

Q, S, DEAE, CM Ceramic HyperD[®] Ion Exchange Sorbents

- High Dynamic Binding Capacity at high flow rates.
- High-efficiency capture from dilute feedstock.
- Rigid, non-compressible sorbent – easy to pack.
- Easy cleaning with sodium hydroxide.
- Regulatory Support Files (RSF) available.

Improve throughput with Ceramic HyperD enhanced diffusion chromatography sorbents.

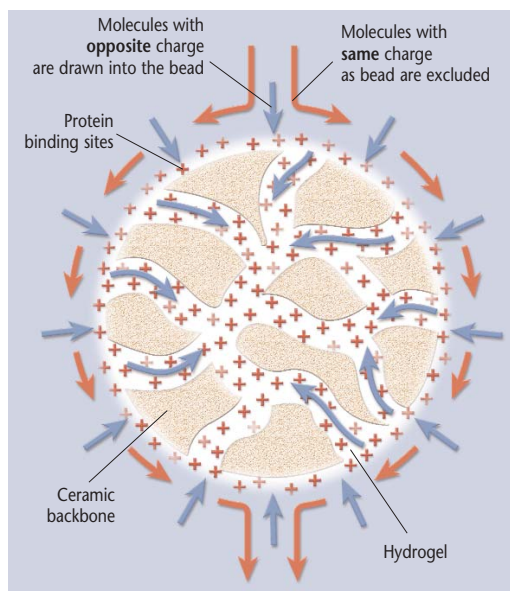
High productivity from rigid, composite sorbents.

Q, S, DEAE, CM Ceramic HyperD ion exchangers are high capacity sorbents specially designed for the efficient and scaleable purification of biomolecules from a variety of feedstocks. Of particular value in process-scale application, these sorbents are designed to maintain high dynamic binding capacity (DBC) under conditions where conventional sorbents display significant capacity or productivity limitations.

- **The "Gel in a Shell" design provides rapid mass transfer and effective binding.**

BioSeptra Ceramic HyperD ion exchangers employ a high-capacity hydrogel polymerized within the gigapores of a rigid ceramic bead. As shown in Figure 1, this design combines the desirable characteristics of a soft, high-capacity hydrogel with the absolute dimensional stability of a rigid ceramic bead. Ceramic HyperD sorbents do not shrink or swell with changes in pH or conductivity. Abundant ion exchange sites in the hydrogel are highly accessible to protein molecules. Proteins 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 macroporous ion exchange sorbents.

Figure 1. The "Gel-in-a-Shell" Design.



Ceramic HyperD sorbents deliver outstanding dynamic capacity and exceptional dimensional stability. This translates into unsurpassed productivity.

- **Capture product at high linear velocity.** Feedstock can be processed rapidly and efficiently. Dynamic binding capacity with Ceramic HyperD ion exchangers is virtually independent of linear velocity at values typically used – or sought – in laboratory or process scale separations. The pronounced flow-dependent behavior associated with traditional ion exchangers is not observed.

TABLE 1: Ceramic HyperD Ion Exchangers Main Properties.

Type of Ceramic HyperD Grade	Q 20	S 20	Q F	S F	DEAE F	CM F
Average particle size (µm)	~20	~20	~50	~50	~50	~50
Dynamic binding capacity (mg/mL) 10% breakthrough at 200 cm/h	BSA ≥ 85 ⁽¹⁾	lysozyme ≥ 85 ⁽²⁾	BSA ≥ 85 ⁽¹⁾	lysozyme ≥ 75 ⁽²⁾	BSA ≥ 85 ⁽¹⁾	IgG ≥ 60 ⁽³⁾
Amount of ionic groups (µeq/mL)	≥ 250	≥ 150	≥ 250	≥ 150	≥ 200	250-400
Working pH	2-12					
Cleaning pH	1-14					
Volumes changes due to pH and ionic strength	Non compressible					
Pressure resistance	20 grade: 200 bar (3,000 psi)			F grade: 70 bar (1,000 psi)		

(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.

• Efficient harvest from dilute feedstock.

