



ProSep[®]-vA High Capacity Chromatography Media

- ▶ Animal free
- ▶ Rigid and incompressible
- ▶ Low back pressure
- ▶ No shrinking or swelling
- ▶ Open, uniform pores
- ▶ Flexible operating conditions

Designed for large-scale purification of therapeutic antibodies

ProSep-vA High Capacity chromatography media with v Protein A is ideally suited as the capture step in the purification of monoclonal, polyclonal and engineered antibodies. Binding capacities in excess of 30 mg/mL are achievable for human monoclonal antibodies and in excess of 40 mg/mL for polyclonal. With dynamic capacity sustained at high flow rates irrespective of column dimensions, the need for a concentration or buffer exchange step prior to loading the affinity column is reduced. ProSep-vA High Capacity media can improve process time, maximize productivity and reduce costs.

Meeting Industry and Regulatory Requirements

Since the introduction of PROSEP-A High Capacity media the demand for therapeutic monoclonal antibodies has increased, necessitating high throughput capability at initial capture. Regulatory authorities have also encouraged the biopharmaceutical industry to remove mammalian derived materials from the processes. ProSep-vA High Capacity media has been developed to fulfill both these requirements. The product is equivalent to its predecessor, PROSEP-A High Capacity media in both functionality and specification. Both are made with natural Protein A derived from *Staphylococcus aureus* but with ProSep-vA High Capacity media no mammalian products are used in its manufacture.

Matrix

ProSep-vA High Capacity media is based on a porous glass matrix that is fully incompressible, highly porous and with a large percentage of open ended, interconnected pores. This allows very rapid mass transfer, resulting in high dynamic capacity. Due to a linear relationship between back pressure and flow rate, the response of a ProSep-vA High Capacity packed column to increased flow rate is entirely predictable over different column lengths and diameters. The absolute rigidity allows operation at flow rates of >1000 cm/h (Figure 1).

Dynamic Binding Capacity

The open interconnected pore structure of ProSep-vA High Capacity media provides rapid mass transfer rates resulting in sharp breakthrough curves (Figure 2) and high dynamic capacities being maintained even at high flow rates (Figure 3). This, coupled with the low pressure drop and the rigid nature of the controlled pore glass base matrix, delivers high throughput and productivity. It is possible to operate at bed lengths greater than 30 cm, providing greater flexibility in column selection and process design and optimization.

Predictable Performance

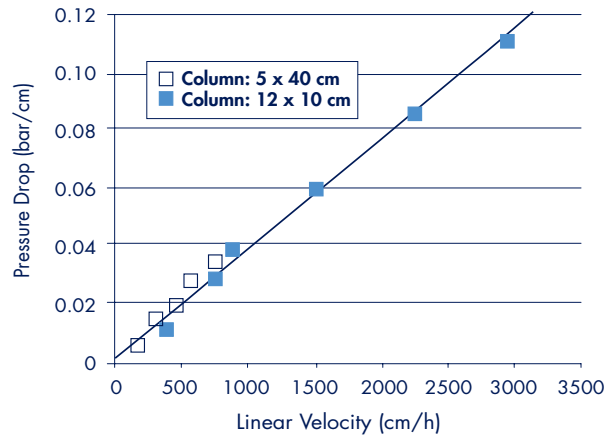


Figure 1. Response of ProSep-vA High Capacity media to increased flow rate.

Breakthrough Curve Comparison

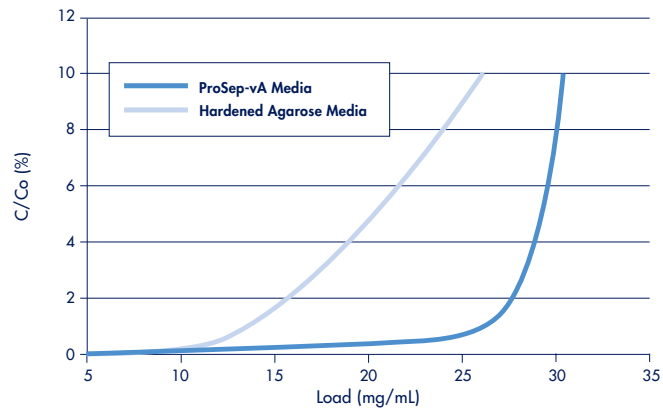


Figure 2. Human polyclonal IgG breakthrough curves, ProSep-vA media vs. hardened agarose media at 500 cm/h in a 19 cm bed height.

