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Product Datasheet

MEP HyperCel Mixed-Mode Chromatography Resin

Hydrophobic Charge Induction Chromatography | HCIC

Introduction

MEP HyperCel mixed-mode chromatography resin is a flexible chromatography resin 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 resin offers a specific mixed-mode or multi-modal separation mechanism, different from conventional ion exchange or affinity mechanisms, and is particularly effective as an alternative to conventional HIC. MEP HyperCel provides significant benefits when used at process scale. In contrast to conventional HIC, MEP HyperCel resin 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 resin is immunoglobulin-selective. It can

be used for direct capture or intermediate purification of IgG from various feedstocks in combination with other 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 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 resin contributes to a simplification of process development, saving of unit operations such as diafiltration or ultrafiltration, and lower waste disposal. Long resin life time is expected due to MEP HyperCel's resistance to harsh cleaning-in-place methods (0.5 to 1 M NaOH, 30 to 60 minutes contact time). All of these factors contribute to lower purifications costs.

Note: For MEP HyperCel 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 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 resins, IgG binding capacity on MEP HyperCel resin is essentially independent of subclass or species. "Weakly-binding" variants (e.g., murine IgG1 or Rat IgG) are well retained. MEP HyperCel resin contributes to HCP removal and virus clearance, and provides 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.

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 contaminant 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 resin'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.)

Protein Binding in No-salt or Low salt Conditions

Several families of "non-antibody" recombinant proteins have been purified using MEP HyperCel resin. In contrast to conventional HIC (e.g., Phenyl or Butyl ligands), protein binding to the resin 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 resin 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 can be used from laboratory to hundred-liter production-scale columns. The resin is available in a variety of packaging configurations as well as convenient PRC prepacked columns of 1 mL and 5 mL designed for resin screening, fast methods optimization and scale up. MEP HyperCel resin is supplied in 1 M NaCl containing 20% ethanol, and custom packaging is available on request.

Table 1: Main Properties of MEP HyperCel Resin

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 µmol/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 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.

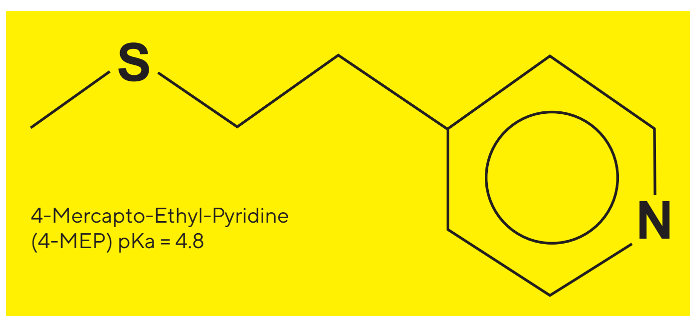


Figure 1: Structure of 4-MEP Ligand

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.

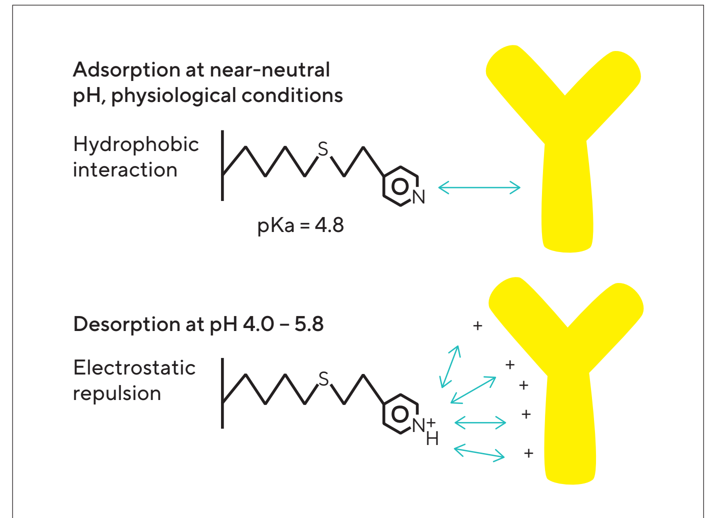


Figure 2: Antibody Binding and Elution on MEP HyperCel Resin

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 resin, the capacity of MEP HyperCel 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 resins, 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 resins, the key parameters influencing the binding capacity of MEP HyperCel include:

Residence Time

The residence time (RT) influences the yield | purity ratio and should be optimized on a case-by-case basis. 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 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 resins, still with lower salt concentrations.

