SARTORIUS

Instructions for Use

Q, DEAE and CM Ceramic HyperD® F

Ion Exchange Chromatography Resins

1 Product Description

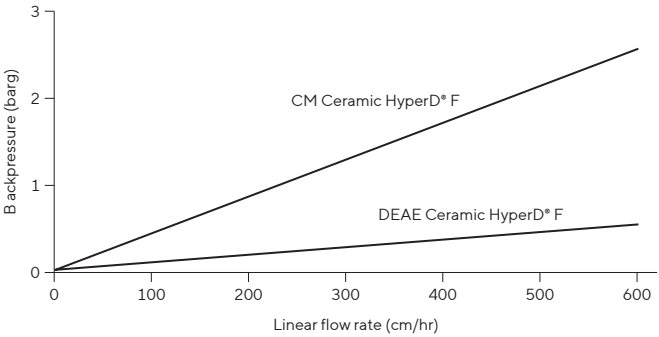
Q, DEAE, CM Ceramic HyperD® F high capacity resins are designed for efficient and scalable purification of biomolecules. They maintain high dynamic binding capacity (DBC) under conditions where conventional resins display significant capacity or productivity limitations, and offer differentiated selectivity compared to other new generation resins. Ceramic HyperD® F resins are manufactured at ISO 9001:2008 and ISO 14001:2004 compliant manufacturing facility. Ceramic HyperD® F resins are used for the production of material for preclinical and clinical trials as well as for the purification of therapeutic proteins in regulatory approved production processes in columns of several hundred liters. Regulatory Support Files (RSF) and column packing support are available upon request. They are provided as a slurry in 1 M NaCl containing 20% ethanol / 1.2 M EDTA. Q and CM resins are also available in 1 mL prepacked PRC columns and RoboColumn®* for rapid selectivity screening under reliable and reproducible conditions.

2 Properties

	Ceramic HyperD® F Resins		
	Q	DEAE	CM
Average particle size (µm)	50	50	50
Dynamic binding capacity (mg/mL) 10% breakthrough, 3 minutes residence time	BSA* 85[(1)]	BSA* 85[(1)]	hu IgG* 113 – 173[(2)]
Amount of ionic groups (µeq/mL)	250	200	213 – 423
Pressure resistance	70 barg (1,000 psig)		
Working pressure	< 3 barg (44 psig)		
Working pH	2 – 12		
Cleaning pH	1 – 14		
Volumes changes due to pH and ionic strength	Non compressible		

Sample: 5 mg/mL: [(1)] BSA* in 50 mM Tris-HCl buffer, pH 8.6. [(2)] hu IgG* in 50 mM sodium acetate, pH 4.7.
* BSA = Bovine Serum Albumin. Hu IgG = Human Immunoglobulins G.

Figure 1: Pressure vs. Flow Rate



Resins packed in a laboratory scale column (15 mm I.D. x 15 cm bed height). Buffer: 50 mM sodium phosphate, pH 6.5 – 7.0 for CM and DEAE Ceramic HyperD® F resins.

Note: The pressure drop is based on Ceramic HyperD® F beads only. Pressure values of the empty system were subtracted to measure values generated by the resin itself.

3 Column Packing

3.1 Packing a Small Column (≤ 5 cm I.D.)

IEX Ceramic HyperD® F resins are compatible with traditional low or medium pressure chromatography columns and equipment. For preliminary laboratory studies, a column equipped with an adjustable piston, of 1 cm I.D. x 20 cm length, facilitates optimal packing. Direct scale-up is accomplished by maintaining bed height constant while diameter is increased. We recommend a chromatography system able to deliver a minimum linear flow rate of 300 cm/h for packing only, 600 cm/h for performing a pressure versus flow rate curve.

3.1.1 Preparing the Resin Slurry

⚠ **Do not use stainless steel paddles or magnetic stirrers as they may damage the beads and create fine particles.**

1. Gently agitate the container to fully suspend the resin.
2. Depending on the desired bed volume, transfer a suitable volume of slurry to a graduated beaker including a "practical" excess of resin (20 to 25% of the desired bed volume are recommended).
3. Allow the resin to settle and remove the supernatant. Add 3 to 5 volumes of packing buffer. It is very important that buffers with a minimum ionic strength of 5 mS/cm using Ceramic HyperD® F resins (i.e., 50 mM sodium phosphate pH 6.5 – 7.0) be used. Sharp increases in column backpressure may occur when water alone is used.
4. Gently agitate the slurry and let the resin settle. Remove the supernatant and add 3 to 5 volumes of fresh packing buffer.
5. Repeat step 4, three times minimum in total.
6. Gently agitate the slurry, pour it into a measuring cylinder and allow the resin to settle.
7. Remove the supernatant and add a volume of packing buffer equal to one-half the volume of settled resin. A slurry of 67% (v/v) — the concentration recommended for packing — is obtained. Alternatively, the slurry concentration can be estimated by centrifuging between 20-g and 200-g about 10 mL of homogenized slurry in a graduated vial tube for 5 minutes. The concentration of slurry expressed as: [resin volume | total volume (resin + supernatant) x 100] can be directly estimated in the tube. The 10 mL slurry can be poured back into the total suspension before packing.