Using Ceramic HyperD ion exchange sorbents, higher dynamic binding capacity is obtained with dilute feedstock than with concentrated feedstock (see Figure 6). This behavior – contrary to that observed with traditional sorbents – illustrates that these novel materials operate by a mechanism that is fundamentally distinct from that associated with classical macroporous sorbents. In many applications, product can be harvested from dilute feedstock without need for preliminary concentration, thereby enhancing the productivity of the overall purification scheme.

• Recover product from feedstock of moderate ionic strength.

The extraordinarily high ligand density present within the hydrogel of the CM Ceramic HyperD F ion exchange sorbent allows for effective binding even when product is present in feedstock of moderate ionic strength. For example, monoclonal antibody has been harvested from feedstock of 19 mS conductivity – equivalent to 180 mM salt. Thus, in many applications feedstock can be applied without preliminary diafiltration or dilution.

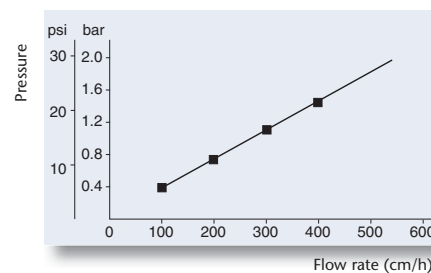
• Absolute dimensional stability, excellent pressure/flow characteristics, easy to pack.

Ceramic HyperD ion exchangers form very stable beds. They do not swell or shrink in response to changes in pH, ionic strength or flow rate. Indeed, the sorbents are stable

TABLE 2. Examples of Volumetric Flow vs. Linear Flow Rate.

Col. Ø	Flow rate (mL/min)		
	100 cm/h	300 cm/h	500 cm/h
9 cm	110	320	533
18 cm	420	1,270	2,120
30 cm	1,180	3,530	5,880
45 cm	2,650	7,950	13,250

Figure 2. Pressure vs. Flow rate Curve on Q Ceramic HyperD F.



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

at pressures up to 70 bar. Since the bead does not deform under fluid flow, the sorbent provides linear pressure/flow behavior, and has characteristics well suited to operation using low-pressure columns and equipment (see Figure 2 and Table 2). For example, in a pilot-scale column (9 cm ID x 16 cm) operated at 500 cm/h, operating pressure generated by the sorbent is less than 2 bar. Equally important, column packing is accomplished quickly and easily owing to the dense nature of the Ceramic HyperD beads.

TABLE 3. Lot-to-lot Consistency of Q Ceramic HyperD F Sorbents.

Lot No.	1	2	3	4	5	6	7	8	9	10	11
Ionizable groups (µeq/mL)	375	329	314	317	322	342	349	349	329	318	338
Dynamic binding capacity for BSA (mg/mL), 10% breakthrough:											
- at 200 cm/h	105	102	97	118	94	103	102	104	97	115	107
- at 600 cm/h	109	93	89	111	89	92	91	89	89	109	96

• **Robust, safe and documented for validation.**

Ceramic HyperD sorbents are manufactured in BioSeptra S.A. ISO 9001 registered manufacturing facility. Extensive experience on batch-to-batch reproducibility (see Table 3) guarantees the reliable performance and supply you need for your process. The sorbents can be treated with NaOH for efficient cleaning and sanitization.

Ceramic HyperD sorbents are used in a number of registered products, as well as in many clinical and preclinical trials, in columns larger than 500 liters. All grades of BioSeptra ion exchange Ceramic HyperD have Drug Master Files (DMF) :

- Q/S Ceramic HyperD 20, F: DMF No. 13241
 - DEAE Ceramic HyperD F: DMF No. 13242
 - CM Ceramic HyperD F: DMF No. 11856
- and for numerous chromatography sorbents, and provides full documentation to support regulatory validation.

Ceramic HyperD is supplied in 1 M NaCl containing 20% ethanol / 1.2 mM EDTA. It is available in a range of package sizes. Custom packaging to meet specific manufacturing requirements is available on request.

The enhanced diffusion concept.

