SARTORIUS

Product Datasheet

HEA and PPA HyperCel Resins

Mixed-mode Chromatography for Protein Separation

Benefits

Take advantage of the benefits mixed mode resins:

- Purify proteins at low ionic strength by direct hydrophobic capture
- Separate challenging mixtures with new ligand selectivities
- Be orthogonal to ion exchange or other chromatography steps

Product Information

HEA and PPA HyperCel resins are industry-scalable chromatography resins designed for protein capture and impurity removal in a biopharmaceutical environment. Operating on a “mixed-mode” mechanism, their behavior is based on a combination of electrostatic and hydrophobic interactions of the proteins with the ligands. HEA and PPA HyperCel resins provide unique selectivities, different from those given by ion exchange or conventional HIC (hydrophobic interaction chromatography), that can be screened to facilitate process development.

For example, the mixed-mode interaction mechanism can be exploited to achieve discrimination of protein isoforms, or proteins having similar or very close isoelectric points, separations which usually cannot be achieved by conventional methods. The resins' mechanical stability allows their use at high flow rates in laboratory to production-scale columns (see Figure 2 for pressure vs. flow rate data).

Product Description

HEA and PPA HyperCel resins are members of a family of chromatography mixed-mode resins, complementing MEP HyperCel (Hydrophobic Charge Induction). HEA and PPA HyperCel carry synthetic ligands, immobilized on HyperCel resin, a mechanically stable base matrix currently used in >100 L columns for the production of proteins.

The ligands include aliphatic (HEA – hexylamine) and aromatic (PPA – phenylpropylamine) amines (see Figure 1), which offer different selectivity and hydrophobicity options (refer to Figures 4 and 5).

Technical Data

Table 1: Main Properties of HEA and PPA HyperCel Resins

Particle Size	80–100 µm (avg)
Bead Composition	High porosity cross-linked cellulose
Dynamic Binding Capacity for BSA (10% breakthrough)[1]	40–60 mg/mL
Ligand: Aliphatic (HEA) Aromatic (PPA)	 Hexylamine Phenylpropylamine
BSA Recovery%	≥ 90
Working pH Cleaning pH	2–12 1–14
Pressure Resistance	< 3 bar (44 psi)
Typical Working Pressure	< 1 bar (14 psi)

[1] Determined using 5 mg/mL BSA in PBS, flow rate: 100 c m/h.

Structure of HEA and PPA Ligands

- HEA HyperCel → n-hexyl substituent

  $-CH_2-(CH_2)_4-CH_3$

- PPA Hypercel → phenylpropyl substituent

  $-CH_2-CH_2-CH_2$ 

Ligand density > 50 µmoles/mL
Critical working ligand pKa = 8

Figure 1

Principles of Operating Mechanism and General Guidelines

(Refer to product insert for details on column packing, buffers and recommendations.)

Protein Binding

Protein binding is usually achieved at neutral pH (i.e., PBS, pH 7.4), principally by hydrophobic interaction. Binding of very basic proteins may require increased pH (pH 9.0) (See lysozyme binding to HEA HyperCel resin, Figure 7).

At salt concentrations recommended for binding, there is limited ion exchange binding. Unlike traditional HIC, binding occurs at low ionic strength, in near physiological conditions. In general, no addition of lyotropic or other salt is required; however in some cases, the addition of moderate quantities of salt (e.g., 0.5 M ammonium sulphate) promotes protein adsorption (see Figure 6).

PPA HyperCel carries an aromatic ligand and has a stronger hydrophobicity than HEA HyperCel. The binding capacity is a function of the protein. For protein models like BSA, typical capacities of 40 to 60 mg/mL are obtained (PBS, pH 7.4, 0.14 M NaCl buffer, flow rate 100 cm/h) for HEA and PPA HyperCel resins. The factors that affect capacity include temperature (see Figure 3), residence time, isoelectric point, hydrophobicity of the target protein, and the quality of column packing. (Using PRC prepacked columns is recommended for screening).