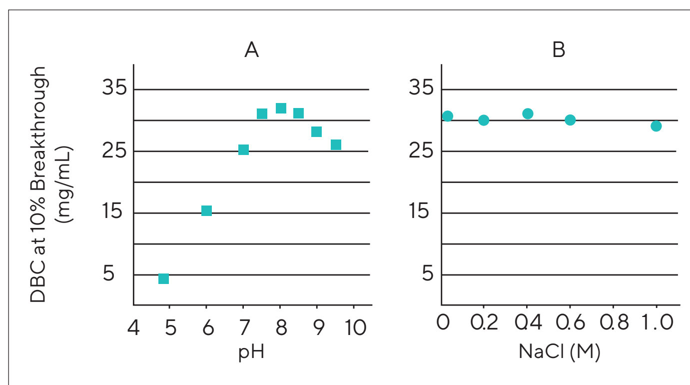


Figure 3: Influence of pH and Ionic Strength on the IgG Binding Capacity of MEP HyperCel Resin

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 × 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 resin therefore supports efficient capture of antibody from highly dilute feedstock, without preliminary concentration.

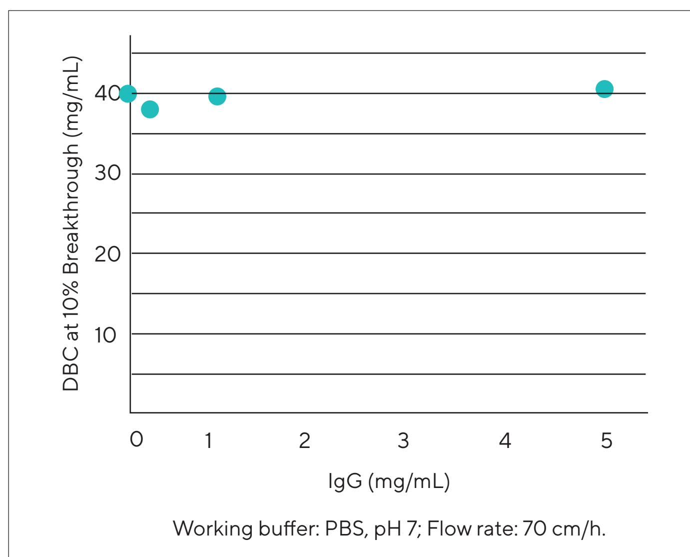


Figure 4: Influence of Human IgG Concentration on the Binding Capacity of MEP HyperCel

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

MEP HyperCel is immunoglobulin-selective, though 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). 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 (See Insert for Use).

Optimization Guidelines to Achieve Best IgG Purity

Initial evaluation of MEP HyperCel resin 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, and the nature and pI of the antibody, 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 is pH 2 to 12. However, for shorter contact times and cleaning in place, MEP HyperCel 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 resin 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 Product Information Insert USD 2518).

Methods Screening and Scale Up

The physical and chemical properties of MEP HyperCel resin are well suited to laboratory, pilot and process scale use. MEP HyperCel 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 along with HEA HyperCel and PPA HyperCel mixed-mode resins that carry aliphatic and aromatic synthetic ligands and provide additional chromatographic selectivities.

At Laboratory Scale or for Methods Development

Efficient separations can be achieved using 96-well filter plates or 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.

Pilot and Process Scale Applications

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

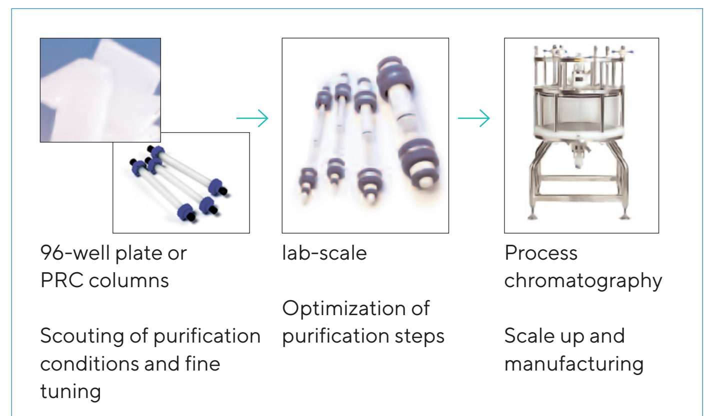


Figure 5: Screening and Scale-up Principles

Resin performance can be first screened using either microfilter plates, then on PRC 1 mL or 5 mL prepacked columns, and transferred to empty lab-scale columns. For manufacturing scale, Sartorius 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.

