



Life Sciences

AcroSep™ Chromatography Columns



Accelerating protein purification and analysis

- ▶ **New “gel-in-a-shell” technology** – Ion exchange resins provide rapid protein purification with high capacity and good resolution.
- ▶ **Flexibility** – More separation and elution options in a single format to provide greater purity.
- ▶ **Versatile** – Luer lock inlet and outlet allows convenient use with syringe, pump, or automated chromatography system.
- ▶ **User-friendly column design** – Color-coded and labeled by chemistry type. Collar is hexagonal so columns will not unexpectedly roll off lab surface.

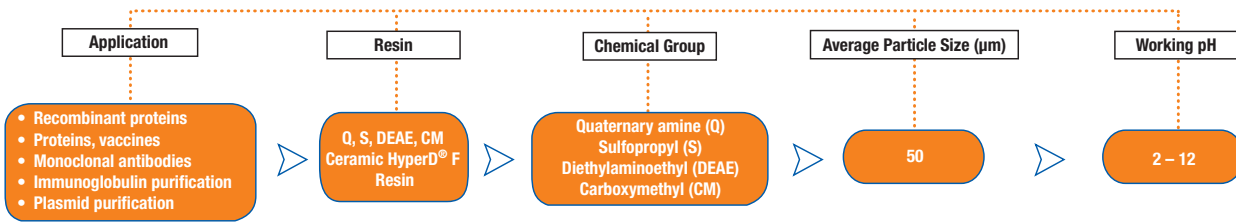
Applications

- ▶ Ion exchange
- ▶ Affinity
- ▶ Mixed-mode
- ▶ Hydrophobic charge interaction
- ▶ Detergent removal

