BIOSEPRA® Protein A Ceramic HYPERD® F

Protein A Ceramic HyperD® F

Affinity Chromatography Sorbent

- Ideal for large-scale antibody purification.
- High binding capacity for human and murine IgGs.
- High selectivity with no non-specific binding.
- Rigid.
- Low backpressure.
- Easy to pack.

BioSepra® Protein A Ceramic HyperD® F sorbent available from Pall® is a high capacity affinity sorbent designed for process-scale purification of immunoglobulins G. The sorbent combines ease of use with high binding capacity and excellent scalability. Antibodies of greater than 95% purity are isolated from cell culture supernatant or ascites fluid in a single chromatographic step. Dilute feedstock (~100 µg IgG/ml) may be applied without preliminary concentration.

BioSepra Protein A Ceramic HyperD F sorbent is prepared using a rigid proprietary ceramic bead. Recombinant Protein A is immobilized to a specially formulated hydrogel within the porous ceramic bead.

The ceramic bead, the hydrogel, and the linkage used for the Protein A coupling are chemically stable over a broad range of conditions. Cleaning-in-place and sanitization procedures using sodium hydroxide may be employed with only limited loss of binding capacity. The unique, multi-point coupling chemistry provides a sorbent that exhibits very low leakage of recombinant Protein A.

The Protein A ligand is of recombinant origin and is produced in strict compliance with cGMP requirements. The rec Protein A is purified by standard chromatographic processes and is never in contact with immunoglobulins.

The rigidity of Protein A Ceramic HyperD F sorbent facilitates operation at high linear velocity. Similarly, bed depth may be increased to provide increased residence time and enhanced capture efficiency.

The sorbent is ideal for production scale purification. The dense ceramic material settles quickly and packs easily, even in large columns. Equally important, the material does not shrink or swell in response to changes in pH, ionic strength or flow rate.

The material is shipped in 1 M NaCl containing 20% ethanol and is available in a range of package sizes. Special packaging to meet specific manufacturing requirements is available on request.

Table 1: Main Properties of Protein A Ceramic HyperD F sorbent.

Particle size	50 µm (av.)
Dynamic binding capacity for hu IgG* (10% breakthrough, 100 cm/h)	> 30 mg/ml
Ligand	Recombinant Protein A
Immobilized Protein A	4 - 5 mg/ml of sorbent
Working pH	2 - 11
Cleaning pH	2 - 13
Volume changes due to pH and ionic strength	Non compressible
Pressure resistance	70 bar (1,000 psi)

* Determined using 10 mg/ml hu IgG in PBS, pH 7.4; Elution with 0.1 M sodium citrate, pH 2.5. Column: 4.6 ID x 100 mm.

• **Capacity**

As with all Protein A sorbents, the dynamic binding capacity is influenced by the composition of the sample, the IgG subclass, and the IgG concentration in the feedstock (figure 1). Nevertheless, Protein A Ceramic HyperD F sorbent exhibits high binding capacities with a variety of samples. Dynamic binding capacity for human IgG exceeds 30 mg/ml (figure 2) at linear velocities ranging from 100 to 300 cm/h.

Table 2 shows binding capacity values for different IgG subclasses from ascites or cell culture supernatant. The capacity ranges from 3.6 mg/ml for IgG_{2a} to 19 mg/ml for IgG_3.

Studies have shown that the best productivity is obtained when the average IgG residence time in the column is about 3-5 minutes (figure 3). A column 15-20 cm deep, operated at 300 cm/h provides a good compromise between high capacity and high linear velocity for process-scale use.

Figure 1. Influence of hu IgG concentration on binding capacity (flow rate: 75 cm/h).

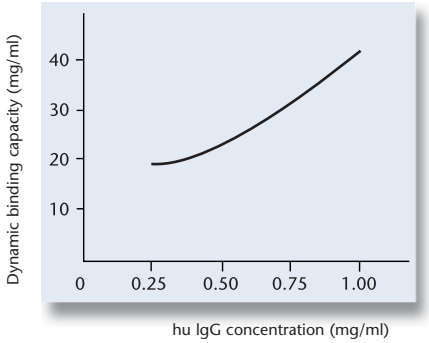


Figure 2. Dynamic hu IgG binding capacity as a function of flow rate (hu IgG: 1 mg/ml).