Traditional macroporous ion exchangers operate on the basis of classical pore diffusion. Pore diffusion is characterized by rapidly decreasing binding capacity with increased flow rate. In contrast, the unique structure of Ceramic HyperD supports a more rapid mechanism of mass transfer, known as enhanced diffusion. Rapid mass transfer overcomes classical flow rate dependence. Since 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 3. Structure of Ceramic HyperD Ion Exchange Sorbents.

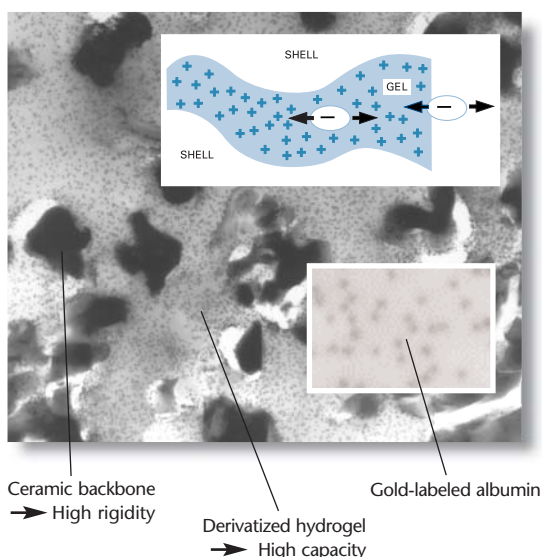
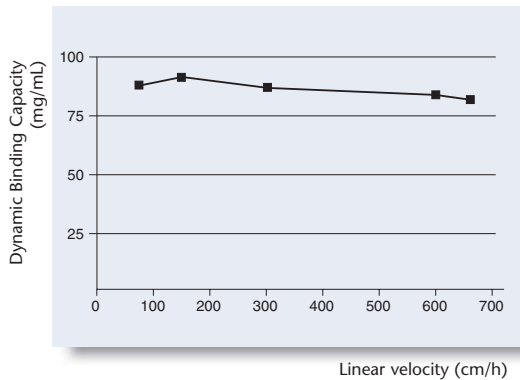


Figure 3. A cross section through the bead shows binding of gold-labeled albumin. Notice that the hydrogel completely fills the pores within the ceramic shell, and that gold-labeled albumin – visible as dense black dots – is distributed homogeneously throughout the hydrogel.

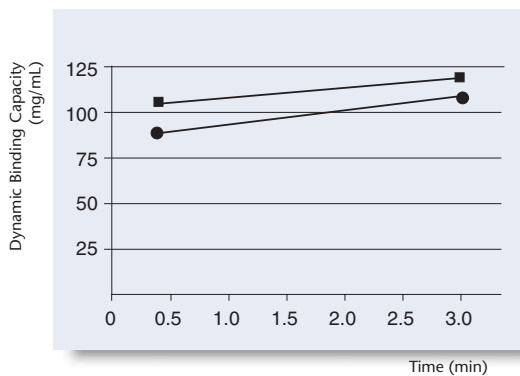
The hydrogel carries an extraordinarily high concentration of ion-exchange functional groups : 150-400 µeq/mL. The average distance between charged sites on the hydrogel is ~20 Å. Thus, a protein molecule within the gel is simultaneously in contact with a large number of ion exchange sites. It remains in contact with a similar number of sites no matter where it moves within the three-dimensional structure of the hydrogel. As a result, the protein is energetically unconstrained and may migrate freely. Protein diffuses rapidly within the hydrogel to give a homogeneous distribution, facilitating uptake of additional material from solution. Under binding conditions, strong attractive electrostatic forces between the

Figure 4. Dynamic Binding Capacity vs. Flow rate on Q Ceramic HyperD F.