Protein Elution

Protein elution is driven by electrostatic charge repulsion, as pH is reduced to values below the pI of the protein and below the pKa of the ligand. Elution is triggered by reducing the pH (from 7 to 2) because some proteins can be eluted without any change in pH just by decreasing the salt concentration. At laboratory scale, optimization can be achieved by descending salt gradient elution experiments, while stepwise elution will be selected at process-scale. This approach can also serve to resolve the target protein from impurities whose hydrophobic characteristics differ. Basic proteins will desorb earlier in the pH gradient or step-elution sequence, followed by more acidic proteins (see Figure 7).

Unlike traditional HIC, the target protein is recovered in dilute buffer, reducing the need for intermediate diafiltration, saving unit operations and contributing to better process economics.

Pressure vs. Flow Rate

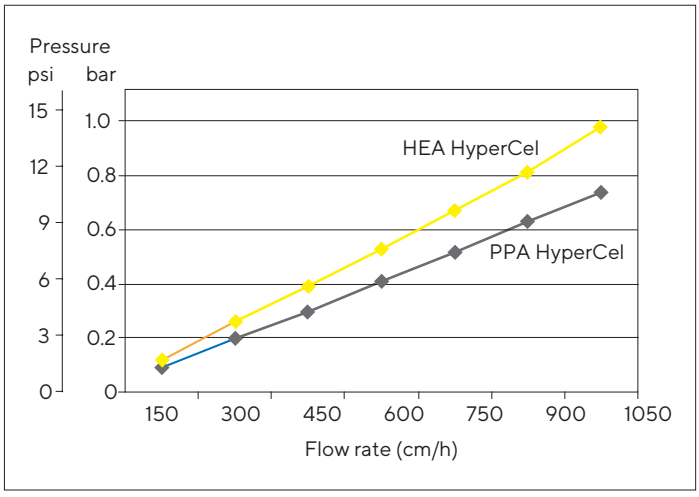

Figure 2: Column: 16 mm I.D. × 20 cm height. HEA and PPA HyperCel resins provide linear pressure/flow behavior and have characteristics well suited to process-scale operations in low pressure columns.

Effect of Temperature on BSA Binding Capacity

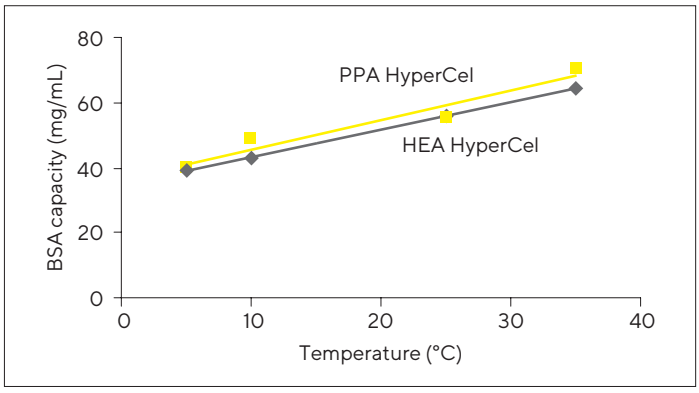

Figure 3: Model protein: BSA (5 mg/mL) bound to HEA and PPA HyperCel resins (7 mL column, 100 cm/h) in PBS, pH 7.4.

This experiment, as shown in Figure 3, demonstrates the dominant hydrophobic interaction component of protein binding to HEA and PPA HyperCel resins. The binding of proteins by HIC is entropy-driven, and the interaction increases with rise of temperature, as shown by the binding capacity increase for BSA. In practice, for robustness and capacity optimization studies, special attention should be given to keep buffer and operation room temperatures consistent.

