

MBI HYPERCEL™

Mixed-mode sorbent for direct capture of antibodies.

- Designed for direct capture of monoclonal and polyclonal IgG.
- Alternative to Protein A sorbents.
- Complement to Hydrophobic Charge Induction Chromatography (MEP HyperCel™).
- No albumin contamination if present in the feedstock.

MBI HyperCel™ is a high capacity, high selectivity, scalable mixed-mode sorbent, specially designed for the capture and purification of monoclonal and polyclonal antibodies from various sources. Complementing recently introduced approaches (HCIC – Hydrophobic Charge Induction Chromatography – on MEP HyperCel™), MBI mixed-mode sorbent provides alternative selectivity characteristics and expands the range of chromatographic options for proteins of various pI values or limited pH-stability. MBI HyperCel™ is particularly suited to separation of IgG sensitive to acidic elution.

MBI HyperCel™ can be used to separate antibodies from cell culture supernatant and from other non conventional sources such as sweet whey, milk from hyper-immunized animals, recombinant expression systems or from human plasma Cohn fractions (obtained by alcohol precipitation).

Mixed-mode ligand complements Hydrophobic Charge Induction Chromatography (MEP HyperCel™).

The MBI HyperCel™ ligand is a complement to the Hydrophobic Charge Induction Chromatography (HCIC) sorbent MEP HyperCel™ (refer to CIPHERGEN® Process Proteomics Product note LPN PN702-002). MBI HyperCel™ can be used alone as a direct capture step, or in a sequence, combined to other standard chromatographic steps (e.g. ion exchange).

Table I. MBI HyperCel™ Main Properties.

| | |
|---|--|
| Particle size | 80-100 µm |
| Dynamic binding capacity for hu IgG (10% breakthrough)* | 20-40 mg/ml |
| Ligand | 2-mercapto-5-benzimidazole sulfonic acid (MBI) |
| Working pH | Adsorption: pH 5.0-5.5 Elution: pH 8.0-9.5 |
| Cleaning pH | 3-14 |
| Pressure resistance | < 3 bar (44 psi) |
| Typical working pressure | < 1 bar (14 psi)** |

* Capacity varies according to the IgG type, the concentration and the residence time, i.e. for human polyclonal IgG (5 mg/ml) in a 50 mM sodium acetate, pH 5.5, capacity is equal to 25 mg/ml for 5 min residence time.

** With buffer, backpressures of <1 bar are obtained at linear flow rates of 500-700 cm/h.

HCIC (MEP) and mixed mode ligands (MBI) represent cost-effective alternatives to Protein A-based sorbents for the capture of antibodies. MEP HyperCel™ binds IgG at neutral pH, and typically elutes at slightly acidic pH (pH 4.0). MBI adsorbs antibodies at mildly acidic pH (typically in the pH range of 5.0-5.5), and IgG is eluted at alkaline pH (8.0-9.5). While operating at physiological ionic strength for IgG capture, MBI HyperCel™ gives the users more options to explore, according to the pI and pH-sensitivity of their specific antibody. This alkaline elution may preserve the biological activity of acidic-sensitive IgGs, or limit aggregation and precipitation during the purification process.



BioSeptra® Process Division

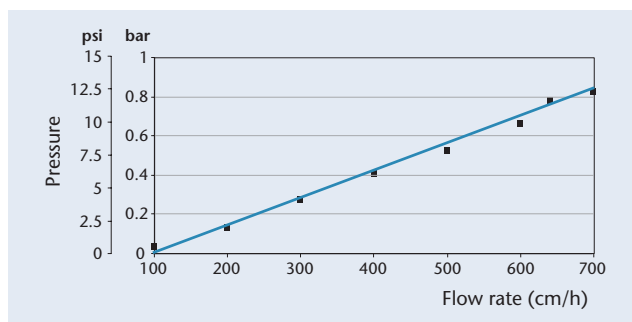
MBI HyperCel™ can be used at both laboratory or process-scale : the sorbent is easy to pack and run, and can be operated at low backpressure (<3 bar) in ml to multi-liter packings. See pressure vs flow rate curve in Figure 1.

