PALL Life Sciences

Product Note

USD 2774

Q, S, DEAE, CM Ceramic HyperD® F Ion Exchange Sorbents

- High dynamic binding capacity at high flow rates
- Truly rigid, non-compressible sorbent
- Salt tolerant CM Ceramic HyperD F sorbent reduces Ultrafiltration (UF)/Diafiltration (DF) requirements

*Filtration. Separation. Solution.*SM

Introduction

Pall Q, S, DEAE, and CM Ceramic HyperD F ion exchangers are high capacity sorbents designed for efficient and scalable purification of biomolecules. They maintain high dynamic binding capacity (DBC) under conditions where conventional sorbents display significant capacity or productivity limitations, and offer differentiated selectivity compared to Pall Q and S HyperCel™ or other new generation ion exchangers.

Ceramic HyperD F sorbents are manufactured at a Pall ISO 9001:2008 and ISO 14001:2004 compliant manufacturing facility.

Ceramic HyperD F sorbents are used in a number of approved therapeutic proteins, as well as in many clinical and preclinical trials, in columns larger than 500 liters. Regulatory Support Files (RSF) and column packing support are available from Pall.

The sorbents are available in a 50 µm grade for pilot to full scale production. They are supplied in a variety of packagings in 1 M NaCl containing 20% ethanol/ 1.2 mM EDTA.

Principles of Operating Mechanism

"Gel-In-A-Shell" Sorbent Design

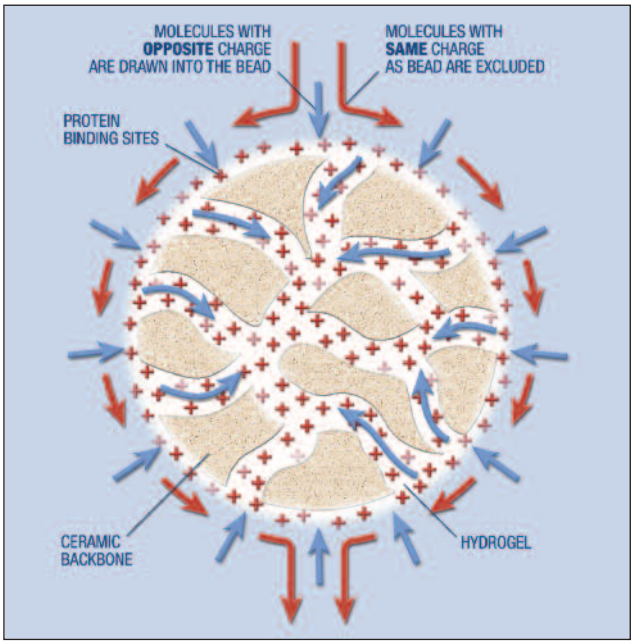
Traditional macroporous ion exchangers operate on the basis of classical pore diffusion, characterized by rapidly decreasing binding capacity with increased flow rate. In contrast, the unique structure of Ceramic HyperD F sorbent supports a more rapid mechanism of mass transfer, known as enhanced diffusion.

Ceramic HyperD F ion exchangers employ a high-capacity hydrogel polymerized within the large pores of a rigid ceramic bead. This design (Figure 1) combines the characteristics of a soft, high-capacity hydrogel with the absolute mechanical stability of a rigid ceramic bead.

Ceramic HyperD F sorbents do not shrink or swell with changes in pH or conductivity; columns with bed heights higher than 20 cm can be used to increase productivity and save plant space and capital investment. Because product is bound throughout the gel-filled pore – not merely at the interior surface of the pore – total binding capacity is enhanced. Binding of protein within the hydrogel is illustrated by the electron micrograph in Figure 2. Abundant ion exchange sites in the hydrogel are highly accessible to proteins. Biomolecules diffuse rapidly within the hydrogel, facilitating rapid uptake of product. This mechanism of mass-transfer – known as enhanced diffusion – allows the sorbent to operate free of the operational constraints typically encountered with conventional ion exchange sorbents.[2]