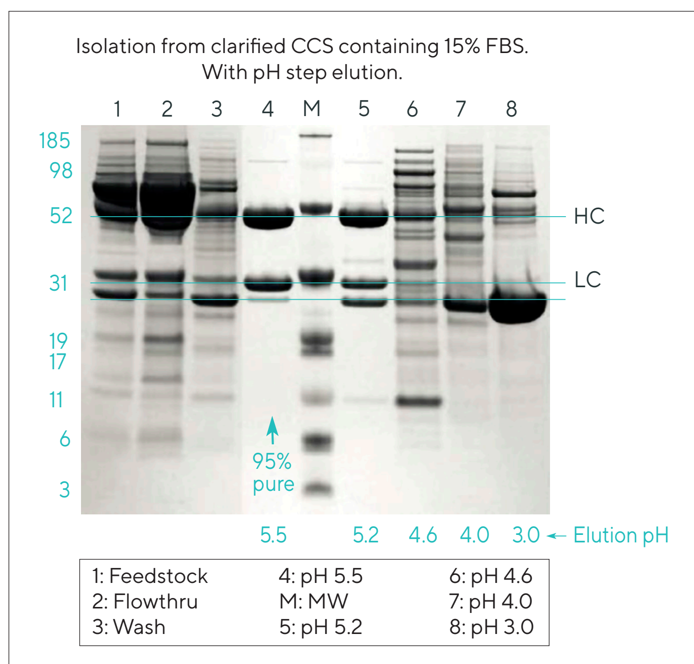


Figure 6: Purification of Rat IgG from "Protein-rich" Feedstock

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

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

MEP HyperCel 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 resin.

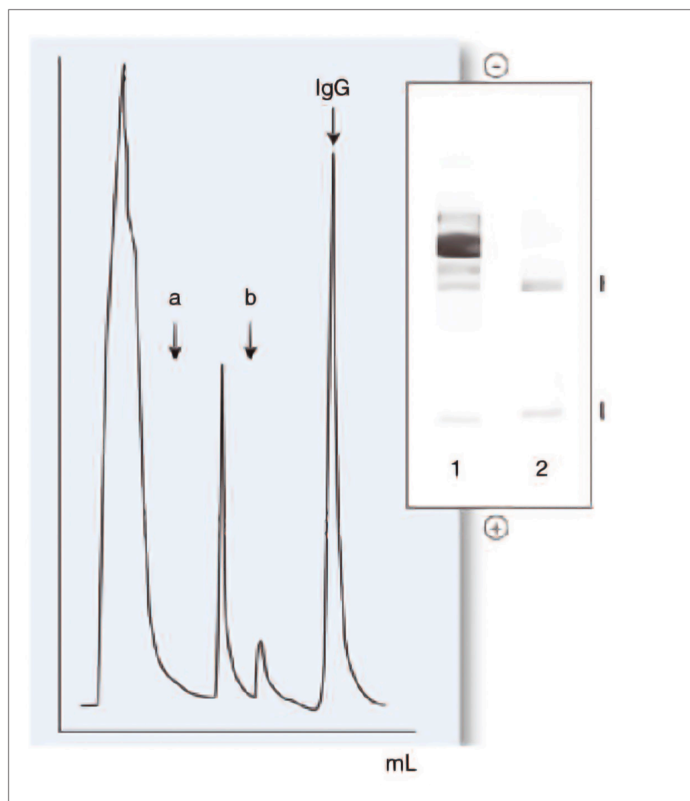
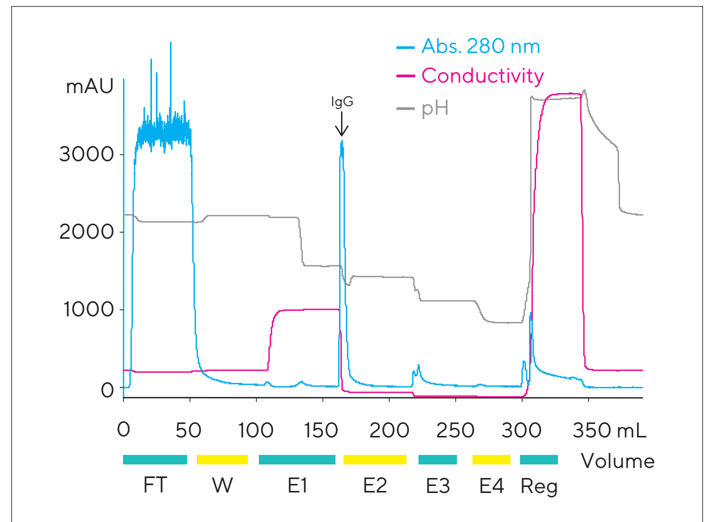