Comparison between HEA and PPA HyperCel, MEP HyperCel, Conventional HIC and Anion Exchange Resins

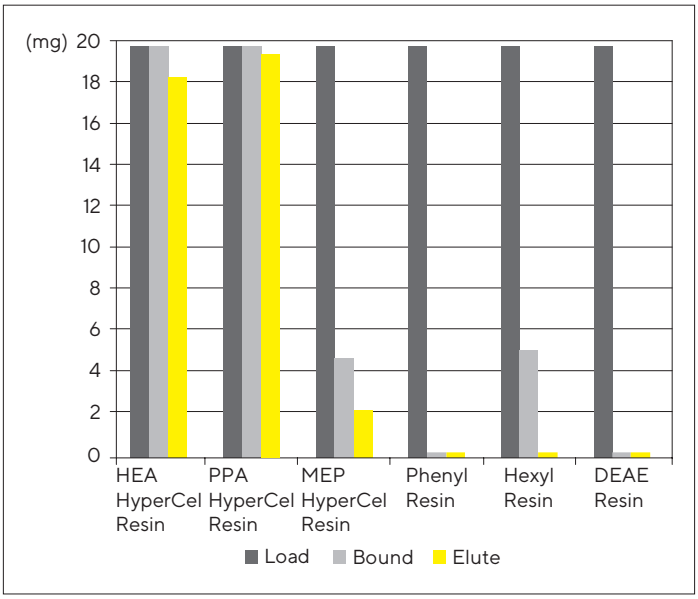

Figure 4: The adsorption | desorption of Bovine Serum Albumin (BSA), in PBS buffer, pH 7.4 on various resins. See text for details.

A series of experiments with various standard proteins was carried out to show the selectivity differences of HEA and PPA HyperCel resins compared to MEP HyperCel resin (HCIC – Hydrophobic Charge Induction Chromatography), conventional HIC resins (Phenyl and Hexyl ligands), as well as anion exchange resins (DEAE). Figure 4 illustrates the adsorption | desorption of Bovine Serum Albumin (BSA), in PBS buffer, pH 7.4. Data shows that BSA is efficiently retained on both HEA and PPA HyperCel resins, but is poorly retained on MEP HyperCel resin. In PBS, without salt addition, low binding of BSA to both Phenyl and Hexyl HIC resins is observed. The anion exchange (DEAE) resin also did not bind BSA at these non-optimal conditions (pH 7.4 and too high salt concentration).

Selectivity Difference between HEA and PPA HyperCel Resins: Adsorption | Desorption of a-chymotrypsinogen A

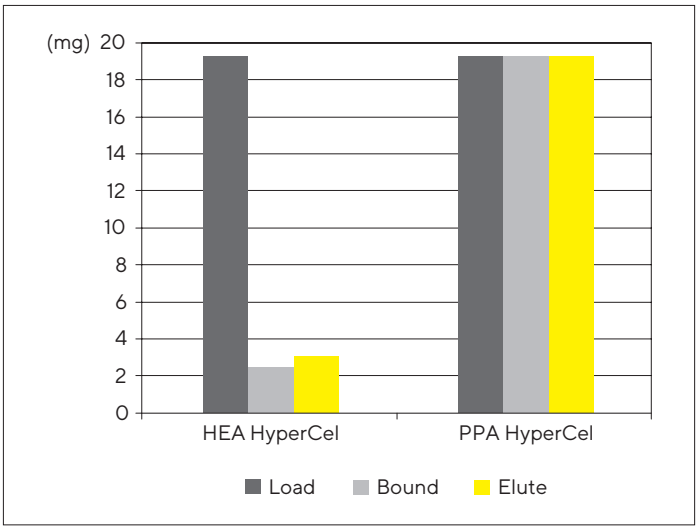

Figure 5: HEA and PPA ligands are of different nature (respectively aliphatic and aromatic). This translates in differences in selectivity for various proteins. Figure 5 illustrates the binding and elution properties for a-chymotrypsinogen A (2 mg/mL, binding in PBS, pH 7.4, 0.14 M NaCl), elution in 0.02 M sodium acetate at pH 5.0, 4.0 or 3.0. Data suggests a stronger binding of the model protein to PPA HyperCel resin, as well as a good recovery (elution at pH 4.0 is the most efficient).