Figure 1

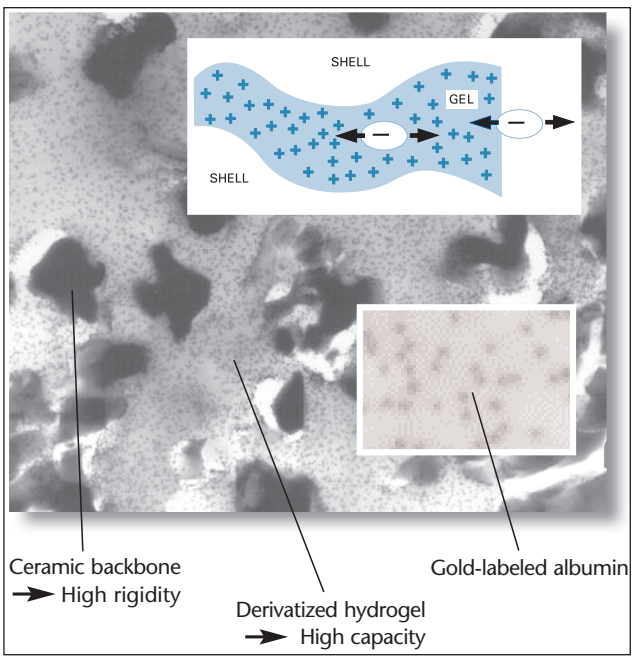
The "Gel-in-a-Shell" Design



Ceramic HyperD F sorbents deliver high dynamic capacity and absolute mechanical stability. This translates into productivity and process economics benefits at manufacturing scale.

Figure 2

Structure of Ceramic HyperD F Ion Exchange Sorbents



Cross section through the bead showing binding of gold-labeled albumin. The hydrogel completely fills the pores within the ceramic shell, and that gold-labeled albumin – visible as dense black dots – is distributed homogeneously throughout the hydrogel.

AcroSep™ and PRC Prepacked Columns For Fast Selectivity Screening

Ceramic HyperD F sorbents offer a different selectivity than Pall HyperCel sorbents or other new generation of ion exchangers.[9] To allow a convenient screening, Ceramic HyperD sorbents are supplied in easy-to-use prepacked columns: two different formats, AcroSep and PRC columns are proposed .

Prepacked 1 mL AcroSep Columns: Ceramic HyperD F ion exchangers are available in AcroSep 1 mL columns for sample preparation and sorbent screening. They can be operated manually in combination with a syringe, or with a chromatography system (semi-automated or automated).

Prepacked PRC Columns for Screening and Process Development: Q and CM Ceramic HyperD F sorbents are available in 1 mL (5 mm ID x 50 mm) prepacked PRC columns for rapid selectivity screening under reliable and reproducible conditions (refer to Pall USD 2492a). PRC columns can be easily connected to standard chromatography systems, and their typical backpressure is < 2.0 barg at 600 cm/hr in 0.1 M NaCl buffer.

Specifications and Main Properties

Table 1

Ceramic HyperD F Ion Exchangers Main Properties

	Type of Ceramic HyperD F Sorbent			
	Q	**S**	**DEAE**	**CM**
Average particle size (µm)	50	50	50	50
Dynamic binding capacity (mg/mL) 10% breakthrough at 200 cm/hr	BSA* ≥ 85[1]	lysozyme ≥ 75[2]	BSA* ≥ 85[1]	IgG ≥ 60[3]
Amount of ionic groups (µeq/mL)	≥ 250	≥ 150	≥ 200	250-400
Pressure resistance	70 barg (1,000 psi)			
Working pH	2-12			
Cleaning pH	1-14			
Volumes changes due to pH and ionic strength	Non compressible			

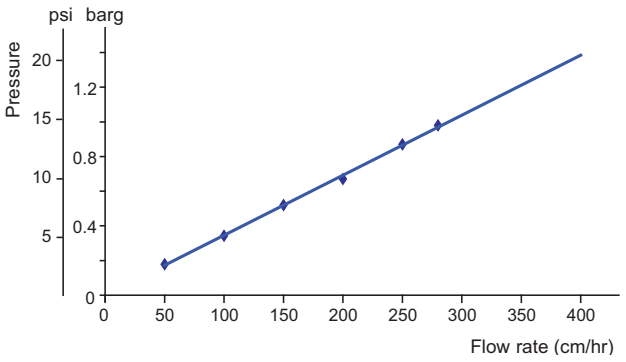
1. Sample: 5 mg/mL BSA in 50 mM Tris-HCl buffer, pH 8.6
2. Sample: 5 mg/mL lysozyme in 50 mM sodium acetate, pH 4.5
3. Sample: 5 mg/mL hu IgG in 50 mM sodium acetate, 100 mM NaCl, pH 4.7
** BSA = Bovine Serum Albumin*