3.1.2 Preparing Column and System for Packing

⚠ **Do not use stainless steel paddles or magnetic stirrers as they may damage the beads and create fine particles.**

1. Prime the solvent delivery system, lines and valves to assure that all air is displaced. Assure that top and bottom frits or nets are fully wetted and free of air. Fill the column with packing buffer and operate the system over a range of flow rates representative of values that will be used during packing and anticipated chromatographic procedures. Record pressure associated with the empty column and system (including detectors, etc...).
2. Determine pressure | flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatography operation.
3. Stop the pump, close the column outlet, and remove the upper flow-adaptor or piston.4. O pen the outlet and drain buffer from the column, leaving 1 – 4 cm of buffer above the bottom frit.

3.1.3 Preparing Column and System for Packing

1. Gently resuspend the slurry and pour it into the column in one continuous motion against the wall of the glass tube to minimize introduction of air bubbles (if the pouring process is done in several motions, gently homo genize the slurry in the column using a plastic rod).
2. Fill with packing buffer (i.e., 50 mM sodium phosphate pH 6.5 – 7.0) up to the top of the glass tube. Allow the suspension to settle so that a layer of clear supernatant ≤ 1 cm is visible at the top of the column.

3. Connect the upper piston to the system and prime with packing buffer to ensure that no air is trapped under the net or frit. Stop the pump and insert the adjustable piston into the column. Tighten it.
4. Open the column outlet and operate the pump at a selected linear velocity of 300 cm/h (~3.9 mL/min for a 1 cm I.D. column) for efficient packing.
5. When the top of the bed stabilizes, stop the pump and untighten the piston. Position the adjustable piston at the top of the packed resin, leaving no visible space between the frit and packed resin at any point around the circumference.
6. Operate the pump again and repeat the adjustment of the piston until no visible space appears under flow (300 cm/h) between the frit and the top bed.

3.1.4 Evaluating Column Performance

Column performance is evaluated by determining column efficiency, expressed as either plates/meter (N/m), or HETP (height equivalent to one theoretical plate). Additionally, the asymmetry factor (AF) is calculated. Required formulas are shown below. Measurements are made as follows:

1. Equilibrate the column with equilibration buffer (i.e., Tris-HCl, pH 8.5 for Q/DEAE Ceramic HyperD® F resins or sodium acetate, pH 4.5 for CM Ceramic HyperD® F resin).
2. Inject a sample of 5% (v/v) acetone in equilibration buffer corresponding to 1% (v/v) of the total column volume (i.e., 100 µL for a column of 10 mm I.D. x 0 – 12 cm length filled with 10 mL resin). Apply a flow rate of 100 cm/h. Record UV absorbance at 280 nm.
3. Alternatively to acetone, 1 – 2 M NaCl in equilibration buffer can be injected (corresponding to 1% (v/v) of the column volume). In that case, the column should be equilibrated in equilibration buffer supplemented with 0.5 M NaCl to prevent salt ions from interacting with the resin. A flow rate of 100 cm/h is applied and conductivity (mS/cm) is recorded instead of absorbance.

Use the following formulas to determine the HETP and the number of plates per meter (N/m):

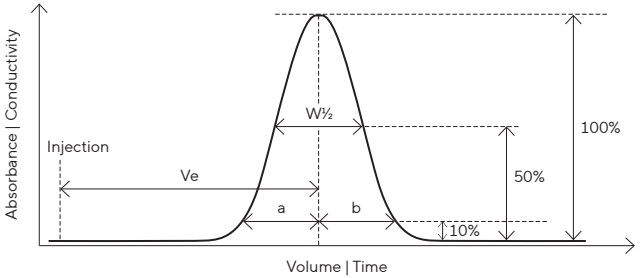
$$N/m = \frac{5.54 \times 100 \times (Ve / W½)^2}{BH}$$

With:
N = Number of theoretical plates
Ve = Elution volume on the chromatogram (cm)
W½ = Width of the acetone peak at half of the height (cm)
BH = Bed height (cm)

$$AF = \frac{b}{a}$$

With:
b = Right section of the peak at 10% peak height
a = Left section of the peak at 10% peak height

Figure 2: Peak Trace in a Typical Test Evaluation of Column Performance



"a" and **"b"** are respectively first and second half peak width at 10% of peak height. Typical values at 100 cm/h for N/m are 1,500 – 3,000 plates/m after packing in sodium phosphate, pH 7.0 and for asymmetry factor (AF), are from 0.8 and 1.8. These values are given as the average of experimental values. More important than the values by itself, the reproducibility of the values over the successive packing operations is critical.