Antibody selectivity of MBI HyperCel® and segregation between IgG and albumin.

2-mercapto-5-benzimidazole sulfonic acid (MBI) – see Figure 2 – used as a ligand for the adsorption of immunoglobulin G was deduced from an extensive number of synthetic ligands described in the literature. From this review, it appeared that heterocycles were among those molecules repeatedly described for the capture of antibodies. The MBI ligand has a sulfonate group present on the aromatic ring which is negatively charged over the recommended adsorption pH range (5.0 - 5.5). Most of the antibodies are positively charged around pH 5.5, whereas albumin is negatively charged and therefore does not interact by ionic effect. The separation between IgG and human albumin as a function of the adsorption pH is shown in Figure 3.

Experimental data based on protein binding capacity as a function of temperature and competition using inhibitors of hydrophobic associations (e.g. polyvinyl alcohol) suggests that hydrophobic associations between the spacer and albumin do not occur. Contrary to HCIC sorbents, the spacer and the ligand head are both hydrophilic in the MBI HyperCel™ sorbent.

Figure 1. Pressure vs. Flow Rate.



Column: 15.6 cm x 2.5 cm I.D., 50 mM sodium acetate, 0.14 M NaCl, pH 5.5.

Figure 2. Structure of the MBI ligand.

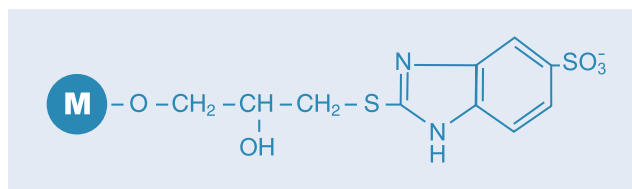
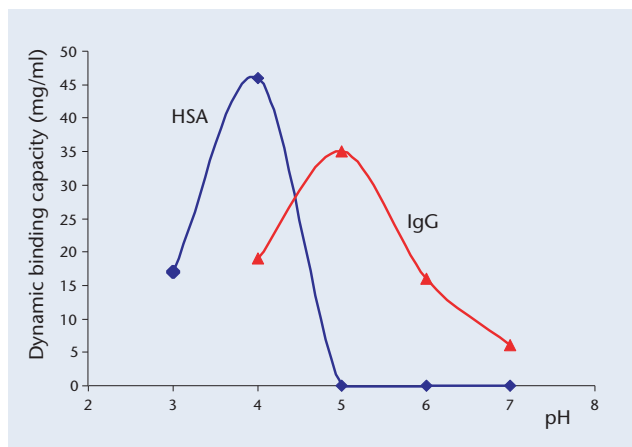


Figure 3. Effect of binding pH on discrimination between human IgG and albumin.



Data using solutions of pure HSA and hu IgG (5 mg/ml) shows that the highest binding capacity for human albumin is obtained at pH 4.0. HSA is not bound at pH values above pH 5.0, where the optimal binding capacities for IgG are found.

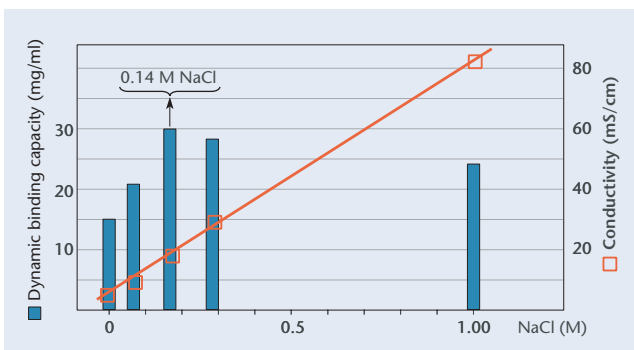
Dynamic binding capacity for IgG.

The capacity of MBI HyperCel™ for IgG depends on multiple parameters: column geometry and flow rate should be adjusted to allow an average residence time around 5 min to get an optimum capacity. Other important parameters include the nature and

concentration of the IgG itself as well as the relative isoelectric points of the IgG and feedstream proteic contaminants.