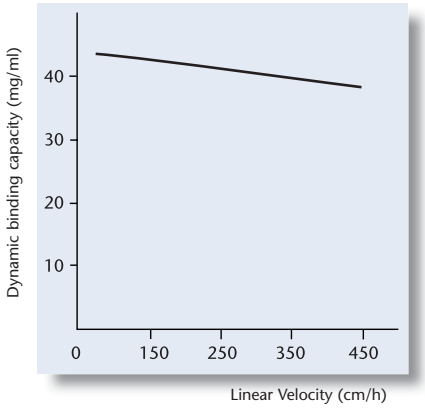
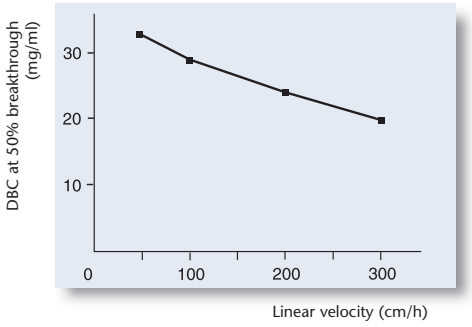
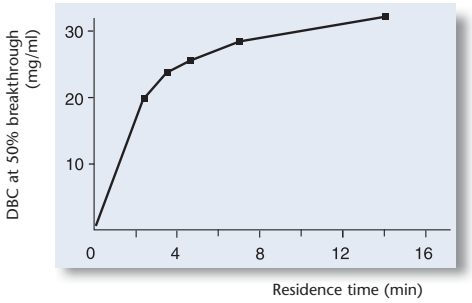


Figure 3. Capacity vs. linear velocity or residence time for hu IgG_1.





Experimental: 2.55 mg/ml hu IgG_1; Column: 1.0 x 11.7 cm.

Table 2. Binding Capacities for IgG Subclasses at 300 cm/h.

Source	IgG conc. in the sample (mg/ml)	Subclass	Capacity (mg/ml)
Cell culture supernatant	0.05	IgG_1	13.4
Ascites fluid	6.34	IgG_1	12.7
Ascites fluid	2.43	IgG_{2a}	3.6
Ascites fluid	4.00	IgG_3	19.0
Ascites fluid	4.10	IgG_{2b}	9.5

Column: 3 mm ID x 100 mm; Volume: 0.7 ml; Loading: 1 M glycine / 2 M NaCl, pH 8.9; Elution: 0.1 M acetic acid, pH 3.

• Purity

During studies with various IgG subclasses and feedstocks, Protein A Ceramic HyperD F sorbent provided high purity product, even at high column loading. Results are summarized in table 3. In all cases, the purity was greater than 98% as determined by SDS-PAGE. No albumin was detected in IgG_1 isolated from ascites fluid. Isolation of antibody from serum-containing cell culture supernatant is illustrated in figure 4. Analysis by SDS-PAGE is shown in figure 5.

Despite the relatively high selectivity of Protein A, isolation of high purity product, suitable for therapeutic use requires further, orthogonal chromatographic steps. Cation exchange chromatography may be conducted using CM or S Ceramic HyperD F sorbents. Typically, the cation exchange procedure is designed to bind IgG while impurities are selectively desorbed. Anion exchange chromatography on Q or DEAE Ceramic HyperD F sorbents may be employed to bind a variety of relatively acidic impurities while the antibody passes unretained in the flowthrough fraction.

• Stability

BioSepra Protein A Ceramic HyperD F sorbent is a dense, rigid, non compressible material. Packing is faster than for conventional Protein A Agarose. The sorbent typically settles in a few minutes, i.e. less than 10 min are needed to pack a 10 ml column.

Protein A Ceramic HyperD F sorbent allows the use of flow rates higher than 300 cm/h at backpressure less than 3 bar. Indeed, the chromatography described in figure 6 is accomplished at less than 0.4 bar at 300 cm/h. The pressure remained consistent for more than 30 cycles.

