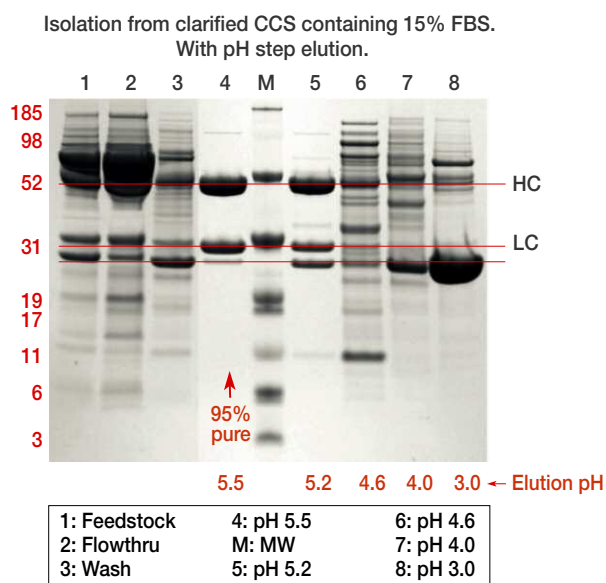
Application Examples

Application 1. Purification of Rat IgG from a “Protein-rich” Feedstock: Principle of Elution Optimization Using Decreasing pH Steps

A protein-rich feedstock (rat IgG in 15% Fetal Bovine Serum content) was selected to illustrate the impact of elution pH on antibody purity (see Figure 6). In a first series of experiments, the IgG fraction was eluted at pH 4.0; however, a broad range of impurities was also eluted at pH 4.0, including a truncated form of free light chain (TFLC), leading to medium (around 75%) purity of the target IgG. Then, a pH-step-elution was conducted at pH 5.5, 5.2, 4.6, 4.0 and 3.0. Using pH 5.5 elution, the IgG eluted purity was increased to 95% (the fraction contained 4% TFLC and was remarkably free of other impurities [Lane 4]). When pH was reduced to 5.2, desorption of an increased concentration of TFLC was prompted (Lane 5). When the pH was reduced to 4.6 and then to pH 4.0 (Lanes 6 and 7), impurity components were eluted. Finally, TFLC was eluted at pH 3.0 (Lane 8). Based on these findings, optimal recovery of the target antibody would be conducted at pH 5.5.

Figure 6

Purification of Rat IgG from “Protein-rich” Feedstock



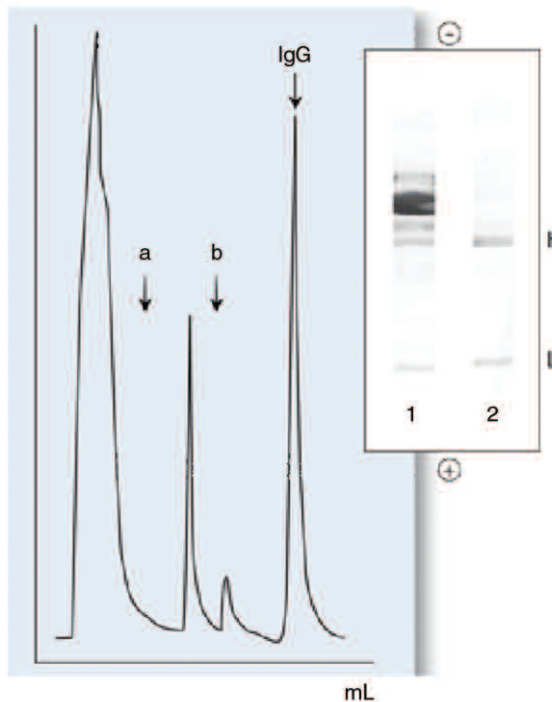
Data Courtesy of J. Ford and D. Conrad, Virginia Commonwealth University

Application 2. Laboratory Scale Purification of Monoclonal IgG from Ascites Fluid

MEP HyperCel sorbent was used to purify IgG from ascites fluid. 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. IgG was 83% pure with 79% yield. Purity of the IgG fraction could be increased by anion exchange chromatography on DEAE Ceramic HyperD® F sorbent.

Figure 7

Immunoglobulin Capture from Ascites Fluid



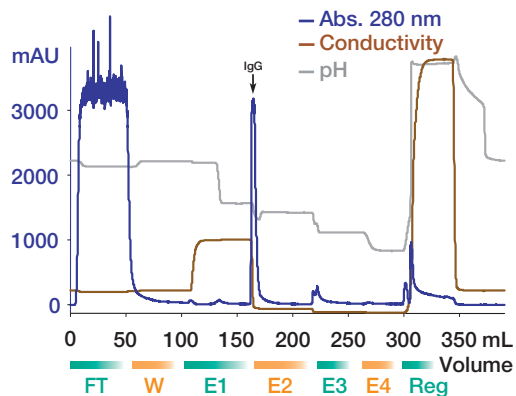
(a), (b) = Contaminant elution peaks after the 2 washing steps; equilibration: 50 mM Tris-HCl, pH 8; elution: 50 mM sodium acetate, pH 4.0; flow rate 70 cm/hr. Washing with water, followed by 25 mM sodium caprylate. SDS-PAGE (reduced conditions) analysis: (1) = crude sample; (2) = purified IgG.