Dynamic Capacity vs. Residence Time

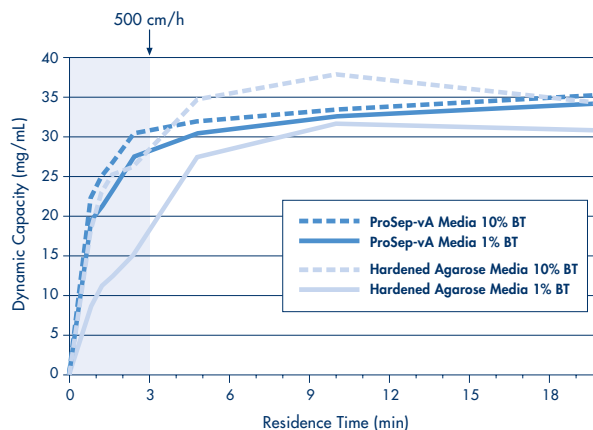


Figure 3. Dynamic capacity vs. residence time, ProSep-vA media vs. hardened agarose media (bed height = 19 cm). Feed: 1 mg/mL human polyclonal IgG.

Reusability

An important consideration when designing a cost effective purification protocol is the number of times chromatographic media can be re-used without loss in performance.

Re-use of PROSEP-A High Capacity media over a number of cycles was studied. Humanized IgG₁ from cell culture supernatant was purified over 100 cycles under the following conditions:

Linear

flow rate: 315 cm/h at
1% breakthrough

Load: Cell culture supernatant

Wash: 0.1M sodium acetate
pH 6.5

Elute: Acetic acid pH 2.5

The column was cleaned every fifth cycle with HCl pH 1.5, followed by 6M guanidine hydrochloride.

No change in performance was observed over 100 cycles of use. Antibody capacity (Table 1), elution profile (Figure 4) and purity (Figure 5) remained consistent throughout the study.

Cleaning and Sanitization

Sustained column performance depends on the use of recommended handling and cleaning procedures. A number of sanitants and cleaning agents are recommended for use with ProSep-vA High Capacity media, for example 1% (v/v) phosphoric acid pH 1.5; 0.3% (v/v) hydrochloric acid pH 1.5; 6M guanidine hydrochloride. These are detailed in the User Instruction Manual that accompanies the product.

Antibody Capacity

Cycle Number	Mean Capacity (mg/mL)
1	14
1 to 10	15
11 to 20	15
21 to 30	15
31 to 40	14
41 to 50	14
51 to 60	15
61 to 70	15
71 to 80	16
81 to 90	15
91 to 100	15

Table 1. Re-use data capacity of PROSEP-A High Capacity media over 100 cycles.

Consistent Performance

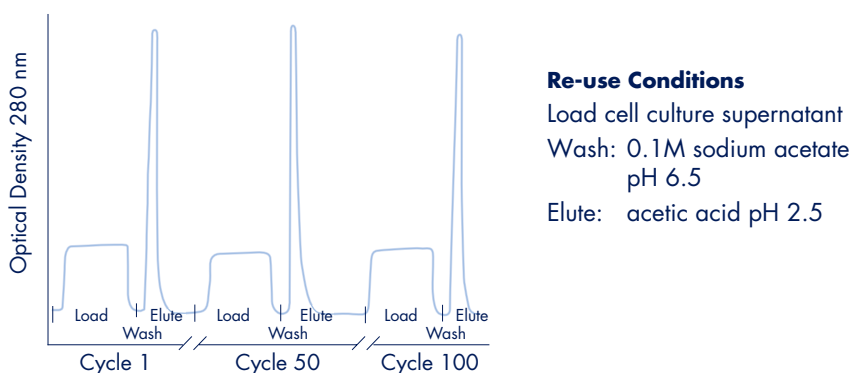


Figure 4. Chromatograms over 100 cycles of re-use.