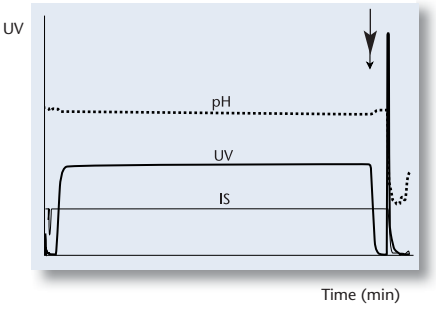
Pressure/flow rate characteristics of Protein A Ceramic HyperD F sorbent facilitate process-scale application. For example, at large scale, bed heights of 20-50 cm can be used at flow rates up to 200 cm/h to maximize capacity of the sorbent by optimising the residence time.

Table 3. Preparative Purification of Monoclonal IgG on Protein A Ceramic HyperD F.

Source	Origin	IgG Subclass	Initial IgG conc. (mg/ml)	Eluted IgG (mg)	Purity SDS-PAGE
Ascites fluid	murine	IgG_1 kappa	6.34	37	> 98%
Ascites fluid	murine	IgG_1*	2.26	20	> 98%
Ascites fluid	murine	IgG_1*	3.86	31	> 98%
Ascites fluid	murine	IgG_{2b}**	2.31	6	> 98%
Ascites fluid	murine	IgG_{2a}**	2.43	10	> 98%
Ascites fluid	murine	IgG_{2a}*	2.86	28	> 98%
Cell culture supernatant	murine	IgG_1*	0.05	15	> 98%
Cell culture supernatant	humanized	IgG_1*	0.20	30	> 98%

Column: 6.6 mm ID x 120 mm; Volume: 4 ml, 10-30 mg IgG per run; Loading: 1 M glycine / 2 M NaCl (*), pH 8.9 or PBS (**); Elution: 0.1 M acetic acid.

Figure 4. Isolation of IgG_1 from serum-containing cell culture supernatant on Protein A Ceramic HyperD F sorbent.



The arrow indicates introduction of elution buffer. Column: 3 mm ID x 100 mm. Loading and wash: 1 M glycine / 2 M NaCl, pH 8.9. Elution: 100 mM acetate buffer, pH 4.6. Linear velocity: 300 cm/h. Sample: 50 ml cell culture supernatant.

Figure 5. SDS-PAGE analysis of feedstock (1), flowthrough (2), recovered IgG_1 (3).

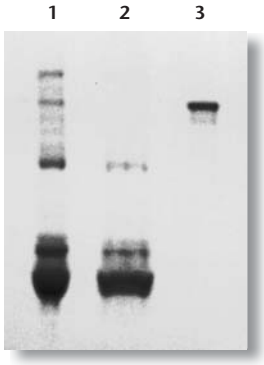
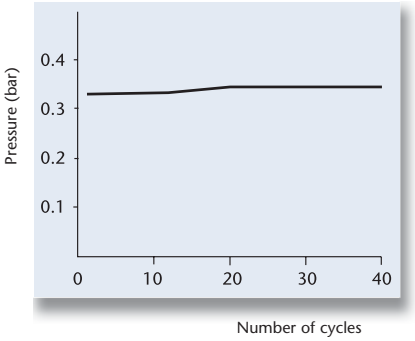


Figure 6. Pressure vs. number of cycles.



hu IgG capacity at 10% breakthrough; Linear flow rate: 300 cm/h; Bed height: 25 cm; Residence time: 5 min; Loading: 35 mg/ml.

• Cleaning

Recombinant Protein A, rich in carboxylic side chains, is coupled to the primary-amine-containing hydrogel via stable secondary-amide linkages. The procedure has been tailored to give optimized multi-point attachment, as illustrated in figure 7.

This unique multi-point coupling chemistry confers high chemical stability.