BSA Binding Capacity of HEA and PPA HyperCel Resins in the Presence of 1.7 M ammonium sulphate

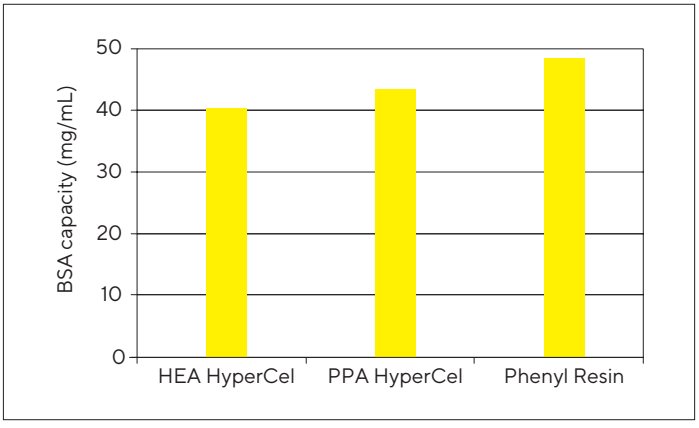

Figure 6: Binding of BSA to HEA and PPA HyperCel under classic HIC binding conditions, 1.7M ammonium sulfate.

As shown in Figure 6, HEA and PPA HyperCel resins can be used in "conventional" hydrophobic interaction conditions, in the presence of lyotropic salt-like ammonium sulphate or other salt. Data shows that BSA binding capacity in these conditions is close to the capacity of a conventional HIC resin (phenyl resin). Lower concentrations of salt (0.5 M ammonium sulphate) have been used to promote stronger adsorption of recombinant proteins (data not shown).

Application Examples

Example 1. Separation of a Protein Mix on HEA and PPA HyperCel Resins

HEA and PPA HyperCel resins have different retentivities and selectivities for proteins and should be screened at early stages during process development. The example in Figure 7 illustrates the chromatographic profiles obtained with a reference protein mixture applied to columns of 1.1 cm I.D. × 7 cm length. A step-elution sequence was performed followed by gradient elution from pH 5.4 to 2.6; all conducted using sodium phosphate | citrate buffers. Data shows that a very basic protein – lysozyme – does not bind to PPA HyperCel resin at pH 7.4, and is found in the flowthrough; in contrast, when raising the binding pH to 10.0 by reducing the ionic repulsion, lysozyme can be retained on HEA HyperCel resin.

In practice, for a protein of unknown pI and hydrophobicity, screening of the two ligands at different pH and salt concentrations is needed, and can be conveniently done by using ready-to-use 1 mL or 5 mL PRC prepacked columns.

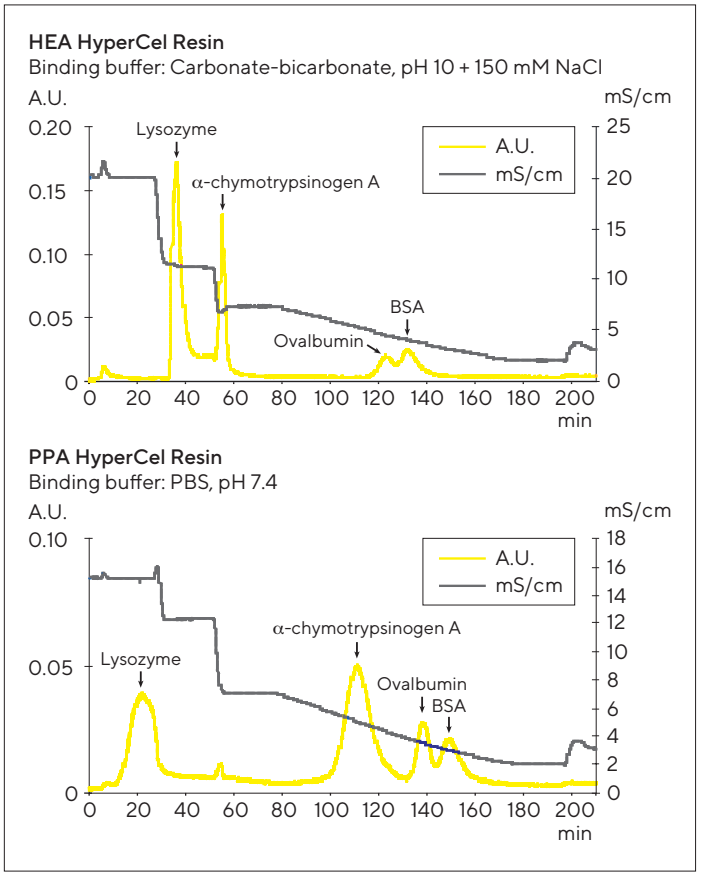

Figure 7: Sample volume: 1 mL; Column volume: 7 mL; Proteins: BSA, ovalbumin, lysozyme, a-chymotrypsinogen A, at a concentration of 2 mg/mL each.