3.2 Packing a Large Column (> 5 cm I.D.)
Please contact technical service.

4 Working Conditions and Basic Protocol
Use only samples and buffers that are previously filtered on a 0.2 µm membrane. It is also important to understand whether changes in pH and ionic strength used during ion exchange chromatography do not cause precipitation of sample components.

4.1 Equilibration
1. After packing, regenerate the column with 5 CV of a high ionic strength solution (i.e., 1 to 2 M NaCl in equilibration buffer). It is recommended to proceed to a cleaning-in-place (CIP) step as described in Section 5, especially if the resin has been previously used with a crude feedstock.
2. Then wash the column with 2 to 3 CV of a concentrated buffer (e.g., 200 mM Tris-HCl or sodium acetate) and run the column in equilibration buffer (i.e., 50 mM Tris-HCl, pH 8.5 for anion exchangers, or 50 mM sodium acetate, pH 4.5 for cation exchangers) until the ionic strength and pH of the buffer at both the outlet and the inlet of the column are identical.

4.2 Sample Application
1. Ensure the absence of bubbles in the sample.
2. Inject the sample into the column through a pump being careful not to introduce air into the column. Once sample injection is complete, continue with the various steps and data recording required by your protocol using a standalone pump or chromatography system. To maximize the dynamic binding capacity, appropriate dilution or diafiltration of the sample may be necessary.

4.3 Working Flow Rate
Because Ceramic HyperD® F resins are rigid and are not diffusion-limited due to the hyperdiffuse properties of the matrix, columns prepared as recommended can be used at high flow rates. For a 10 cm bed height column, 300 cm/h correspond to 2 minutes residence time. Higher flow rates can be used, depending on the optimal residence time and column and system pressure limits.

4.4 Choice of Elution Gradient and its Slope
For preliminary studies, a 10 CV linear NaCl gradient from 0 to 1 M salt is helpful as a first determination of separation conditions and resolution. Optimization of elution conditions can be achieved through adjustments to the gradient slope, utilization of salt steps, and changes in sample load.

5 Regeneration and Cleaning
Regenerate the column with 2 to 4 CV of 1 to 2 M NaCl, followed by a cleaning-inplace (CIP) with 5 CV of 0.5 M sodium hydroxide, 60 min contact time at room temperature. After CIP, neutralize the column with 2 to 3 CV of a concentrated buffer (e.g., ≥ 200 mM Tris-HCl or sodium acetate) to prepare for the next cycle, and pump 2 to 3 CV of storage solution onto the column before putting a packed column into storage between campaigns.

6 Thermal Stability and Storage

Temperature of use	2 – 30 °C
Storage temperature	2 – 30 °C (2 – 8 °C once opened)
Storage solution between runs	Neutral buffer containing bacteriostatic agents such as 20% (v/v) ethanol, 1 M NaCl and 1.2 mM EDTA
⚠	Product must never be frozen
i	Product is shipped at ambient temperature

7 Ordering Information

Ceramic HyperD® F Bottled Resin

Pack size	Part Number		
	Q Resin	DEAE Resin	CM Resin
25 mL	20066-031	20067-039	20050-035
100 mL	20066-023	20067-021	20050-027
1 L	20066-015	20067-013	20050-019
5 L	20066-064	20067-054	20050-050
10 L	20066-056	20067-047	20050-043

Prepacked Columns

Description	Part Number
PRC Columns	
PRC Prepacked Column Q Ceramic HyperD® F 5 x 50, 1 mL, 1/pkg	PRC05X050QCHDF01
PRC Prepacked Column CM Ceramic HyperD® F 5 x 50, 1 mL, 1/pkg	PRC05X050CMCHDF
RoboColumn®*	
RoboColumn®* CM Ceramic HyperD® F 200 µL, row of 8	SR2CMCHDF
RoboColumn®* CM Ceramic HyperD® F 600 µL, row of 8	SR6CMCHDF

* RoboColumn is a trademark of Repligen GmbH

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