

# Rapid Protein Separations Using 1 mL Pre-Packed AcroSep™ Ion Exchange Chromatography Columns

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## Abstract

Biology-based research laboratories of all types frequently need purified proteins that are not readily available and must be custom purified. The challenge for protein purification is time. Method development can be slow, chromatography systems busy, and purification runs long. Ion exchange (IEX) chromatography is one of the most commonly used modes of protein separation. The performance of Q, DEAE, S, and CM IEX on the Ceramic HyperD® F bead is characterized using model proteins in pre-packed AcroSep 1 mL column formats. Data generated using an automated chromatography system and manual syringe-based methods demonstrate that the resolution and high binding capacities of these resins are maintained even at very high flow rates. High speeds are possible due to a non-compressible, porous ceramic bead structure while high capacity results from the hydrogel infused throughout the ceramic bead. Good IEX performance at high speed has several potential advantages including shorter run times on automated chromatography systems, better results when using the column with a syringe which is typically run at higher speeds, and the ability to test a greater number of purification optimization parameters in a specified period of time. These advantages can result in greater productivity in the lab. Thus, we believe that Pall Life Sciences' 1 mL pre-packed AcroSep IEX chromatography columns are a strong addition to the chromatography product choices for scientists engaged in protein separation and purification.



## Materials and Methods

### Pall AcroSep 1mL Pre-packed IEX HyperD F Columns

- CM Ceramic HyperD F, PN 20050-C001
- S Ceramic HyperD F, PN 20062-C001
- Q Ceramic HyperD F, PN 20066-C001
- DEAE Ceramic HyperD F, PN 20067-C001

### Resolution Testing Proteins and Buffers

Q and DEAE HyperD F resolution was demonstrated with a solution of 4 mg/mL bovine serum albumin (BSA) + 2 mg/mL conalbumin in load/wash buffer (50 mM Tris-HCl, pH 8.5, conductivity ~5 ms/cm). A mix of ribonuclease A (1.5 mg/mL) + lysozyme (0.4 mg/mL) was used for CM resolution testing (20 mM sodium phosphate pH 6.8, ~13 ms/cm). S resolution was performed with 0.5 mg/mL trypsinogen + 3 mg/mL lysozyme in load/wash buffer (50 mM sodium acetate, pH 4.5, ~8 ms/cm). 1 M NaCl was added to load buffer for elution for all resins, except CM, which used 0.5 M NaCl. Each column was tested at 1, 4, 8, 12, 15, 20, and 25 mL/min. CM was also tested at 2 mL/min.

Table 1  
Resolution Testing Chromatography System Set Up (AKTA® Explorer)

IEX	Equilibrate	Inject	Wash	Elution Buffer Gradient	Strip	Re-Equilibration
Q	10.4 mL	3 mL	20.8 mL	0-40% over 40 mL	10.4 mL	5.2 mL
DEAE	10.4 mL	0.5 mL	20.8 mL	0-50% over 25 mL	10.4 mL	5.2 mL
S	10.4 mL	1 mL	20.8 mL	0-100% over 10 mL	26 mL	10.4 mL
CM	10.4 mL	2 mL	20.8 mL	30-90% over 40 mL	5.2 mL	5.2 mL

### Dynamic Binding Capacity (DBC) Determination

For DBC testing, place 5 mg/mL protein (BSA for Q and DEAE, IgG for CM, and lysozyme for S) in load/wash buffer. Buffer as described above, Tris pH 8.5 for Q and DEAE, Acetate pH 4.5 for S and CM. 1.0 or 1.5 M NaCl was added to load/wash buffers (CM 1.5 M, 1 M for all others) for elution and stripping steps.

Table 2  
DBC Chromatography System Method (AKTA Explorer)

IEX	Equilibrate	Inject	Wash	Strip	Re-Equilibration
Q	10.4 mL	50 mL	20.8 mL	10.4 mL	5.2 mL
DEAE	10.4 mL	50 mL	20.8 mL	10.4 mL	5.2 mL
S	10.4 mL	75 mL	20.8 mL	26 mL	10.4 mL
CM	15.6 mL	50 mL	20.8 mL	0-100% over 40 mL	5.2 mL

### Determination of Dynamic Binding Capacity

DBC Formula:  $DBC = [C \times (V_L - V_0)] / CV$

C = Concentration of load

$V_L$  = Volume at 10% breakthrough

$V_0$  = Void volume determined from bypass run

CV = Column volume (1.04 mL)

### Syringe Method Resolution Test on Q Ceramic HyperD F

- BSA and Cytochrome C (Sigma) each at 2.5 mg/mL in running buffer (50 mM Tris HCl, pH 8.5, 5 ms/cm)
- Flow rates of 1 and 12 mL/min. A syringe pump was used throughout the experiment to ensure accurate control of flow rate. 1 mL fractions collected.
- Method
  1. Equilibrate the column with 5 mL of Tris buffer.
  2. Load 1 mL of protein solution (5 mg total protein).
  3. Wash column with 5 mL of Tris buffer.
  4. Run 5 mL of elution buffer over the column to elute bound protein.
  5. Strip column with 5 mL of 2 M NaCl in Tris buffer.
  6. Equilibrate the column with 5 mL of Tris run buffer. Columns run in triplicate.