Operating Flow Rates

The rigid ceramic skeleton of Ceramic HyperD F sorbent allows work at high linear velocities (typically over 300 cm/hr) with low or moderate backpressures (typically less than 3 barg) and without compression or shrinkage. Standard low pressure chromatography pumps and columns can be used. Figure 3 shows pressure vs. flow rates curves for Q Ceramic HyperD F sorbent. Column packing is accomplished quickly and easily owing to the dense nature of the Ceramic HyperD F beads.

Figure 3

Pressure vs. Flow Rate Curve on Q Ceramic HyperD F Sorbent



Column: 9 cm ID x 16 cm; Buffer: 50 mM Tris-HCl, 0.5 M NaCl, pH 8.6

Dynamic Binding Capacity (DBC) and Residence Time (RT)

Ceramic HyperD F ion exchangers can be operated at a high linear velocity or short RT. There is only a modest decline in DBC for bovine serum albumin (BSA) as linear velocity is increased from 50 cm/hr to more than 650 cm/hr (see Figure 4). At a RT of only 0.4 minute, DBC for BSA is over 85 mg/mL at 10% breakthrough for Q Ceramic HyperD F sorbent. As shown in Figure 5, there is only modest reduction in DBC as RT is reduced from 3 to 0.4 minute. DBC values ranging from ~85 to 120 mg BSA/mL were achieved. The high DBC of Ceramic HyperD F sorbents permits operation using columns of moderate volume and reduces buffer volume requirements, resulting in process productivity benefits.

Figure 4

DBC vs. Flow Rate on Q Ceramic HyperD F Sorbent

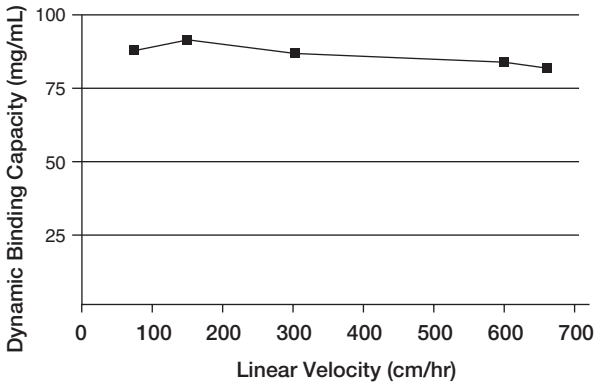
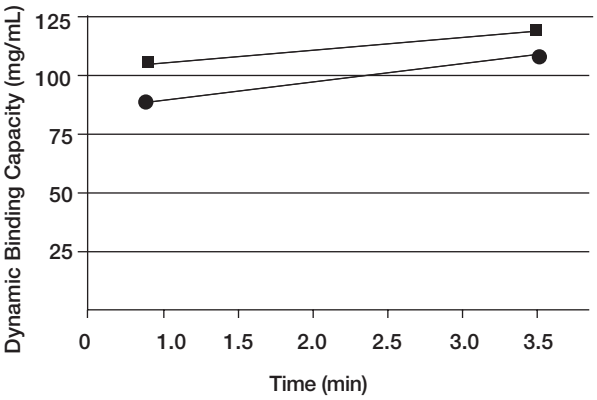


Figure 5

DBC vs. RT of Q Ceramic HyperD F Sorbent



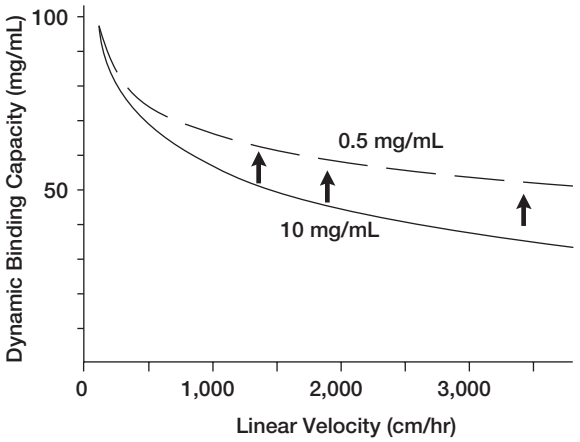
DBC at 10% (●) and 50% (■) breakthrough for BSA (0.5 mg/mL) in 50 mM Tris-HCl, pH 8.6