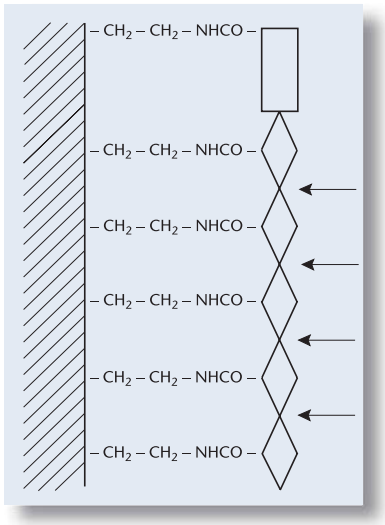
Cleaning with sodium hydroxide from 1 to 100 mM can be performed repeatedly. Modest decreases in binding capacity can occur over time depending upon the concentration of sodium hydroxide and the total contact time. Alternatively, cleaning may be conducted using 6 M guanidine hydrochloride for some/all cleaning cycles.

• Ligand leakage

As a consequence of the Protein A coupling chemistry, the ligand leakage is low. An independent study of Protein A Ceramic HyperD F sorbent showed less than 10 ng Protein A per mg of purified hu IgG (average value).
A non-competitive ELISA procedure was used to determine native Protein A in the IgG eluate. These findings reflect the high stability of Protein A Ceramic HyperD F sorbent.

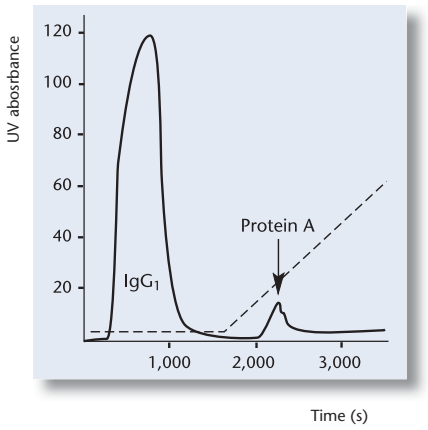
In many pharmaceutical applications, an anion exchange procedure is employed to assure removal of trace quantities of leached rec Protein A. Q Ceramic HyperD F sorbent is well suited to this application. The procedure is conducted under conditions which promote dissociation of the IgG-Protein A complex. As illustrated in figure 8, IgG is unretained, while the relatively acidic Protein A (pI < 5) is bound. The study was performed using IgG_1 spiked with 107 µg of rec Protein A per mg of IgG_1.

Figure 7. Tentative schematic structure of immobilized Protein A on Ceramic HyperD.



The arrows represent proteolysis sensitive locations of Protein A between sub-units responsible for Fc binding.

Figure 8. Separation of spiked Protein A from IgG_1 using a Q Ceramic HyperD F column.



Sample: IgG_1 (7.9 mg) purified from a cell culture supernatant using Protein A Ceramic HyperD F sorbent spiked with 850 µg Protein A. As required, the mobile phase can contain up to 2 M urea and 50% ethylene glycol. A sodium chloride gradient up to 2.6 mS can be employed.

Applications

Optimum binding conditions depend upon the IgG subclass to be purified. In most applications, binding is conducted under physiological conditions. For weakly interacting immunoglobulins (e.g. murine IgG_1), efficient binding is accomplished at higher pH (~ 8.5 - 9.0) using a mobile phase augmented with glycine and sodium chloride.

Elution is normally accomplished at acidic pH (2.5 - 3.0). Milder pH can be used for weakly adsorbed IgGs such as mouse IgG_1.

Once conditions have been optimized, scale up is accomplished by increasing the column diameter while maintaining the optimum bed height and linear velocity.

Detailed recommendations for use can be found in the Product Insert which is supplied with the product.

Protein A Ceramic HyperD F sorbent is well suited to isolation and purification of IgG from ascites fluid, cell culture supernatant, transgenic milk, and various animal sera. Other applications include :

- Separation of IgG subclasses (e.g. Protein A does not interact with human IgG_3).
- Separation of Fc fragments from a mixture of Fc and Fab fragments obtained after enzymatic hydrolysis.
- Purification of humanized or mouse monoclonal antibodies.
- Separation of immune complexes.
- Purification of enzyme conjugates.
- Removal of bovine IgG from hybridoma cell culture.

References

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Ordering Information

Product	Cat. No.	Size
Protein A Ceramic HyperD F	20078-036	5 ml
	20078-028	25 ml
	20078-010	100 ml
	20078-044	1 L
	20078-051	5 L
	20078-069	10 L

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