### 1 Dimensional Gels – SDS-PAGE

- 25-50 µg of total protein (reduced) loaded in each lane.
- 4-12% BisTris Criterion gels (BioRad) run with MES buffer and stained with GelCode Blue (Pierce).

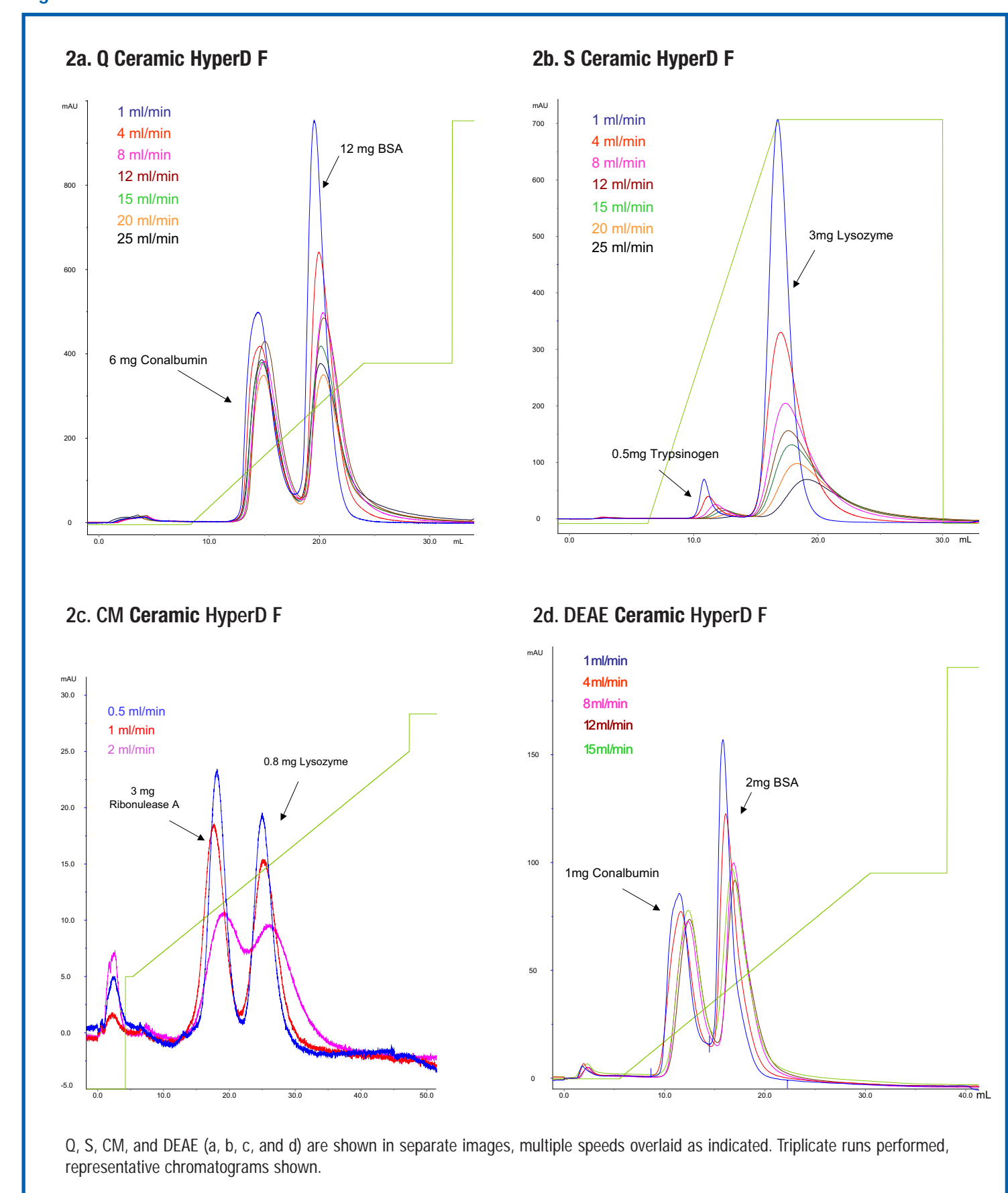
## Results

### Resolution of AcroSep 1 mL Pre-Packed IEX HyperD F Columns

An overlay of the resolution chromatograms from the Q, S, CM, and DEAE runs is shown in Figure 2a-d. The elution gradient is also shown. All three columns were tested up to 25 mL/min although not all data is shown.