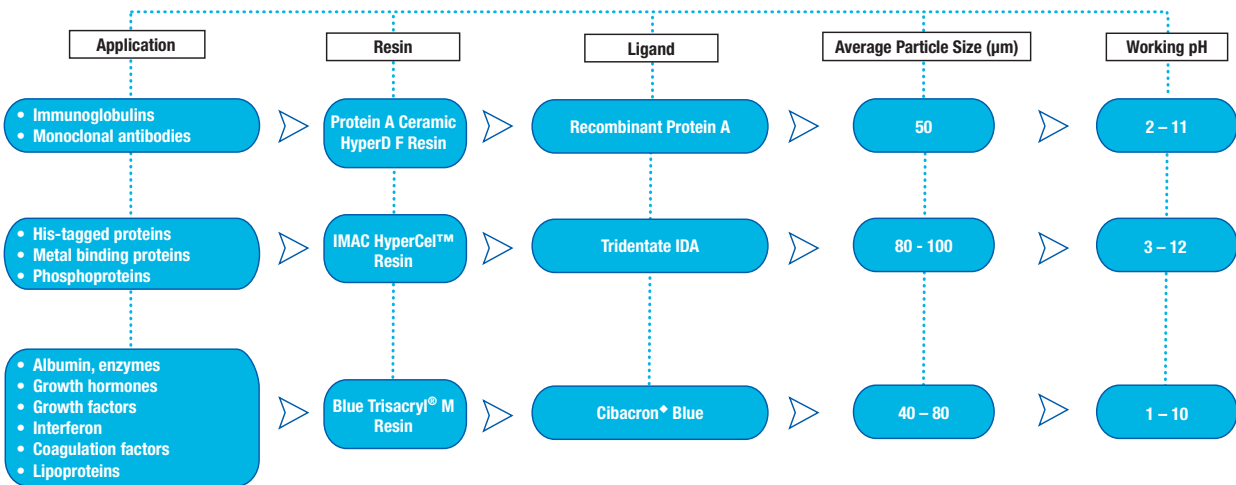
Filtration. Separation. Solution.SM

How to Choose the Best Chromatography Resin for Your Application

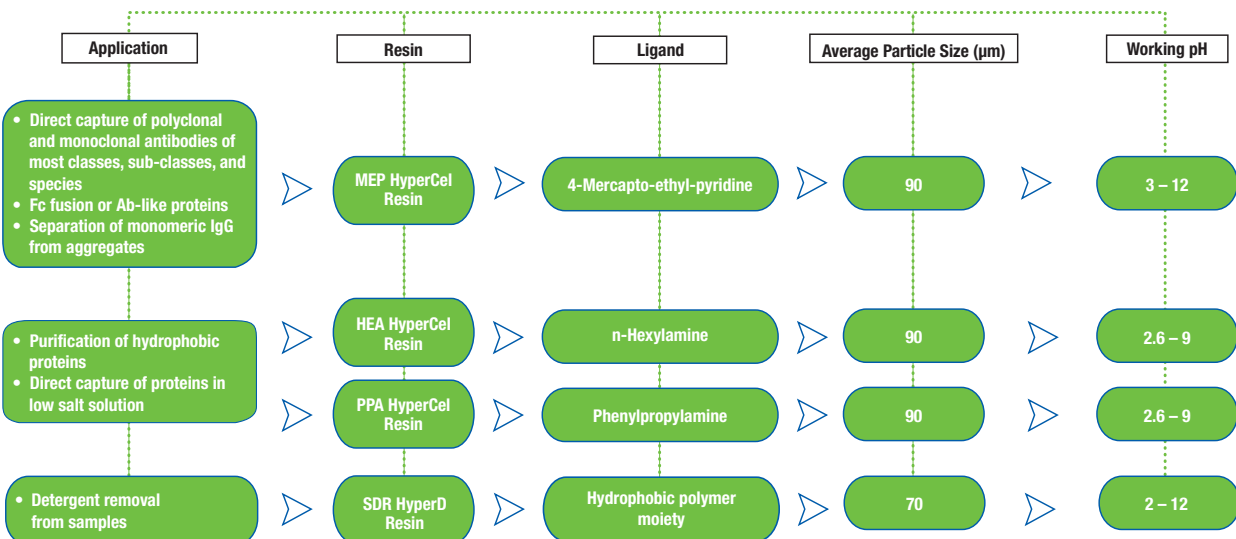
Ion Exchange Chromatography



Affinity Chromatography



Mixed-Mode Resin Chromatography



AcroSep Selection Guide and Color Coding

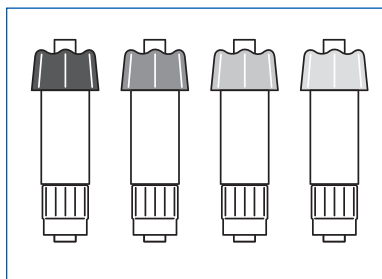
Type of Purification	Resin	Color
Ion Exchange, Weak Cation	CM Ceramic HyperD F	Green
Ion Exchange, Strong Cation	S Ceramic HyperD F	Blue
Ion Exchange, Strong Anion	Q Ceramic HyperD F	Red
Ion Exchange, Weak Anion	DEAE Ceramic HyperD F	Orange
Affinity	Protein A Ceramic HyperD F	Pearl
Affinity	IMAC HyperCel	Cobalt Blue
Affinity	Blue Trisacryl M	Dark Blue
Mixed-Mode	MEP HyperCel	Purple
Mixed-Mode	HEA HyperCel	Black
Mixed-Mode	PPA HyperCel	Yellow
Mixed-Mode	SDR HyperD	Natural



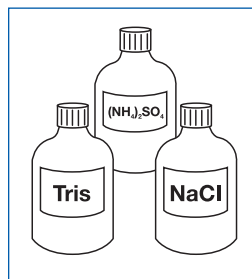
Methodology

AcroSep 1 mL chromatography columns are pre-packed with Pall chromatography media for fast, convenient protein separations. The column, which uses a luer lock connector

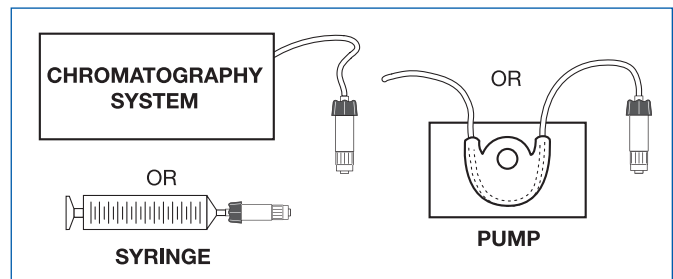
design, is compatible with a syringe, peristaltic pump, or chromatography instrument (e.g., AKTA* Explorer).



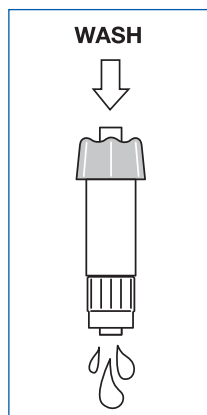
1. Choose appropriate column chemistry.



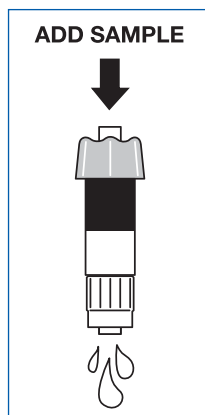
2. Choose appropriate equilibration, loading, and elution buffers.



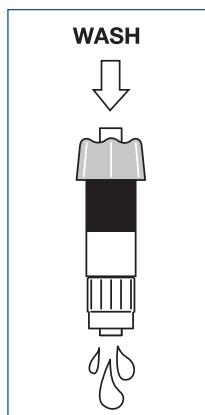
3. Attach column to pre-primed system (e.g., syringe, automated chromatography system, pump).



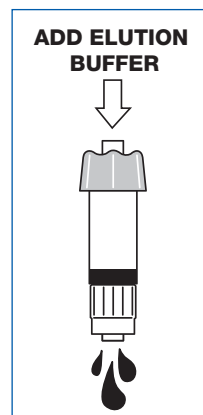
4. Wash and equilibrate column. For IMAC HyperCel only, load with metal and wash again.



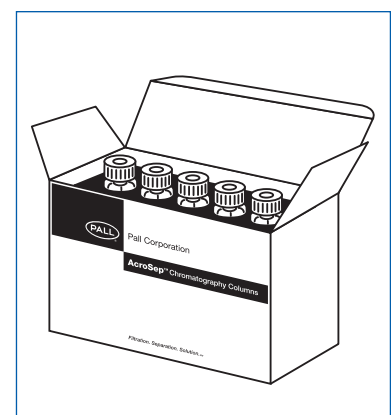
5. Add sample.