MBI HyperCel™ has a dynamic capacity of 20-40 mg/ml with concentrated hu IgG (standard residence times around 5 min and adsorption in 50 mM acetate, 0.14 M NaCl, pH 5.5). Higher capacities may be observed by increasing the residence time.

Figure 4. IgG dynamic binding capacity vs. ionic strength.



Hu IgG (5 mg/ml) in 50 mM sodium acetate, pH 5.5 containing 0 - 0.07 - 0.14 - 0.28 or 1 M NaCl.

1. Influence of pH.

The adjustment of the adsorption pH is a critical parameter to achieve the optimum purity and capacity performance. The MBI ligand does not normally co-adsorb albumin at pH 5.5 (pH usually recommended to start experiments). With pure concentrated IgG, the capacity of MBI at pH 5.5 is in the range of 30 mg/ml, as shown in Figures 3 and 5.

However, with crude feedstreams, this "standard" recommended adsorption pH of 5.5 may not lead to the best purity/capacity ratio. Therefore, a pH adjustment (typically in the pH range of 5-6) is recommended (refer to Table II).

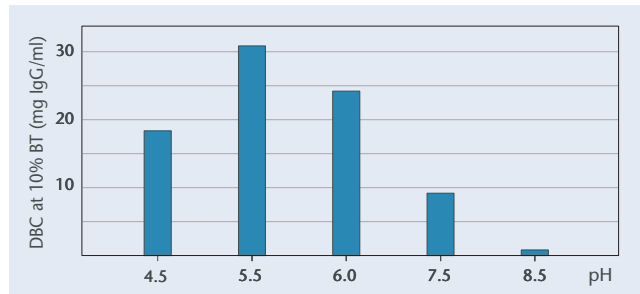
Table II. Guidelines for adsorption pH optimization according to feedstream composition and IgG concentration.

| Type of feedstream | Recommended binding pH |
|-----------------------------------|------------------------------|
| No albumin present | pH 5.0 |
| Albumin present IgG <250 µg/ml | pH 5.0 - pH 5.5 optimization |
| Albumin present IgG ≥250 µg/ml | pH ≥ 5.5 |

2. Influence of ionic strength.

MBI HyperCel™ is suited to direct capture of IgG at physiological ionic strength. The optimal capacity is typically obtained in the presence of 0.14 M NaCl, as shown in Figure 4.

Figure 5. IgG dynamic binding capacity vs. pH.



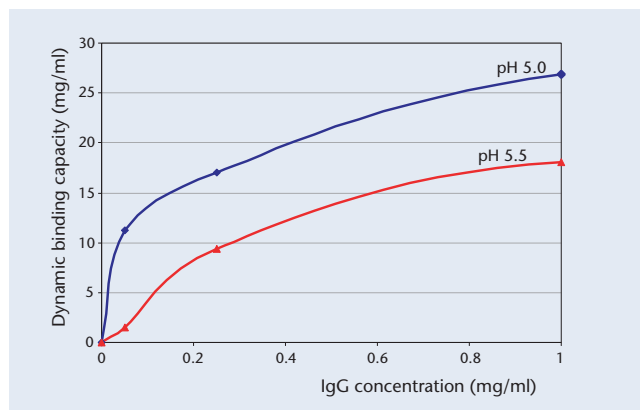
5 mg/ml hu IgG in 50 mM sodium acetate, 0.14 M NaCl, pH 5.5. Conductivity 17.5 mS/cm. Flow rate 100 cm/h.

3. Influence of IgG concentration.

MBI HyperCel™ capacity and purity performance may vary according to the IgG concentration in the feedstream. When using human polyclonal IgG at concentrations from 0.05 to 1 mg/ml, capacity at pH 5.0 was found higher than at pH 5.5, as shown in Figure 6.

When MBI HyperCel™ is used to capture dilute IgG (<250 µg/ml) from serum-supplemented feedstream, a binding pH optimization is recommended to find the best purity/capacity compromise (see Figure 9 and Table II).

Figure 6. Binding capacity as a function of IgG concentration.