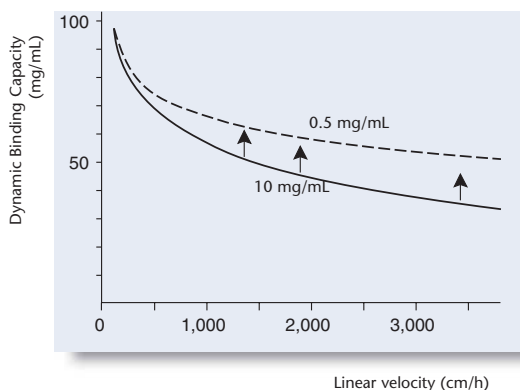
Column: 6.6 cm ID x 16 cm; BSA (5 mg/mL) in 50 mM Tris-HCl, pH 8.6; Dynamic binding capacity at 10% breakthrough.

Figure 5. Binding Capacity vs. Residence Time of Q Ceramic HyperD F.



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

Figure 6. Binding Capacity of S Ceramic HyperD F vs. Sample Concentration.



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

highly substituted hydrogel and the protein drive entry of protein into the gel.

The productivity-enhancing operational characteristics that this novel structure and mechanism provides are illustrated below.

Go fast without sacrificing capacity : higher loads, better throughput.

Ceramic HyperD ion exchangers deliver high dynamic binding capacity at high linear velocity. As shown in Figure 4, there is only a modest decline in DBC for BSA as linear velocity is increased from 50 cm/h to more than 650 cm/h. This behavior is further demonstrated by data in Table 1, comparing DBC values determined at 200 and 600 cm/h.

For a broad-based measure of productivity, many process-developers prefer to examine the influence of residence time on DBC. This approach allows assessment of sorbent characteristics without reference to details of column geometry. At a residence time of only 0.4 min, DBC for BSA is over 85 mg/mL at 10% breakthrough for Q Ceramic HyperD F. As shown in Figure 5, there is only modest reduction in DBC as residence time is reduced from 3 min to 0.4 min. DBC values ranging from ~85 to 120 mg BSA/mL were achieved over the range of conditions studied. The inherently high binding capacity of Ceramic HyperD sorbents permits operation using columns of moderate volume. By reducing bed volume requirements, buffer volume requirements may also be reduced.

High flow velocity, short residence time, reduced bed volume, reduced buffer volume – all of these factors support high productivity and enhanced process economics.

Simplify the process by eliminating preliminary concentration of dilute feedstock.

With traditional macroporous sorbents, dynamic binding capacity declines if protein concentration in the feedstock is reduced. In contrast, Ceramic HyperD sorbents provide higher binding capacity with dilute feedstock. This unique behavior is a function of enhanced diffusion, and arises because the absolute rate of uptake into the sorbent is independent of protein

concentration in the feedstock.

This useful behavior is illustrated in Figure 6. Over a broad range of linear velocity values, higher DBC is observed for feedstock containing 0.5 mg hu IgG/mL than for that containing 10 mg hu IgG/mL. With Ceramic HyperD used for product capture, it is possible to reduce or eliminate the need for preliminary concentration of feedstock. Indeed, a column of Ceramic HyperD can serve as a device for both concentration and initial purification of the target protein.

Simplify the process by eliminating preliminary diafiltration or dilution of feedstock containing moderate concentrations of salt.

With its highly substituted hydrogel, the CM Ceramic HyperD F ion exchange sorbent binds effectively even in the presence of moderate concentrations of salt. As shown in Figure 7, IgG₁ was harvested from 31 L of clarified cell culture supernatant (CCS) using a 330 mL column of CM Ceramic HyperD F.

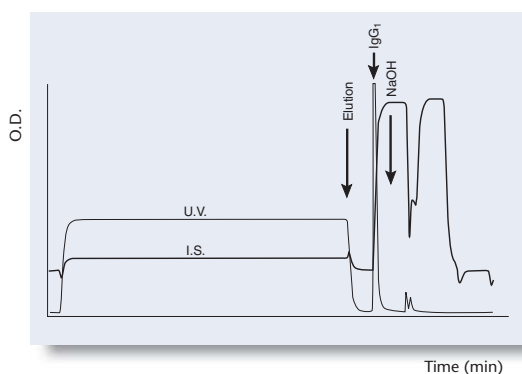
Prior to loading, the pH of the CCS was adjusted to pH 4.7. Conductivity of the feedstock was 19 mS/cm, equivalent to about 180 mM sodium chloride. The concentration of IgG in the feedstock was modest : 150 µg/mL. At a linear velocity of 260 cm/h, loading was accomplished in 112 min, and chromatography was complete in 164 min. Residence time was only 1 min. Isolated IgG was >90% pure.