6. Wash column.



7. Elute sample with appropriate elution buffer.



8. Clean, store, and reuse column, where appropriate.

For more information on methodology, please refer to the specific AcroSep chromatography column product information insert.

AcroSep Chromatography Columns for Ion Exchange

Rapid, high capacity protein purification



Features

- ▶ Patented Ceramic HyperD F ion exchange chromatography resin features “gel-in-a-shell” technology, providing rapid protein purification with high capacity.
- ▶ Rapid and efficient. Perform faster runs with good resolution and capacity, increasing overall throughput.
- ▶ High resolution provides distinct separation of proteins for increased purity.
- ▶ Easy optimization. Convenient variety pack offers four different ion exchange column chemistries: S, Q, DEAE, and CM.

Applications

- ▶ Efficient and reliable small-to-medium scale purification of proteins for structural, functional, and other analyses.
- ▶ Easily screen multiple ion exchange chemistries.
- ▶ Ideal for optimization studies of protein purification schemes using small sample volumes prior to scale-up.

Specifications

Materials of Construction

Column Housing, Cap, Plug, and Adapter: Polypropylene
Column Frit: Polyethylene

Specs	CM Ceramic HyperD F	S Ceramic HyperD F	Q Ceramic HyperD F	DEAE Ceramic HyperD F
Function	Weak cation exchanger	Strong cation exchanger	Strong anion exchanger	Weak anion exchanger
Color Code	Green	Blue	Red	Orange
Particle Size	50 µm (avg.)	50 µm (avg.)	50 µm (avg.)	50 µm (avg.)
Working pH	2-12	2-12	2-12	2-12
Cleaning pH	1-14	1-14	1-14	1-14
Ion Exchange Capacity ¹	> 60 mg/mL ²	> 75 mg/mL ⁴	> 85 mg/mL ³	> 85 mg/mL ³

(1) Dynamic binding capacity (DBC) determined at 10% breakthrough, 200 cm/h with 1.66 mL sorbent packed in a column of 5 mm ID and 100 mm height using the following:

(2) 5 mg/mL human IgG in 50 mM sodium acetate buffer, 100 mM NaCl, pH 4.7

(3) 5 mg/mL BSA in 50 mM Tris-HCl buffer, pH 8.6

(4) 5 mg/mL lysozyme in 50 mM sodium acetate buffer, pH 4.5

Column Geometry

Column Volume: 1.04 mL

Bed Height: 1.48 cm (0.58 in.)

Bed Diameter: 0.94 cm (0.37 in.)

Device Dimensions

Diameter: 1.6 cm (0.6 in.)

Length (Without Plugs): 4.8 cm (1.9 in.)

Connections

Inlet: Threaded female luer lock

Outlet: Rotating male luer locking hub

Recommended Flow Rate

1-4 mL/min

Back Pressure

Maximum: 3 bar (300 kPa, 43.5 psi)

Storage

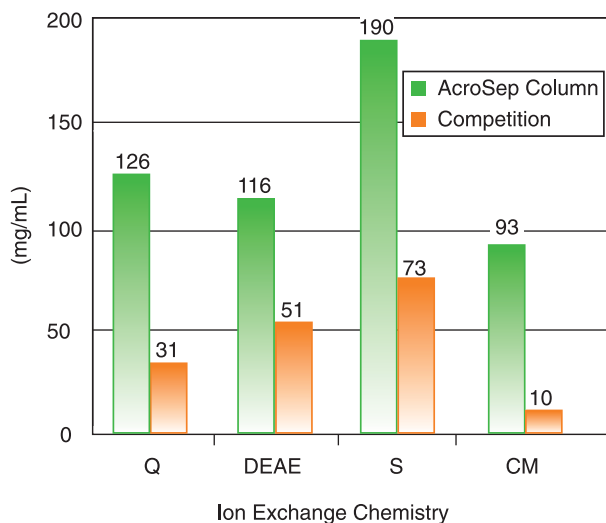
2-30 °C (36-86 °F)

2-8 °C (36-46 °F) after initial use

Performance

Figure 1

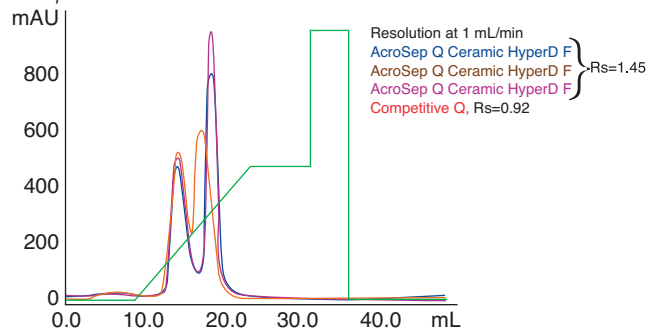
AcroSep DBC Exceeds Competition in All Ion Exchange Chemistries



Data shows up to nine times higher DBC of Pall AcroSep columns over competitive columns at increased flow rates. Each average is derived from three replicates of ≥ 8 AcroSep columns or three replicates of two competitive columns. The proteins were at a concentration of 5 mg/mL and include BSA for Q and DEAE, Lysozyme for S, and IgG for CM. Flow rate at 3.56 mL/min with 10% breakthrough.