0.05, 0.25 and 1 mg/ml hu IgG in 50 mM sodium acetate, 0.14 M NaCl, pH 5.0 or 5.5 containing 0.14 M NaCl.

Elution : recovery and purity factors

Purity values of 80-95 % (SDS-PAGE) are typical with crude feedstreams. Higher purities (95-98%) can be obtained in a single step when using protein-free cell culture supernatants. Elution is performed with alkaline buffers, in the pH range of 8.0 - 9.5. The nature of the elution buffer may influence the recovery, typically 50 mM sodium bicarbonate, pH 9.0 containing 0.14 M NaCl is recommended.

Stability, cleaning and regulatory support for industrial applications.

MBI HyperCel[™] is stable to repeated cleaning with 1 M sodium hydroxide. In most cases, 30 to 60 min wash procedures using 0.5-1 M NaOH are sufficient for cleaning and column regeneration.

Ligand and sorbent toxicity: a study performed according to ISO 10993-5 (1992) standard showed the absence of toxicity of the MBI HyperCel[™] sorbent.

For industrial applications, a validation regulatory package is available, please contact CIPHERGEN.

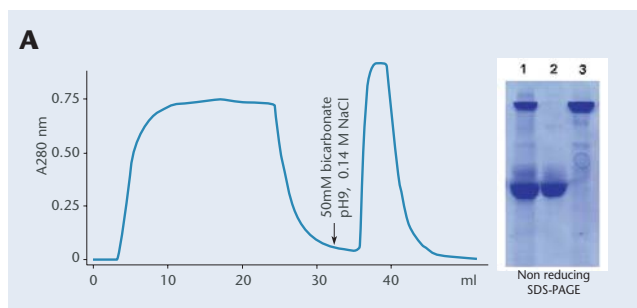


APPLICATION EXAMPLES

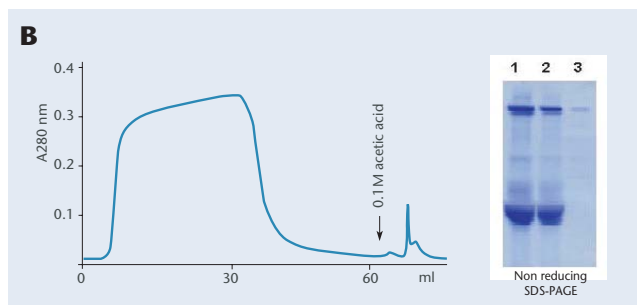
Example 1. Purification of Rat monoclonal IgG from a hybridoma cell culture containing 10% serum – a comparison with Protein A.

MBI HyperCel[™] allows an efficient capture of Rat IgG in the presence of fetal bovine serum, in contrast with Protein A: IgG purity was estimated higher than 95% by SDS-PAGE. Albumin from serum was not bound by MBI HyperCel[™], no trace of IgG was found in the MBI HyperCel[™] column flowthrough.

Figure 7. Separation of Rat IgG on MBI HyperCel[™] (A) and on a Protein A-based sorbent (B).



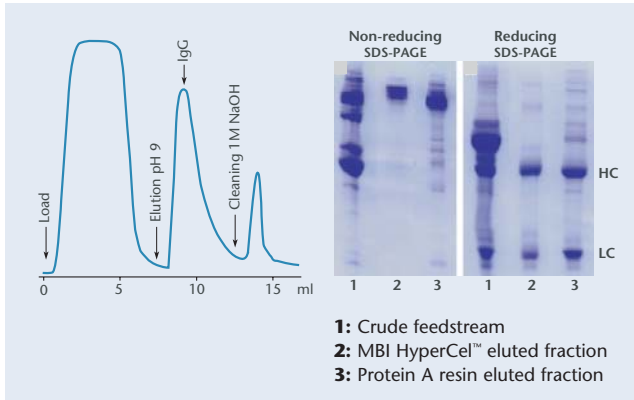
Load: 19 ml sample, adjusted to pH 5.2 by addition of acetic acid.
Wash: 50 mM acetate buffer, 0.14 M NaCl, pH 5.2.
Elution: 50 mM bicarbonate buffer, 0.14 M NaCl, pH 9.0.
1 = Crude feedstock. 2 = Column flowthrough. 3 = Eluate.