Example 2. Separation of Recombinant GST (glutathione S transferase) from filtered E.coli Lysate on HEA HyperCel PRC Prepacked Columns of 1 mL, 5 mL and 2 × 5 mL Connected in Series

The example in Figure 8 illustrates the reproducibility of a real feedstock separation using PRC prepacked columns of different volumes (1 mL and 5 mL) and connected in series (2 × 5 mL). The lysate was diluted 10-fold with 50 mM Tris-HCl pH 8.0 buffer, and elution carried by pH steps at pH 5.0, 4.0 and 3.0. Data shows that the chromatograms are perfectly overlayed, illustrating the consistency of the separations on the three PRC columns or assemblies tested.

Purity analysis by SDS-PAGE showed an equivalent purity level for the eluted fractions in all cases (see Figure 9).

Scale up of rGST on PRC Prepacked Columns

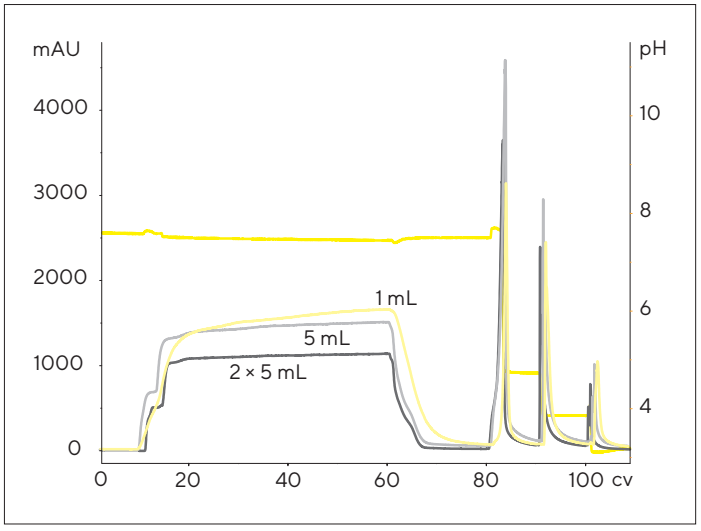

Figure 8

SDS-PAGE of the elution fractions (NuPAGE™ Novex™ 4–12% Bis-Tris gel, Coomassie staining)

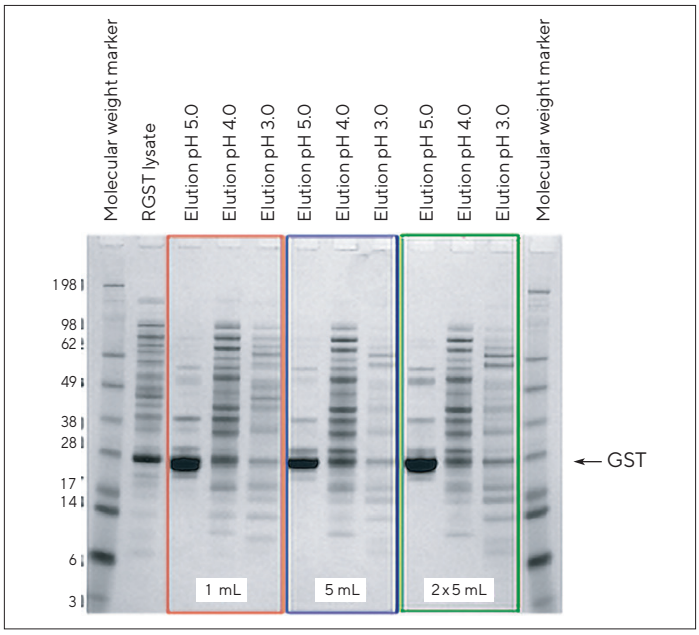

Figure 9

Example 3. Separation of Partially-purified Polyclonal IgG from Major Plasma Impurities on HEA HyperCel Resin

The objective of this experiment was to separate a target IgG from contaminants present in the feedstream. Three different mixed-mode resins – HEA, PPA, and MEP HyperCel resins – were tested.