Eliminating the need for preliminary diafiltration or dilution will simplify the process and enhance productivity of the scheme.

Achieve high flow velocities using standard chromatography equipment.

The rigid ceramic skeleton of Ceramic HyperD F allows work at high linear velocities (typically over 300 cm/h) with low or moderate backpressures (typically less than 3 bar) without compression or shrinkage. Standard low pressure chromatography pumps and columns can be used, contributing to overall process economy. Figure 2 shows pressure vs. flow rates curves for Q Ceramic HyperD F, and Table 2 gives examples of volumetric flow rates of different column diameters.

Figure 7. One-step Capture of Mouse IgG₁ from CCS on CM Ceramic HyperD F.



IgG₁ 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/h.

Stability and Cleaning.

• Chemical stability.

Ceramic HyperD F ion exchangers can be easily 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 of Ceramic HyperD F.

Other chemical agents such as 20% ethanol/1 M acetic acid mixtures can also be used for cleaning in place (for other chemical agents, contact our Technical Service).

• Thermal stability.

The cation exchange Ceramic HyperD sorbents are stable over a wide range of temperatures. They can be autoclaved (121°C for 20 min). Caution needs to be taken with the anion exchangers however, due to the progressive degradation of the tertiary and quaternary amine groups.

Regulatory and Validation Support.

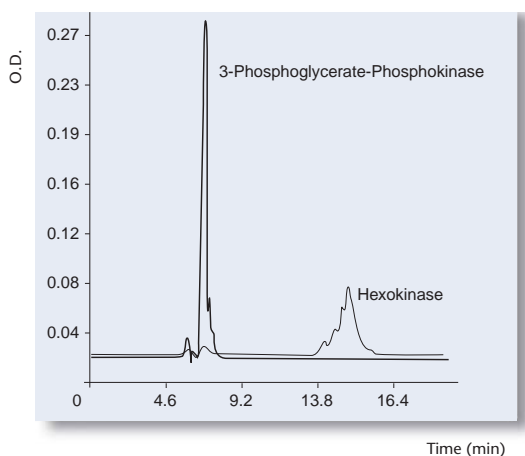
Ceramic HyperD ion exchange sorbents are manufactured under ISO 9001. They are available in a 20µm grade ("20") for pilot scale and polishing applications and a 50µm grade ("F") for full scale production. Regulatory support can be provided to customers to help qualify the material and

the chromatographic purification in regulated processes.

Ceramic HyperD F sorbents are fully validated products, presently used in the clinical production of several biopharmaceuticals, including full scale manufacturing of FDA-registered products.

An example of the typical batch-to-batch consistency of Ceramic HyperD sorbents is shown in Table 3. The data are part of the Regulatory Support File for Q Ceramic HyperD F.

Figure 8. Purification of Hexokinase and 3-phosphoglycerate-Phosphokinase on Q Ceramic HyperD 20.



Column: 0.5 cm ID x 10 cm (1.7 mL); Adsorption, washing, equilibration in 50 mM Tris-HCl / Tris base, pH 7.2; Elution by 0 to 1 M NaCl gradient; Protein concentration: 1 mg/mL; Linear velocity: 1,223 cm/h (4 mL/min).

Applications.

Ceramic HyperD F sorbents are ideally suited for purification of biomolecules in research, scale-up and full-scale pharmaceutical manufacturing.

The Ceramic HyperD 20 particle size is more adapted for polishing steps or rapid separations when a higher resolution is required.

- Direct capture of biomolecules from a variety of feedstocks.
- Polypeptides, IgG, albumin purification.
- Large-scale purifications.
- Purification of monoclonal antibodies from ascites or cell culture.
- Plasmid purification.
- Process polishing steps.
- Rapid high resolution purification (20µm grade).