- AcroSep Q columns (Figure 2a) show similar protein separation (valley between the protein peaks) up to 25 mL/min. There is some broadening of the BSA peak at higher speeds.
- AcroSep S (Figure 2b) columns also perform well with baseline resolution at all speeds. However, there is pronounced peak broadening with increased speed.
- AcroSep CM columns (Figure 2c and data not shown) show the largest decrease in resolution with increased speed. The protein separation is quite good at 0.5 and 1 mL/min, but the resolution decreases significantly at 2 mL/min and further at higher flow rates.
- AcroSep DEAE column performance (Figure 2d and data not shown) is strong at high speeds. The protein separation is achieved at all speeds, but resolution decreases with increased flow rate. There is some peak broadening as well.

Figure 2

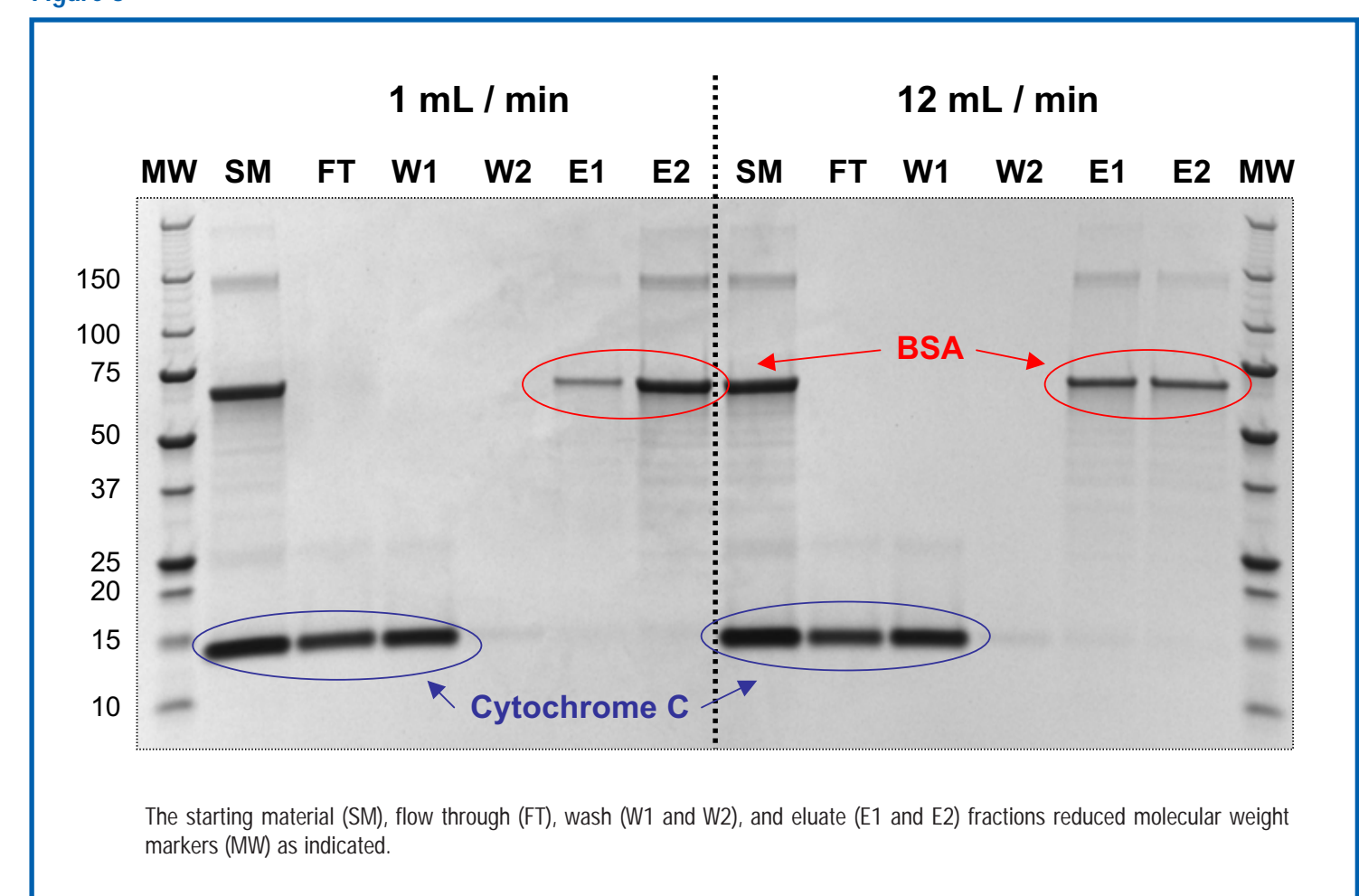


### SDS-PAGE Analysis of Q HyperD F AcroSep Column Protein Fractions (Manual Method With Syringe)

Although IEX is rarely used with a syringe for protein purification because gradient elution cannot be performed, this method is an option when stepwise elution will achieve the desired protein separation. An example of this, using the Q HyperD F column and two proteins, Cytochrome C which does not bind and BSA which binds well, is shown in Figure 3.