Figure 10 shows only the chromatogram obtained with HEA HyperCel resin, which gave the best results in this case; the IgG (main elution, E2) was well separated from HSA (elution E3). Additionally, molecular-mass based analysis using mass spectrometry (data not shown) demonstrated resolution from lower molecular weight contaminants (elution E1), as well as a partial separation of IgA, co-eluting with the HSA peak.

HEA HyperCel Resin Chromatogram

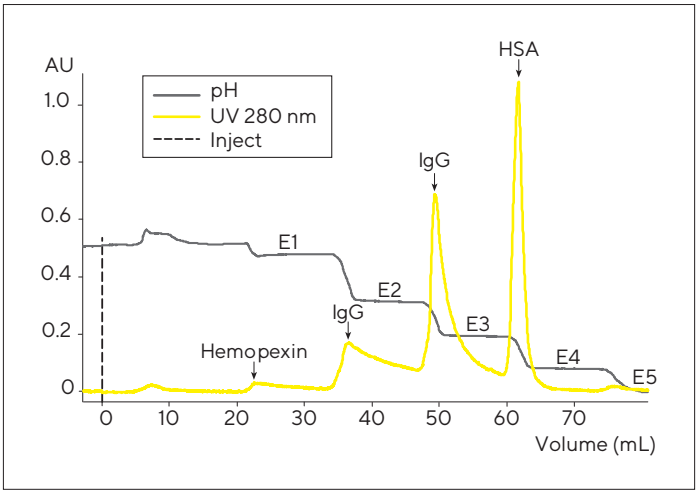

Figure 10: Column: 0.66 cm I.D. × 7 cm height; Resin volume: 2.4 mL. Run at 100 cm/h; Equilibration in PBS, pH 7.4; Loading 5 mL of a partially-purified human polyclonal IgG (60% purity) at 3.8 mg/mL, pH 8.4, and 8.3 mS/cm; Wash with 5 CV in PBS; Elution in 0.2 M sodium phosphate/100 mM citric acid, pH 7.0 (Elution 1), pH 5.4 (Elution 2), pH 4.4 (Elution 3), pH 3.4 (Elution 4), pH 2.6 (Elution 5); Regeneration in 1M NaOH.

Example 4. Separation and Resolution of Low Molecular Weight Recombinant Protein Isoforms on PPA HyperCel Resin

Sample Courtesy of Dr. Giovanni Magistrelli, NovImmune, Plan les Ouates, Switzerland

This example illustrates the unique resolution power of mixed-mode chromatography resins (PPA HyperCel resin in this case) to capture and discriminate isoforms of recombinant proteins expressed at low concentrations in cell culture supernatants.

The supernatant was loaded on the PPA HyperCel resin column at pH 7.4 (PBS), and fractions eluted at pH 5.0, 4.0 and 2.6.

Figure 11 shows a SELDI-MS analysis (confirmed by ELISA assays, not shown) indicating that a first population of isoforms with molecular weight (MW) between 8.0 and 8.3 kDa are eluted first and another population of isoforms with MW > 8.5 kDa are eluted next. Proteins with minor differences can therefore be at least partially separated using mixed-mode chromatography.