Figure 7: Immunoglobulin Capture from Ascites Fluid

(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 can achieve one-step IgG purification with similar levels of purity and yield to Protein A resins, even when the CCS contains high amounts of albumin as major contaminant.



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.)

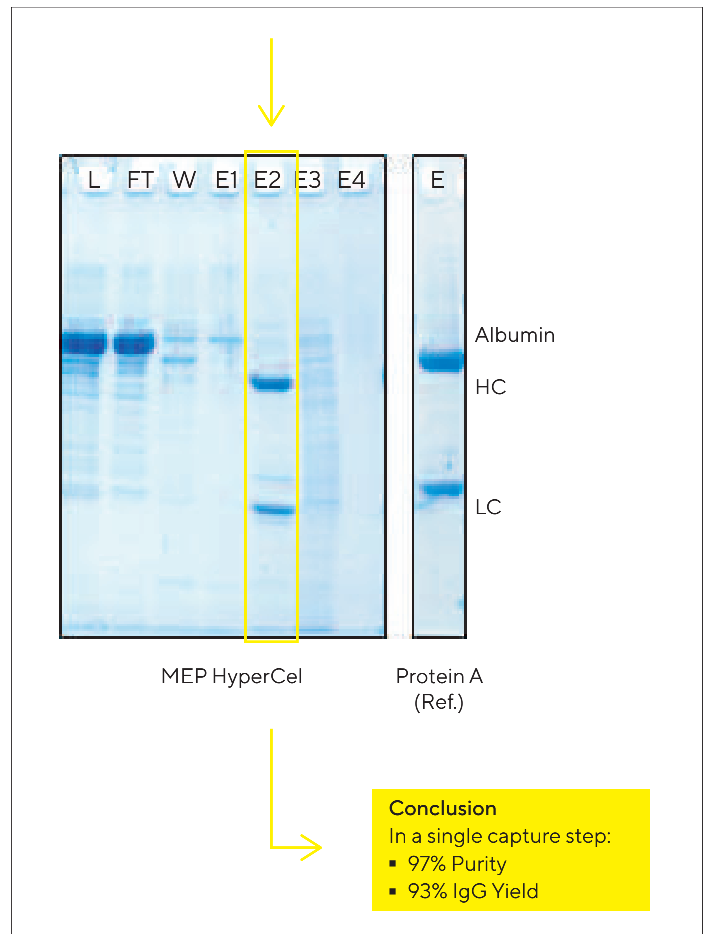


Figure 8: One-step Purification of IgG1 from Albumin-rich CHO Cell Culture Supernatant

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

MEP HyperCel 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.

A further chromatographic step using ion exchange chromatography on CM Ceramic HyperD F cation exchange resin reduced the HCP content further (data not shown).

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

MEP HyperCel resin 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 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 capture	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 as HIC Alternative (Replacement of a Butyl Ligand)

	Conventional Process Including a HIC (Butyl) Step	Process Including MEP HyperCel 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
12035-010	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
PRC05X050MEPHCEL	PRC Column 5 × 50 MEP HyperCel	Prepacked 1 mL of resin
PRC08X100MEPHCEL	PRC Column 8 × 100 MEP HyperCel	Prepacked 5 mL of resin
SR2MEP	Robocolumn MEP HyperCel 200 µL	row of 8
SR6MEP	Robocolumn MEP HyperCel 600 µL	row of 8

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