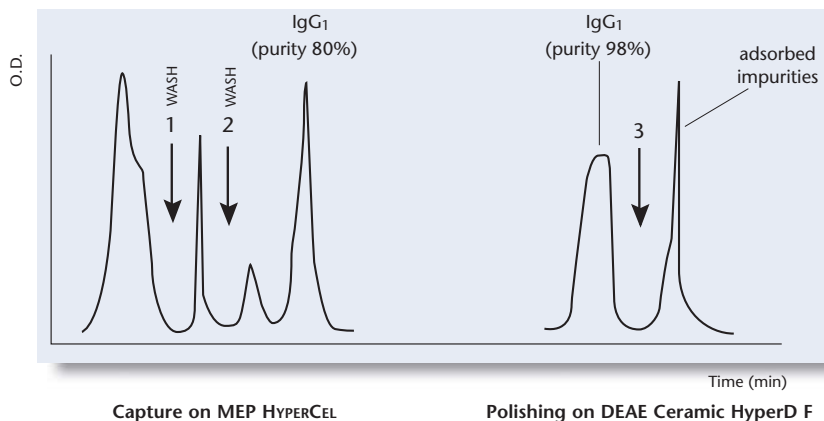
- **Direct one-step Capture of an IgG₁ from diluted cell culture supernatant (CCS) on CM Ceramic HyperD F.**

CM Ceramic HyperD F can be applied for a direct, one-step capture of monoclonal antibody from cell culture supernatant (see Figure 7).

- **Rapid purification of yeast proteins on Q Ceramic HyperD 20.**

The 20µm grade allows rapid method development for enzyme separation using a salt gradient (see Figure 8).

Figure 9. Two-step Purification of IgG₁ from Ascites Fluid on MEP HYPERCEL followed by DEAE Ceramic HyperD F.



MEP HYPERCEL column: First wash with 50 mM Tris-HCl buffer, pH 8, second wash 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 IgG₁ 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/h. IgG do not bind, adsorbed impurities are eluted by 1 M NaCl (arrow 3).

• **Polishing step on DEAE Ceramic HyperD F after monoclonal antibody capture on MEP HyperCel.**

DEAE Ceramic HyperD F has been used in a two-step process for a polishing step to purify a mouse IgG₁ from ascites fluid (see Figure 9). The first step is a capture of the IgG₁ on a MEP HyperCel column (Hydrophobic Charge Induction Chromatography – HCIC –), which results in a good initial capture of the IgG₁ (93%).

A purity of 98% for the IgG₁ is achieved in two steps.

• **More Regulatory Support information.**

Regulatory Support Files are updated periodically. Please contact your local representative.

• **Information from Regulatory Support Files.**

- Long term storage data,
- Material Safety Data Sheets,
- Lot-to-lot consistency information,
- Detailed Quality Control procedures,
- Chemical stability in various media,
- Cleaning in place (CIP) studies,
- Extractives and leachables quantification.

References

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Ordering Information

Product	Cat. No.	Size
Q Ceramic HyperD 20	20040-051	5 mL
	20040-044	25 mL
	20040-036	100 mL
	20040-028	500 mL
	20040-010	1 L
S Ceramic HyperD 20	20038-055	5 mL
	20038-048	25 mL
	20038-030	100 mL
	20038-022	500 mL
	20038-014	1 L
Q Ceramic HyperD F	20066-098	5 mL
	20066-031	25 mL
	20066-023	100 mL
	20066-015	1 L
	20066-064	5 L
	20066-056	10 L
S Ceramic HyperD F	20062-089	5 mL
	20062-030	25 mL
	20062-022	100 mL
	20062-014	1 L
	20062-048	5 L
	20062-055	10 L
DEAE Ceramic HyperD F	20067-070	5 mL
	20067-039	25 mL
	20067-021	100 mL
	20067-013	1 L
	20067-054	5 L
	20067-047	10 L
CM Ceramic HyperD F	20050-084	5 mL
	20050-035	25 mL
	20050-027	100 mL
	20050-019	1 L
	20050-050	5 L
	20050-043	10 L



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