Figure 2

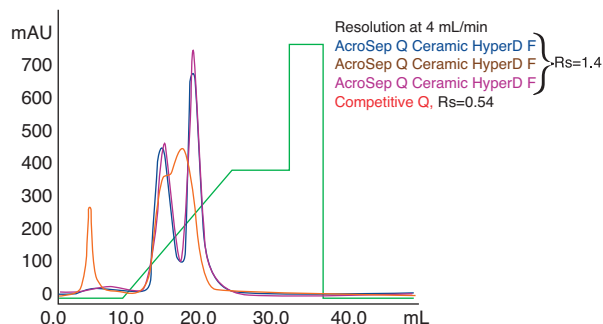
AcroSep Columns Provide Better Resolution Than Competitive Columns



$R_s = [(VR_2 - VR_1) / 2(WH_2 - WH_1)] / 2.354$, R_s = Resolution, VR_1 = Retention of Protein 1, VR_2 = Retention of Protein 2, WH_1 = Peak width at half height of Protein 1, WH_2 = Peak width at half height of Protein 2.

Figure 3

AcroSep Columns Maintain Good Resolution as Flow Rate Increases to 4 mL/min



$R_s = [(VR_2 - VR_1) / 2(WH_2 - WH_1)] / 2.354$, R_s = Resolution, VR_1 = Retention of Protein 1, VR_2 = Retention of Protein 2, WH_1 = Peak width at half height of Protein 1, WH_2 = Peak width at half height of Protein 2.

As shown in Figure 1, Pall AcroSep ion exchange columns have a high DBC even at increased flow rates. Figure 2 illustrates that AcroSep columns provide better resolution than competitive columns at 1 mL/min. Resolution is maintained when flow rates are increased, as shown in Figure 3.

Ordering Information

Part Number	Description	Color Code	Column Volume	Pkg
20050-C001	CM Ceramic HyperD F	Green	1 mL	5/pkg
20062-C001	S Ceramic HyperD F	Blue	1 mL	5/pkg
20066-C001	Q Ceramic HyperD F	Red	1 mL	5/pkg
20067-C001	DEAE Ceramic HyperD F	Orange	1 mL	5/pkg
IEXVP-C001	(1) each: Q, S, CM, and DEAE Ceramic HyperD F	Mixed	1 mL	4/pkg

AcroSep Chromatography Columns for Affinity Purification

For the purification of a wide variety of proteins and antibodies



Features

Protein A Ceramic HyperD F Resin

- ▶ Achieves > 90% recovery of IgG in a single step.
- ▶ Recombinant Protein A immobilized to specially formulated hydrogel within a ceramic bead offers high selectivity with low non-specific binding and low levels of Protein A leaching.

IMAC HyperCel Resin

- ▶ Provides single-step purity under both native and denaturing conditions.
- ▶ IMAC HyperCel resin uses tridentate IDA (iminodiacetic acid) chelating ligand immobilized on the HyperCel bead resulting in a stable and robust resin for small-to-large scale protein separation.
- ▶ Flexible, uncharged IMAC HyperCel resin offers the option to select specific metal ions to achieve high purity and yield of target proteins.

Blue Trisacryl M Resin

- ▶ Trisacryl macroporous base matrix allows good diffusion and improves exchange kinetics.
- ▶ Cibacron blue dye ligand selective for enzymes and albumin.
- ▶ Enhanced stability due to ligand coupling mechanism prevents dye leakage.

Applications

Protein A Ceramic HyperD F Resin

- ▶ Designed for IgG affinity purification from multiple sample types.
- ▶ Purification of Fc fusion and Ab-like proteins.
- ▶ Depletion of IgG from plasma and serum.

IMAC HyperCel Resin

- ▶ Ideal for purification of His-tagged proteins.
- ▶ Purification of metal binding proteins or other proteins capable of metal ion coordination.

Blue Trisacryl M Resin

- ▶ Optimized for depletion of albumin from plasma and serum samples.
- ▶ Good for purification of some enzymes.

Specifications

Materials of Construction

Column Housing, Cap, Plug, and Adapter: Polypropylene
Column Frit: Polyethylene

Media	Color Code	Particle Size	Cleaning pH	Capacity
Protein A Ceramic HyperD F	Pearl	50 μm (avg.)	2-13	> 30 mg/mL ¹
IMAC HyperCel	Cobalt Blue	80-100 μm	3-14	40-70 $\mu\text{mol Cu}^{2+}$ /mL
Blue Trisacryl M	Dark Blue	40-80 μm	1-10	10-15 mg/mL ²

(1) 10% breakthrough, 100 cm/hr, determined using 10 mg/mL human IgG in PBS, pH 7.4, elution in 0.1 M sodium citrate, pH 2.5, column 4.6 ID x 100 mm.

(2) Binding capacity using 5 mg/mL human albumin in PBS. Column dimensions – 1.6 cm ID x 3 cm bed height; residence time (Tr) = 7.26 min.