Dynamic Binding Capacity and Sample Concentration

Ceramic HyperD F sorbents provide higher DBC with dilute feedstock. This behavior is independent of protein concentration in the feedstock, as illustrated in Figure 5. Over a broad range of linear velocity values, higher DBC values are observed for feedstocks containing 0.5 mg hu IgG/mL than for those containing 10 mg hu IgG/mL. Use of Ceramic HyperD F sorbent at capture step reduces or eliminates the need for preliminary concentration of feedstock.

Figure 6

Binding Capacity of S Ceramic HyperD F Sorbent vs. Sample Concentration



Column: 0.2 cm ID x 15 cm; Sample: Hu IgG in 50 mM acetate, pH 4.6

Chemical Stability and Cleaning in Place (CIP)

Ceramic HyperD F ion exchangers can be sanitized using NaOH (i.e., 5 column volumes of 0.5 M NaOH for 1 hour contact time at room temperature). Data from Regulatory Support Files demonstrate long-term resistance (over 200 cycles) and no significant modification of the sorbent performance. Other chemical agents such as 20% ethanol/1 M acetic acid mixtures can also be used.

Virus clearance data have shown that viral clearance performance of Q Ceramic HyperD F sorbent was not affected after more than 200 cycles of CIP with 0.5 M NaOH.[8]

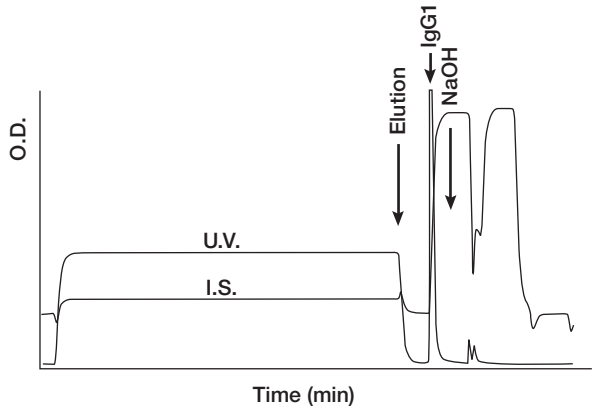
Application Examples

Example 1. Direct one-step capture of an IgG_1 from diluted cell culture supernatant (CCS) on CM Ceramic HyperD F sorbent

CM Ceramic HyperD F sorbent can be applied for a direct, one-step capture of monoclonal antibody from CCS. Prior to loading, the pH of the CCS was adjusted to pH 4.7 at a conductivity of 19 mS/cm (equivalent to about 180 mM NaCl). The concentration of IgG in the feedstock was low : 150 µg/mL. IgG was purified (>90%) at only 1 minute RT (260 cm/hr).

Figure 7

One-step Capture of Mouse IgG_1 from CCS on CM Ceramic HyperD F Sorbent



IgG_1 purity: 90%; Column: 9 cm ID x 5.2 cm (330 mL); Load: 31 L CCS 100-150 µg/mL adjusted to pH 4.7; Equilibration and post-load wash: 50 mM sodium acetate, 0.1 M NaCl, pH 4.7; Elution: same buffer + 1.5 M NaCl; Duration: 164 min; Residence time: 1 min; Linear velocity: 260 cm/hr.