Load: Direct sample load on Protein A Ceramic HyperD[®] F. Wash: PBS. Elution: 0.1 M acetic acid.

Example 2. Purification of monoclonal IgG₁ from ascites fluid.

Figure 8. Mouse Monoclonal IgG₁ purification on MBI HyperCel™.



MBI HyperCel™ chromatography resulted in an efficient capture of Mouse IgG from a complex mixture such as ascites fluid. Purity was higher than that obtained on Protein A Ceramic HyperD® F and albumin was not bound by MBI HyperCel™.

Example 3. Use of MBI HyperCel in a three-step sequence as alternative to Protein A for clinical grade IgG purification.

MBI HyperCel™ was evaluated as a potential replacement to a conventional Protein A sorbent in a three-step “generic” purification. IgG purity, host cell impurity removal and Protein A leakage were evaluated. Data suggests that MBI HyperCel™ capture step is compatible with the needs of preclinical antibody purification.

Table III. Use of MBI HyperCel™ in a three-step sequence as alternative to Protein A for clinical grade IgG purification.

| Process | IgG purity (%) | HCP (ng/mg IgG) | Protein A (ng/mg IgG) |
|---------------------------------|----------------|-----------------|-----------------------|
| MBI HyperCel + IEX1 + IEX2 | 99.3 | 3.52 | NR |
| Protein A sorbent + IEX1 + IEX2 | 99.9 | < 0.64 | < 0.15 |

Data courtesy of Dr. Philippe Marschal, Novartis Pharma AG.
IEX = Ion Exchange step, HCP = Host Cell Proteins, NR = Non Relevant.

Example 4. Capture of Mouse monoclonal IgG₁ from a low expression hybridoma cell supernatant – Influence of adsorption pH.

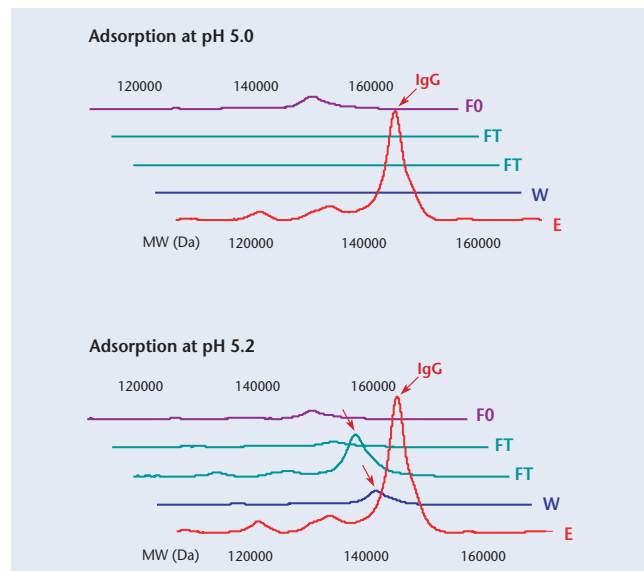
Low expression Mouse IgG₁ cell culture supernatant (150 µg/ml) was loaded on MBI HyperCel™ at two different adsorption pH (5.0 and 5.2). Figure 9 shows the RC-SELDI-MS analysis of chromatography fractions on NP20 ProteinChip® Array.

The data shows that the capture of this low IgG₁ titre can be performed on MBI HyperCel™ at both pH 5.0 or 5.2, but differences in recoveries exist according to the binding pH.

When adsorption is performed at pH 5.2, some loss of IgG is found in the flowthrough (FT) and in the wash (W).

In contrast, when pH 5.0 is used during the adsorption step, a better capture is observed. IgG is eluted in one fraction (pH 9.0 elution), and no IgG loss is found. This data highlights the importance of fine-tuning the pH in order to achieve the best capture and recovery efficiencies when using dilute IgG.