- SDS-PAGE analysis of the fractions from protein separation at 1 and 12 mL/min show that there is very little difference in separation, with Cytochrome C (~12 kDa, blue oval) in the flow through and BSA (~66 kDa, red oval) eluting in the first 2 fractions.

Figure 3

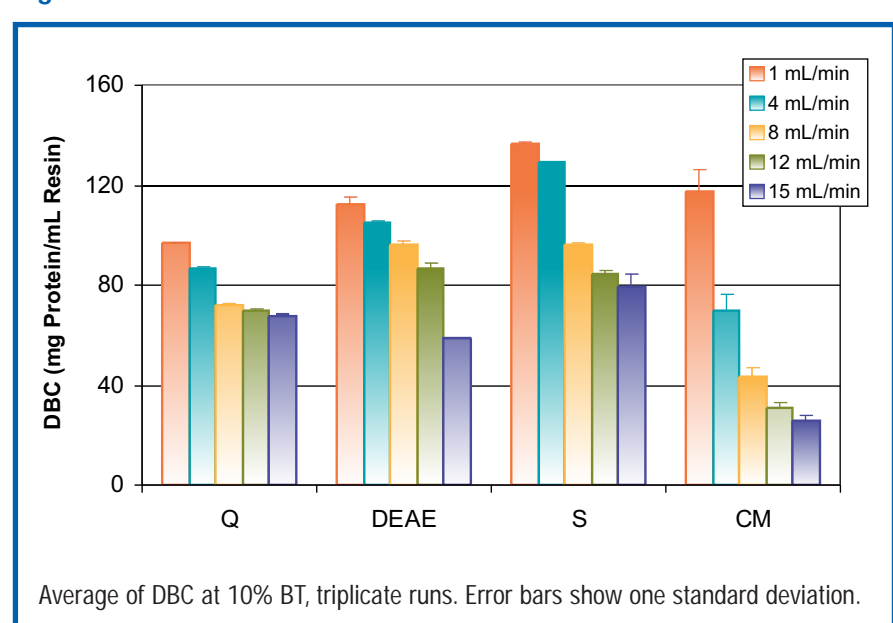


## Results

### DBC of AcroSep 1 mL Pre-Packed IEX HyperD F Columns

The binding capacity data for all four IEX columns up to 15 mL/min is shown in Figure 1. At 1 mL/min, all of the IEX columns show very high binding capacity. As expected, capacity decreases with increasing speed. Despite these decreases, the capacity is still quite high at all speeds tested. Of the four IEX types tested, Q loses the least capacity at 1 and 15 mL/min (30%, 97 mg/mL vs. 67 mg/mL), consistent with exceptional results from the Q high speed resolution tests. In these tests, CM shows the highest decrease in performance at higher speeds although its capacity is still very high at 4 mL/min (~70 mg/mL).

Figure 1



## Conclusions

The AcroSep IEX columns and resins have the unique ability to maintain good protein separation and high binding capacity at very high flow rates. This property can be exploited by purification scientists to improve overall productivity and/or lab performance resulting from more protein separation experiments per 24 hour period. This characteristic of Pall IEX resins reflects the combined attributes of rapid IEX interaction with proteins and a non-compressible, high capacity bead structure. With model proteins:

- Although reductions in DBC do occur at higher speeds, the capacity of these resins is so high at 1 mL/min that in most cases the loss in capacity will not affect the final purification result.
  - Q HyperD F protein resolution data indicates minimal change from 1 to 25 mL/min.
  - S HyperD F data show good protein separation to 25 mL/min although there is significant peak broadening.
  - CM HyperD F shows good resolution and capacity at 1 mL/min, decreases in both at 2 or 4 mL/min, respectively.
  - DEAE HyperD F shows no significant decrease in protein separation until speeds are above 12 mL/min. There is a small amount of peak broadening with increasing speeds.
  - The AcroSep IEX columns can be used for effective protein separation in manual mode with a syringe.
- In combination, these data indicate that the IEX resins in 1 mL AcroSep columns can potentially result in improved lab productivity by increasing the number of proteins purified during any period of time.