Viral and DNA Clearance

An independent GLP study showed the effectiveness of PROSEP-A High Capacity media in the clearance of viruses and DNA (Table 2).

Viral Clearance

Three model viruses were used in the study: Herpes Simplex, an enveloped, double stranded DNA virus; Murine Leukemia, an enveloped, single stranded RNA virus, and Polio virus, a non-enveloped, single stranded RNA virus.

Cell culture supernatants were spiked with virus and applied to the columns. Antibody from the supernatants was purified in a typical purification cycle. Virus concentrations were measured in the flow through, wash fractions and eluants from each matrix.

PROSEP-A High Capacity media gave a significantly higher clearance of Herpes Simplex virus than Protein A agarose media. All other viral clearance results were comparable.

DNA Clearance

This study was carried out using endogenous DNA in the cell culture supernatant and results were comparable. PROSEP-A High Capacity media 2.35 logs clearance and Protein A agarose 2.75 logs clearance.



Key:

- Lane
1. Molecular weight markers*
 2. Feedstock
 3. IgG₁ purified after 1 cycle
 4. IgG₁ purified after 50 cycles
 5. IgG₁ purified after 100 cycles
 6. Molecular weight markers*

* (Molecular weight markers:
14.4, 20.1, 30, 43, 67 and 94 Kd)

Figure 5. SDS-PAGE gel of a purified IgG (overloaded) to show any variations over 100 cycles.

Virus	PROSEP-A High Capacity Media	Protein A Agarose Media
HSV1	7.1 logs	4.4 logs
MuLV	6.4 logs	6.9 logs
Polio	5.9 logs	4.5 logs
Total antibody purified	33.9 mg	21.7 mg

Table 2. Viral clearance results.

Storage and Handling

ProSep-vA High Capacity media is supplied in 0.1M acetate buffer, pH 5.2 and a suitable preservative.

During use, ProSep-vA High Capacity media may be stored in phosphate buffered saline (PBS) or other suitable buffer containing a preservative. The acceptable environmental storage temperature for ProSep-vA High Capacity media is between 2–8 °C.

Specifications

Product	Limit
Static binding capacity for human polyclonal IgG adsorbed in PBS pH 7.4 and eluted in 0.1M glycine/HCl pH 2.0.	≥ 40 mg/mL
Static binding capacity for mouse monoclonal IgG ₁ adsorbed in 1M glycine/NaOH pH 8.6 containing 0.15M NaCl and eluted.	≥ 10 mg/mL
Protein A leakage/mg mouse IgG ₁ . Leakage is determined by measuring the quantity of Protein A in the eluted mouse IgG ₁ using a sensitive immunoassay method.	≤ 15 ng/mg

Manufacturing Standards and Quality Assurance

Millipore recognizes the importance of providing regulatory support and meeting industry quality standards. All ProSep products are manufactured in a facility certified to internationally recognized standard BS EN ISO 9001 and subjected to routine independent surveillance audits.

Ordering Information

Media	Quantity	Catalogue Number
ProSep-vA High Capacity	2 mL	113 115 822
ProSep-vA High Capacity	10 mL	113 115 824
ProSep-vA High Capacity	50 mL	113 115 826
ProSep-vA High Capacity	100 mL	113 115 827
ProSep-vA High Capacity	500 mL	113 115 829
ProSep-vA High Capacity	1 liter	113 115 830
ProSep-vA High Capacity	5 liter	113 115 833
ProSep-vA High Capacity	10 liter	113 115 835

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