Figure 9. Capture of Mouse monoclonal IgG₁ from a low expression feedstream – Influence of adsorption pH (RC-SELDI-MS analysis of eluted fractions from MBI HyperCel™ column).



Sample: 150 µg/ml IgG₁ in cell culture supernatant, protein-free medium; 40 ml of cell culture supernatant (CCS) are filtered and loaded on an MBI HyperCel™ column (10 cm x 0.3 cm ID). Equilibration buffer: 50 mM sodium acetate, 0.14 M NaCl, pH 5.0 or 5.2. Elution buffer: 50 mM sodium bicarbonate, 0.14 M NaCl, pH 9.0. Column regeneration and cleaning: 1 M NaOH (10 CV). Linear flow rate : 150 cm/h; Residence time: 4 min. Analysis performed on normal phase (NP20) ProteinChip® Arrays. F0 = load, FT = flowthrough, W= wash, E= elution.

Example 5. Purification of polyclonal IgG from Human plasma Cohn fractions on MBI HyperCel™ and comparison with a Protein A sorbent and MEP HyperCel™.

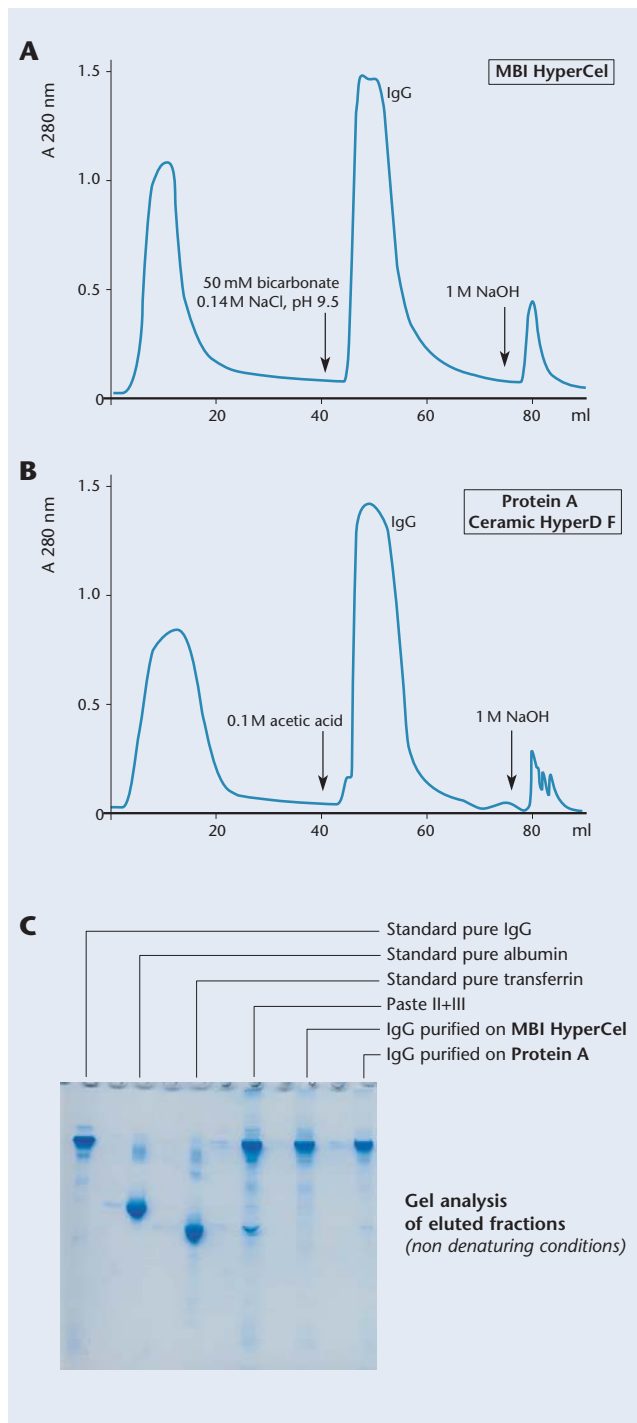
Figure 10 shows the separation of polyclonal IgG from human plasma Cohn Fraction II+III on MBI HyperCel™ and Protein A Ceramic HyperD® F. Fraction II+III is a protein-rich, 19% ethanol precipitated fraction, containing mainly immunoglobulins, transferrin and α and β -globulins. IgG are estimated to represent about 40% of total soluble proteins in this fraction.