Application 3. One-step Capture of Monoclonal Mouse IgG1 from “Protein-rich” (Albumin Containing) CHO (Chinese Hamster Ovary) Cell Culture Supernatant (CCS)

The example in Figure 8 demonstrates that MEP HyperCel sorbent can achieve one-step IgG purification with similar levels of purity and yield than Protein A sorbents, even when the CCS contains high amounts of albumin as major contaminant.

Figure 8

One-step Purification of IgG1 from Albumin-rich CHO Cell Culture Supernatant



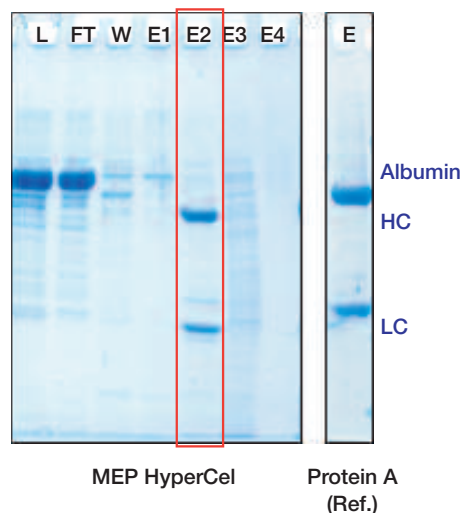
Equilibration/Wash: PBS pH 7.4

Elution: E1: 100 mM Na Ac pH 5.5, 0.5 M NaCl

E2: 50 mM Na Ac pH 5.0

E3; E4: 50 mM Na Ac pH 4.0; 3.0

Flow rate: 80 cm/h (Residence time = 7.5 min.)



CONCLUSION
In a single capture step:
• 97% Purity
• 93% IgG Yield

Application 4. Contaminant (HCP and DNA) Removal During a MAb Capture Step from CHO Cell Culture on MEP HyperCel Sorbent

MEP HyperCel sorbent was used to capture a MAb from a protein-free CHO cell culture supernatant. Results (Table 3) demonstrate a very efficient DNA removal (> 4.7 Log) and a 100-fold reduction in HCP. Further chromatographic step using ion exchange chromatography on Pall CM Ceramic HyperD F cation exchange sorbent did reduce the HCP content further (data not shown).

Application 5. Evaluation of MEP HyperCel Sorbent as a HIC Alternative for the Purification of an *E. coli* Recombinant Protein: Summary of Process Benefits

MEP HyperCel sorbent was used as a replacement of a Butyl resin in an *E. coli* recombinant protein purification sequence. Results summarized in Table 4 demonstrate that used at either step 2 or step 3 in the process, MEP HyperCel sorbent could reduce the amount of salt required for protein binding, resulted in better capacity and purity, and eliminated the need for the final time-consuming size exclusion step needed in the conventional first generation process.

Table 3

Contaminant Removal from CHO Cell Culture

Fraction	IgG Recovery (%)	IgG (ng/mL)	HCP (ppm)	HCP (Log10 reduction)	HCP (ng/mL)	DNA (ppm)	DNA (Log10 reduction)
Start Feedstock	100	92000	102000	-	705	781	-
MEP HyperCel Sorbent	93	8600	1200	1.9	< 0.1	< 0.014	> 4.7

DNA assay using Quant-IT[®] PicoGreen[®] dsDNA assay kit (Invitrogen); HCP assay using ELISA kit (Cygnus Technologies).

Table 4

Purification of E. coli Recombinant Protein Using MEP HyperCel Sorbent as HIC (Hydrophobic Interaction Chromatography) Alternative (Replacement of a Butyl Ligand)

	Conventional Process Including a HIC (Butyl) Step	Process Including MEP HyperCel Sorbent as HIC Replacement
Number of Chromatographic Steps in the Process	4 (including final size exclusion)	3 (saves the final size exclusion)
Salt Concentration Required for Protein Binding	3.5 M NaCl	2 M NaCl
Binding Capacity	Low	Good (10X higher than the HIC conventional resin)
Robustness	Not applicable	Excellent (11 fermentation runs)
Purity (C4 HPLC Assay)	Requires final SEC after the HIC step	High

HIC = Hydrophobic Interaction Chromatography

SEC = Size Exclusion Chromatography

Ordering Information

Product No.	Description	Size
12035-069	MEP HyperCel	5 mL
12035-010	MEP HyperCel	25 mL
12035-028	MEP HyperCel	100 mL
12035-036	MEP HyperCel	1 L
12035-040	MEP HyperCel	5 L
12035-044	MEP HyperCel	10 L
Available upon request	MEP HyperCel	> 10 L
PRC05X050MEPHCEL01	PRC Column 5x50 MEP HyperCel	Prepacked 1 mL of sorbent
PRC08X100MEPHCEL01	PRC Column 8x100 MEP HyperCel	Prepacked 5 mL of sorbent

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