Column Geometry

Column Volume: 1.04 mL
Bed Height: 1.48 cm (0.58 in.)
Bed Diameter: 0.94 cm (0.37 in.)

Device Dimensions

Diameter: 1.6 cm (0.6 in.)
Length (Without Plugs): 4.8 cm (1.9 in.)

Connections

Inlet: Threaded female luer lock
Outlet: Rotating male luer locking hub

Recommended Flow Rates

Protein A Ceramic HyperD F: 0.2-1 mL/min
IMAC-Metal Loading Step: 3-6 mL/min
IMAC-Protein Capture Step: 0.5-1 mL/min
Blue Trisacryl M: 0.2-1.0 mL/min

Back Pressure

Maximum: 3 bar (300 kPa, 43.5 psi)

Storage

2-8 °C

Performance

Protein A Ceramic HyperD F Resin

Figure 4

High Purity IgG in a Single Purification Step

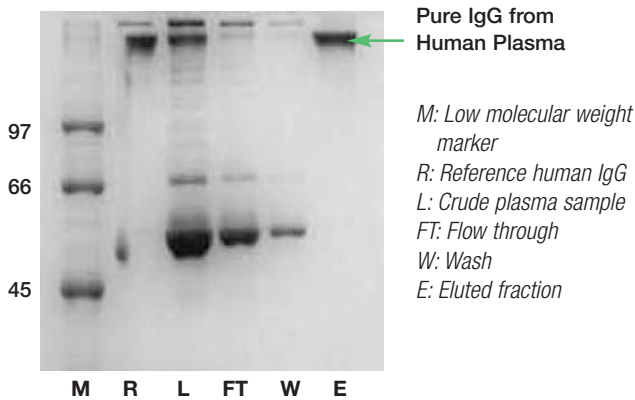
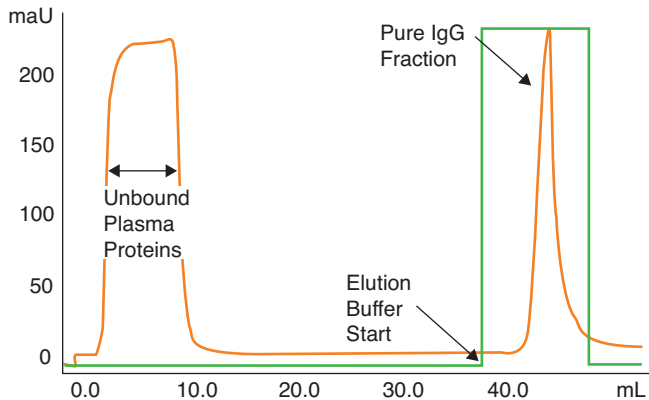
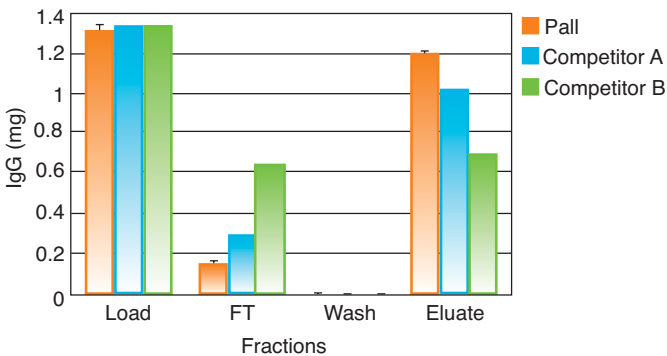


Figure 4 illustrates a chromatographic separation of IgG from human plasma and subsequent analysis of different fractions by non-reducing SDS-PAGE. Greater than 98% purity for IgG is achieved in this single step affinity purification. (Flow rate: 0.2 mL/min for loading, 1 mL/min all other steps.)

Figure 5

AcroSep Columns Provide Greater Capacity of IgG Compared to Competitive Columns



Recovery of IgG from human plasma quantified by ELISA. Data in Figure 5 shows higher recovery of IgG with AcroSep Protein A Ceramic HyperD F columns as compared to competitive columns. Average value is derived from duplicate readings. (Flow rate: 0.2 mL/min for loading, 1 mL/min all other steps.)