In Figure 10A, IgG was captured on MBI HyperCel™ in 50 mM acetate, 0.14 M NaCl at pH 5.5 and totally desorbed in 50 mM bicarbonate, 0.14 M NaCl at pH 9.5. A cleaning step using 1 M sodium hydroxide resulted in elution of a large amount of non-specifically adsorbed proteins.

Figure 10B shows the same separation performed on a Protein A Ceramic HyperD® F sorbent, where IgG were adsorbed in PBS and eluted by 0.1 M acetic acid, pH 3.0.

Figure 10C shows the SDS-PAGE of the eluted fractions in non-reducing conditions: purity of polyclonal antibodies was estimated to be about 90% in the MBI HyperCel™ eluate, and about 95% with the Protein A sorbent.

Figure 10. Purification of polyclonal antibodies from human plasma Cohn fraction.



Purification on MBI HyperCel: Equilibration: 50 mM acetate, 0.14 M NaCl, pH 5.5; Elution: 50 mM bicarbonate buffer, 0.14 M NaCl, pH 9.5
Purification on Protein A Ceramic HyperD F: Equilibration: PBS ; Elution: 100 mM acetic acid, pH 3.0.
 Column dimensions: 11 mm ID x 65 mm (6.5 ml sorbent); Protein load injected around 120 mg, linear flow rate: 50 cm/h.
 A column cleaning was performed in each case using 1 M sodium hydroxide.
 Sample courtesy of Dr. Andrea Buchacher, Octapharma, Austria.

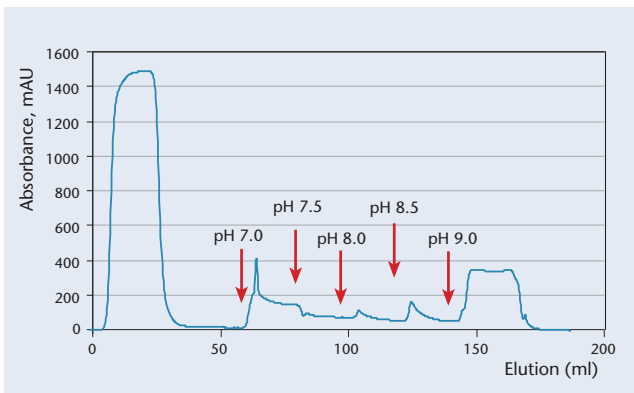


BIOSEPRAR[®] MBI HYPERCEL[™]

Example 6. Separation of IgG light and heavy chains.

Figure 11A shows the chromatographic profile corresponding to the purification of a humanized monoclonal IgG₁ expressed in CHO cells which were grown in a protein-free cell culture medium. Figures 11B and 11C show RC-SELDI-MS profiles of the fractions collected along the chromatography: heavy and light chains are collected at pH 7.0 and 7.5, whereas the intact antibody was primarily eluted at higher pH (8.0 - 8.5).

Figure 11A. Purification of a monoclonal humanized IgG_{1α} from CHO cells in a protein-free medium on MBI HyperCel™ chromatography sorbent.



Conditions (Figures 11A, 11B & 11C) : 1 cm ID column; 7 ml of sorbent; Flow rate: 100 cm/h. Equilibration/Wash: 50 mM sodium acetate with 0.14 M NaCl, pH 5.2. Step elutions using 50 mM Tris buffer at pH 7.0, 7.5, 8.0, 8.5 and 9.0.