SELDI Molecular Mass Analysis (Da) of the Fractions Eluted at 115 mL (grey) and 125 mL (orange) from HEA HyperCel Resin

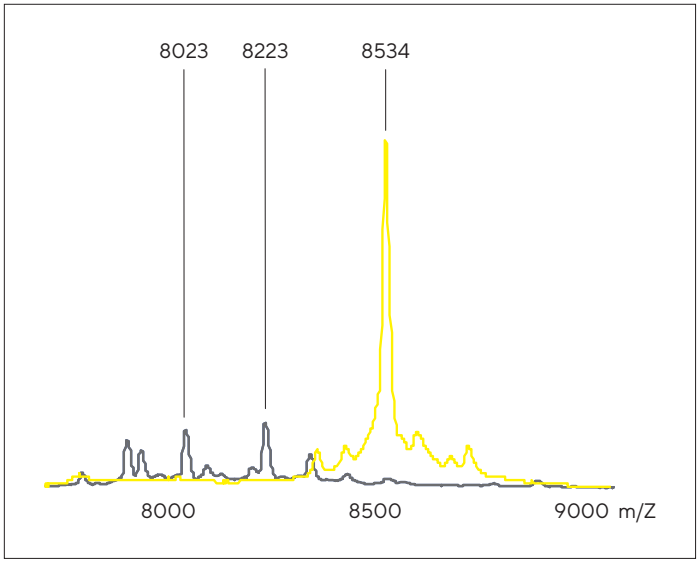

Figure 11

Example 5. Purification of Recombinant F(ab')$_2$ Fragment on HEA HyperCel Resin

Courtesy of X. Santarelli and J. Pezzini, ESTBB, Bordeaux, France

HEA HyperCel resin was used as a capture chromatography step to purify a recombinant F(ab')$_2$ fragment obtained through baculovirus expression in SF9 insect cells. Sample was loaded on HEA HyperCel resin at pH 6.0, 4.0 and 2.0, and analysis done by SDS-PAGE, ELISA and BCA assays. Results shown in Figure 12 indicate that no protein was found in the flowthrough or elution at pH 6.0. The F(ab')$_2$ fragment eluted at pH 4.0 (recovery 82%, 39-fold purification factor), while HCP (Host Cell Proteins) eluted at pH 2.0.

Analysis of Load and Elution

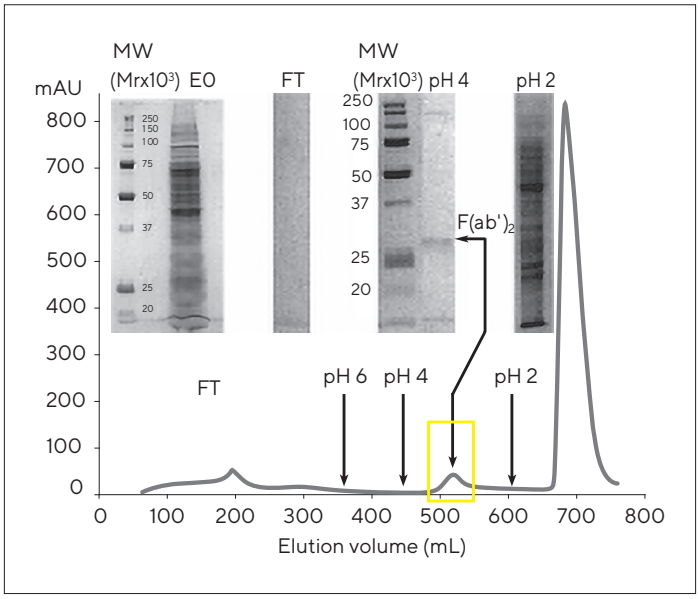

Figure 12: The fractions identified on the chromatogram during load and elution were analyzed using ELISA (not shown) and SDS-PAGE to identify and quantify the F(ab')$_2$.

Ordering Information

	HEA HyperCel Resin	PPA HyperCel Resin
25 mL	20250-026	20260-025
100 mL	20250-033	20260-030
1 L	20250-041	20260-040
5 L	20250-042	20260-045
10 L	20250-056	20260-052
1 mL PRC Prepacked Column, 5 mm ID × 50 mm	PRC05X050HEAHCEL	PRC05X050PPAHCEL
5 mL PRC Prepacked Column, 8 mm ID × 100 mm	PRC08X100HEAHCEL	PRC08X100PPAHCEL
Robocolumn 200 µL, row of 8	SR2HEA	SR6PPA
Robocolumn 600 µL, row of 8	SR6HEA	SR6PPA

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

DIR: 2578760-000-01
Status: 07 | 2020