IMAC HyperCel Resin

Figure 6

High Purity Protein in a Single Step Using Native Conditions

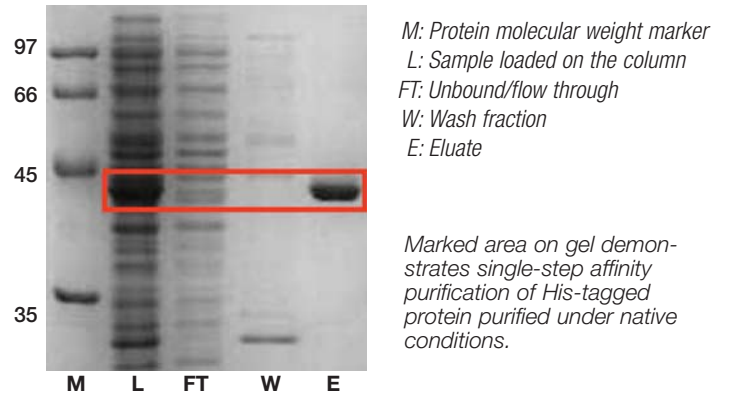
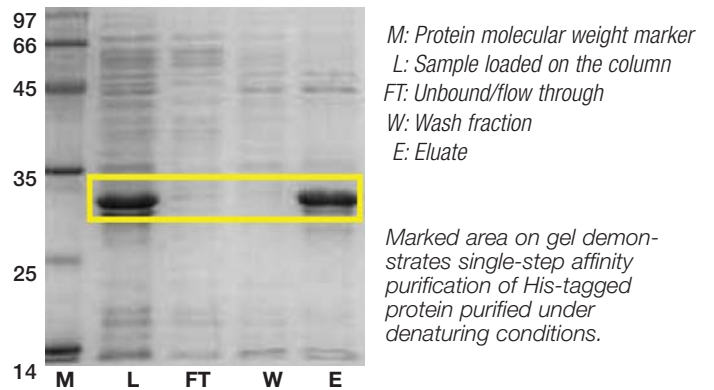


Figure 7

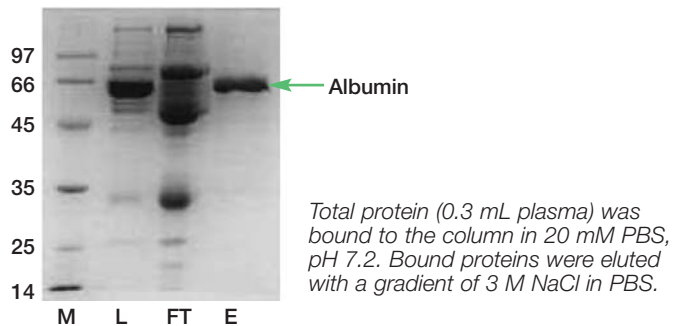
High Purity Protein in a Single Step Using Denaturing Conditions



Blue Trisacryl M Resin

Figure 8

Purification of Albumin From Human Plasma



Ordering Information

Part Number	Description	Color Code	Column Volume	Pkg
20078-C001	Protein A Ceramic HyperD F	Pearl	1 mL	5/pkg
20093-C001	IMAC HyperCel	Cobalt Blue	1 mL	5/pkg
25896-C001	Blue Trisacryl M	Dark Blue	1 mL	5/pkg

AcroSep Chromatography Columns for Mixed-Mode

Flexible chemistries provide better selectivity and more purification options



Features

MEP HyperCel Resin

- ▶ Stability of resin and ligand allows up to 200 cycles of purification.
- ▶ Broad species and isotype binding capabilities for antibodies.
- ▶ Elution at higher pH as compared to conventional antibody purification with Protein A.
- ▶ Ligand structure and density of MEP HyperCel resin provides effective binding in the absence of lyotropic agents or salts.

HEA and PPA HyperCel Resin

- ▶ HyperCel cellulose bead provides high porosity, chemical stability, and low non-specific interaction.
- ▶ Binding based on hydrophobic interactions and elution on the basis of electrostatic repulsion.
- ▶ Binding typically at physiological pH with no need to use high salt, unlike conventional hydrophobic interaction chromatography (HIC).
- ▶ HEA and PPA HyperCel resins offer effective discrimination of proteins having similar or very close isoelectric points.

SDR HyperD Resin

- ▶ Composite structure: silica bead filled with hydrophobic polymer ensures efficient capture and retention of detergents.
- ▶ Effective removal of a wide range of ionic and non-ionic detergents.
- ▶ Stable in acidic, polar organic, and oxidizing solutions.

Applications

MEP HyperCel Resin

- ▶ Antibody capture and purification.
- ▶ Purification of Fc fusion proteins and Ab-like molecules.
- ▶ Easily screen multiple mixed-mode chemistries.

HEA and PPA HyperCel Resin

- ▶ Direct hydrophobic capture and purification.
- ▶ Compatible upstream and downstream of ion exchange and other chemistries for enhanced purification.
- ▶ Easily screen multiple mixed-mode chemistries.

SDR HyperD Resin

- ▶ High efficiency removal of ionic and non-ionic detergents prior to downstream applications.
- ▶ Efficient detergent removal for target proteins > 60 kD.

Specifications

Materials of Construction

Column Housing, Cap, Plug, and Adapter: Polypropylene
Column Frit: Polyethylene

Media	Color Code	Particle Size	Cleaning pH	Capacity
MEP HyperCel	Purple	80-100 μm	3-14	> 20 mg/mL ¹
HEA HyperCel	Black	80-100 μm	1-14	40 mg/mL ²
PPA HyperCel	Yellow	80-100 μm	1-14	40 mg/mL ²
SDR HyperD	Natural	40-100 μm	2-12	> 90 mg/mL ³

(1) DBC determined using 5 mg/mL IgG in PBS; flow rate 60 cm/hr.; column dimension = 1.1 cm ID x 7 cm column; residence time (Tr) = 5.68 min.

(2) DBC at 10% breakthrough; 5 mg/mL BSA in PBS; flow rate 50 cm/hr.; column dimension = 1.6 cm ID x 3.75 cm; (Tr) = 4.51 min.