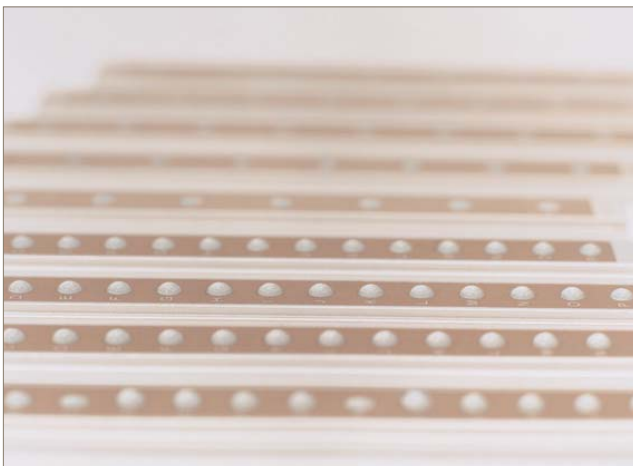


Figure 11B. Purification of a monoclonal humanized IgG_{1α} from a CHO cell line grown in protein-free medium (stirred reactor tank). Analysis using RC-SELDI-MS of collected fractions during chromatography on MBI HyperCel™ on an NP 20 ProteinChip® Array.

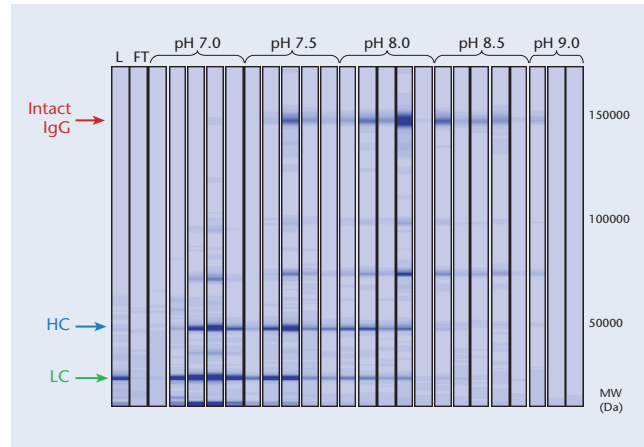
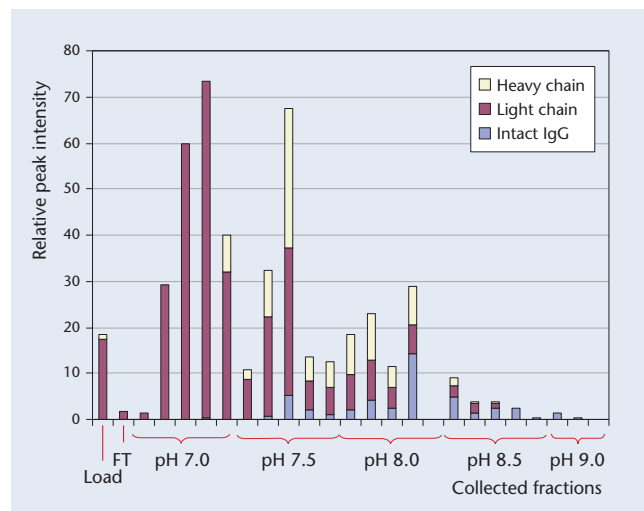


Figure 11C. Separation of light and heavy chains from intact antibody using pH step elution on MBI HyperCel™.



Ordering Information

| Product | Cat. No. | Size |
|--------------|-----------|--------|
| MBI HyperCel | 20194-069 | 5 ml |
| | 20194-010 | 25 ml |
| | 20194-028 | 100 ml |
| | 20194-036 | 1 L |

Bulk quantities available on request.

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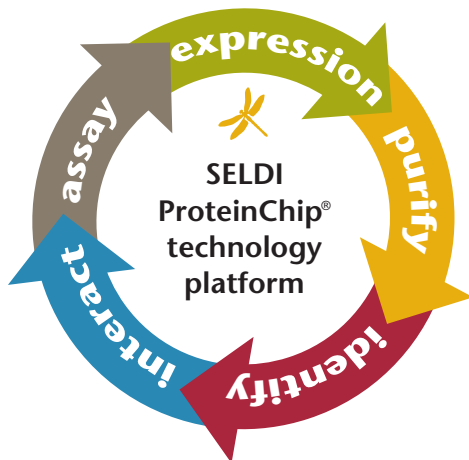
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