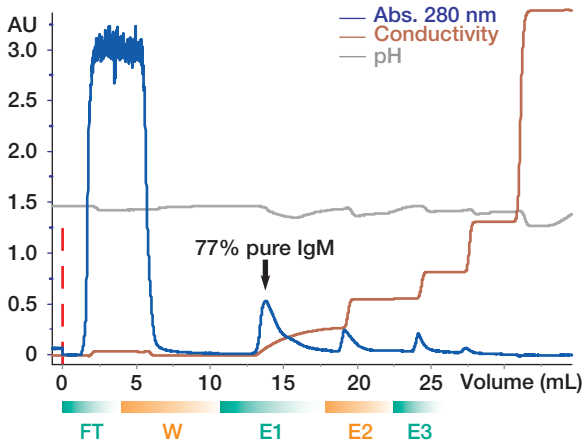
Example 2. Purification of mouse IgM from cell culture supernatant on CM Ceramic HyperD F sorbent

IgM are relatively difficult molecules to purify. Figure 8 shows the application of the purification of a concentrated mouse IgM CCS on CM Ceramic HyperD F sorbent, resulting in 77% step purity (analysis by SEC HPLC).

For details, refer to Pall application note USD 2410.

Figure 8

Purification of a Concentrated Mouse IgM CCS on CM Ceramic HyperD F Sorbent



Load at pH 5.5 : 4 mL after a 4-fold dilution. Equilibration + Wash: 100 mM sodium acetate, pH 5.5.

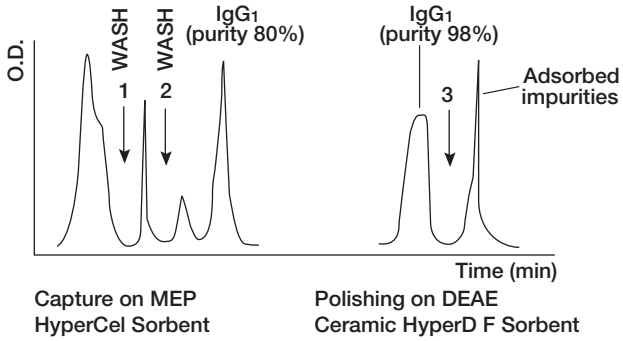
Elution by steps: Equilibration buffer + 0.1 M NaCl (E1); + 0.2 M NaCl (E2); + 0.3 M NaCl.(E3); Flow rate: 43 cm/hr (RT: 7 min.)

Example 3. Polishing step on DEAE Ceramic HyperD F ion exchanger after monoclonal antibody capture on MEP HyperCel mixed-mode sorbent

DEAE Ceramic HyperD F sorbent was used in a two-step process for a polishing step to purify a mouse IgG_1 from ascites fluid. The first step was a capture of the IgG_1 on a Pall mixed-mode MEP HyperCel column, which resulted in a good initial capture of the IgG_1 (93%). A purity of 98% for the IgG_1 was achieved in two steps.

Figure 9

Two-step Purification of IgG_1 from Ascites Fluid on MEP HyperCel Sorbent Followed by DEAE Ceramic HyperD F Sorbent



MEP HyperCel column: Wash 1 with 50 mM Tris-HCl buffer, pH 8, Wash 2 with 25 mM sodium caprylate in same buffer (arrow 1), followed by a water wash (arrow 2), to remove albumin. Elution with 50 mM sodium acetate, pH 4.0. The IgG_1 enriched fraction is added with Tris base up to pH 8.8 and ionic strength of 7.4 mS/cm, and injected onto the DEAE Ceramic HyperD F column. Wash with same buffer to collect the antibody. DEAE Ceramic HyperD F column: 0.6 cm ID x 10 cm; Equilibration: 50 mM Tris-HCl, pH 8.8; Linear velocity: 160 cm/hr. IgG does not bind, adsorbed impurities are eluted by 1 M NaCl (arrow 3).

Ordering Information

	Ceramic HyperD F Sorbent			
Pack Size	Q	S	DEAE	CM
5 mL	20066-098	20062-089	20067-070	20050-084
25 mL	20066-031	20062-030	20067-039	20050-035
100 mL	20066-023	20062-022	20067-021	20050-027
1 L	20066-015	20062-014	20067-013	20050-019
5 L	20066-064	20062-048	20067-054	20050-050
10 L	20066-056	20062-055	20067-047	20050-043

PRC Prepacked Columns

Part Number	Description	Pkg
PRC05X050QCHDF01	Q Ceramic HyperD F 5x50, 1 mL	1/pkg
PRC05X050CMCHDF01	CM Ceramic HyperD F 5x50, 1 mL	1/pkg

AcroSep Prepacked Columns

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

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