(3) DBC determined using 5 mg/mL Triton[®] X-100 at flow rate 3.5 mL/min (300 cm/hr) in PBS pH 7.2.

Column Geometry

Column Volume: 1.04 mL
Bed Height: 1.48 cm (0.58 in.)
Bed Diameter: 0.94 cm (0.37 in.)

Device Dimensions

Diameter: 1.6 cm (0.6 in.)
Length (Without Plugs): 4.8 cm (1.9 in.)

Connections

Inlet: Threaded female luer lock
Outlet: Rotating male luer locking hub

Recommended Flow Rates

MEP, HEA, PPA: 0.2-4.0 mL/min
SDR: 1.0-4.0 mL/min

Back Pressure

Maximum: 3 bar (300 kPa, 43.5 psi)

Storage

2-8 °C

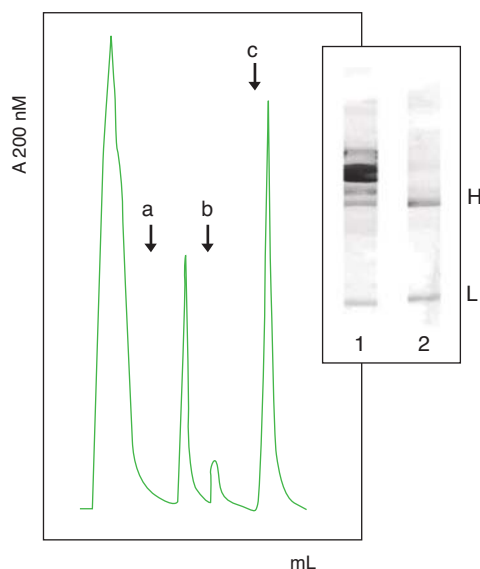
Performance

MEP HyperCel Resin

IgG was isolated from ascites fluid by loading clarified samples diluted with an equal volume of equilibration buffer in order to reduce viscosity (Figure 9). Column was equilibrated with 50 mM Tris-buffer, pH 8.0. After loading, the column was washed with the same buffer. The first large peak contains proteins that flow through the column, the next two peaks are contaminants that are removed from the column with water and caprylate washes. The final peak is the eluted antibody, also shown in lane 2 of the SDS-PAGE data.

Figure 9

High Purity of Monoclonal IgG From Ascites Fluid Using MEP HyperCel Resin

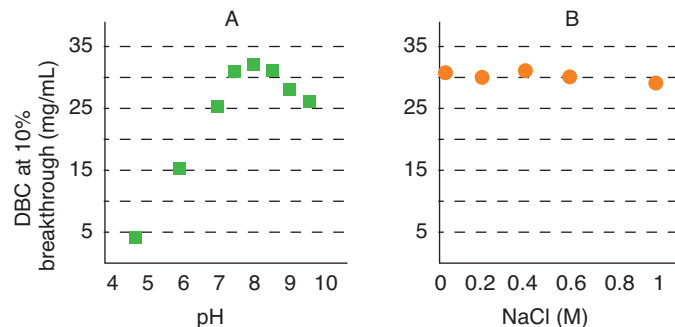


Column run at flow rate of 70 cm/h with 50 mM Tris-HCl, pH used for binding and 50 mM acetate, pH 4 used for elution. In the curve, arrows indicate water (a) and sodium caprylate (b) washes. Arrow c indicates start of elution buffer. SDS-PAGE analysis (reduced conditions): 1 = crude sample; 2 = purified; H = heavy chain; L = light chain.

Data showing the influence of pH and ionic strength on IgG binding capacity (Figure 10) indicate that a wide range of sample pH and salt concentrations are compatible with good MEP performance.

Figure 10

Influence of pH and Ionic Strength on the Binding Capacity of MEP HyperCel Resin



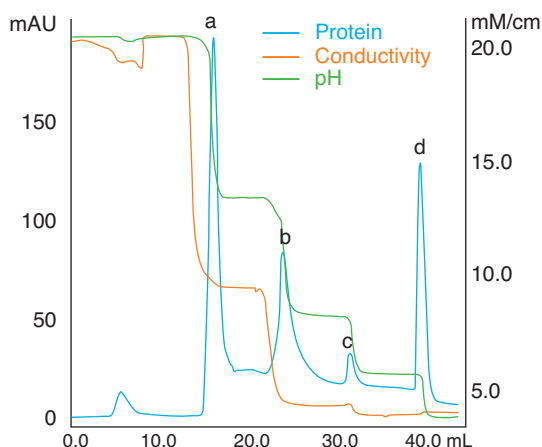
IgG capacities obtained at 10% breakthrough on MEP HyperCel resin vs. pH (A) and ionic strength (B) of the binding buffer. Experimental conditions: column = 1.1 cm (0.4 in.) ID X 9 cm (3.5 in.); sample = IgG (2 mg/mL); flow rate = 90 cm/hr.; Tr = 7.8 min.

HEA and PPA HyperCel Resin

The following chromatograms (Figure 11 and 12) are an illustration of resolution of a mixture of four proteins – Lysozyme, α -Chymotrypsinogen A, α -Chymotrypsinogen B, and Bovine Serum Albumin (BSA). Binding occurs largely through hydrophobic interaction while elution results from electrostatic repulsion resulting from pH change and reduced conductivity.

Figure 11

Distinct Separation of Standard Protein Mixture Using AcroSep HEA Columns



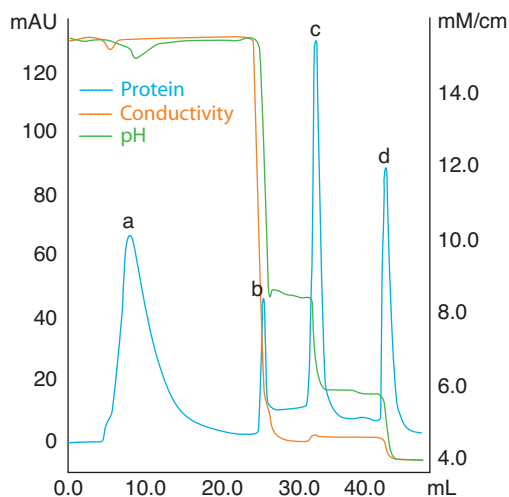
Peak a - Lysozyme
 Peak b - α -Chymotrypsinogen A
 Peak c - α -Chymotrypsinogen B
 Peak d - BSA

Flow rate: 0.2 mL/min for sample injection and 0.5 mL/min for other steps. Carbonate buffer, pH 10.0 with 150 mM salt used for binding. Elution using mixed phosphate and citrate buffers of desired pH.

Performance (continued)

Figure 12

Distinct Separation of Standard Protein Mixture Using AcroSep PPA Columns



- Peak a - Lysozyme
- Peak b - α -Chymotrypsinogen A
- Peak c - α -Chymotrypsinogen B
- Peak d - BSA

Sample injection occurred at 0.2 mL/min then increased to 0.5 mL/min for the remainder of the procedure. PBS, pH 7.2 used for binding of proteins. Elution carried out using citrate buffers of respective pH and conductance as shown.

Demonstration of Dual Mode Behavior

Ligands in HEA and PPA resin promote binding of proteins by virtue of hydrophobic interaction in the presence of very low amounts of salt. Though pKa of both resins is the same (8.0), there is selective binding of proteins between the resins. Lysozyme does not bind completely to PPA resin using PBS. However, complete binding of lysozyme is achieved on HEA HyperCel by increasing the pH.

In the case of HEA HyperCel resin (Figure 11), decrease in conductivity results in elution of lysozyme while elution of α -Chymotrypsinogen A is achieved by a decrease in conductance as well as pH. Figure 12 demonstrates that further elution of proteins is achieved purely on the basis of a decrease in pH.

In the case of PPA HyperCel resin (Figure 12), a very small portion, < 10% of the basic protein lysozyme (pI=11.0), binds to the PPA resin. Elution is achieved by both a decrease in conductivity and pH. Since the resin is highly hydrophobic, proteins are retained very strongly. α -Chymotrypsinogen A is eluted at a lower pH compared to HEA HyperCel resin.

Conclusion

IgG can be bound to MEP HyperCel resin with low to high salt concentrations. An easy two-step cleaning procedure with water followed by sodium caprylate provides extremely pure monoclonal IgG from ascites fluid. Elution of antibodies can be carried out at a higher pH than traditional methods to preserve integrity.

HEA and PPA HyperCel resins provide binding mechanisms based on hydrophobic interaction which can be influenced by pH as well as ionic strength. PPA HyperCel is more hydrophobic than HEA HyperCel. Each resin is able to bind specific proteins based on column conditions.

SDR HyperD Resin

Table 1

Average DBC at Different Flow Rates (mg/mL)

Breakthrough	1 mL/min		3 mL/min		5 mL/min	
	10%	50%	10%	50%	10%	50%
Triton X-100	93.8	112.2	81.0	113.2	55.0	88.7
Tween* 20	47.4	88.6	20.6	51.5	10.5	34.0
NP 40	104.3	122.4	83.0	115.1	61.2	100.8

Ordering Information

Part Number	Description	Color Code	Column Volume	Pkg
12035-C001	MEP HyperCel	Purple	1 mL	5/pkg
20250-C001	HEA HyperCel	Black	1 mL	5/pkg
20260-C001	PPA HyperCel	Yellow	1 mL	5/pkg
20033-C001	SDR HyperD	Natural	1 mL	5/pkg

Additional Information

Related Literature

- ▶ PN 33453 Pall Chromatography Media Poster

Related Products

- ▶ AcroPrep™ Filter Plates
- ▶ Nanosep® MF Centrifugal Devices
- ▶ Enchant™ Multi-protein Affinity Separation Kit
- ▶ Sample prep syringe filters
- ▶ Minimate™ TFF System
- ▶ BioTrace™, Biodyne®, and FluoroTrans® Transfer Membranes
- ▶ Vivid™ Plasma Separation Membrane
- ▶ Bulk chromatography resins for all AcroSep chromatography columns, as well as Ultrogel® AcA, Trisacryl GF, Heparin HyperD M, Lysine HyperD, and HA Ultrogel resin.



Life Sciences

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Fax: 514-332-0996
800-808-6268 (in Canada)

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