Data Sheet

ProSep®–vA High Capacity Chromatography Media

Designed for large-scale purification of therapeutic antibodies

ProSep®-vA High Capacity media is suited as capture step in the purification of therapeutic antibodies. It was the first commercially available animal free protein A resin, designed for the large-scale purification of monoclonal, polyclonal and engineered antibodies.

The larger pore size of ProSep®-vA High Capacity media allows for usage for higher molecular weight MAb's. 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 exhibits the following features:

1. Animal free
2. Rigid and incompressible
3. Low back pressure
4. Open, uniform pores

Benefits

- Improve process time
- Maximize productivity
- Flexible operating conditions

Matrix

ProSep®-vA High Capacity media is based on a highly porous glass matrix that is fully incompressible, 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 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 antibody mass transfer rates, resulting in sharp breakthrough curves (Figure 2). This also results in high dynamic capacities being maintained even at high flow rates (Figure 3). This, coupled with the low pressure drop of the rigid nature of the controlled pore glass base matrix, delivers high throughput and productivity. It is possible to operate at bed heights greater than 30 cm, providing higher flexibility in column selection, process design and optimization.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

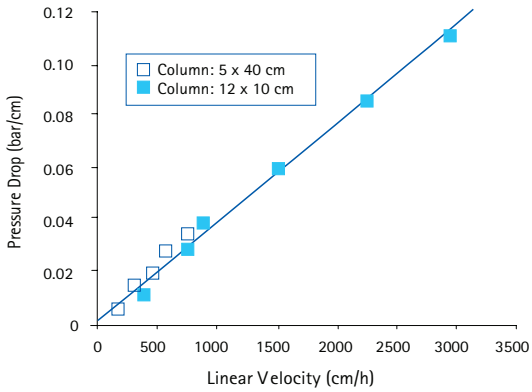


Figure 1. Predictable Performance

Pressure response from resin bed of ProSep®-vA High Capacity media to increased flow rate.

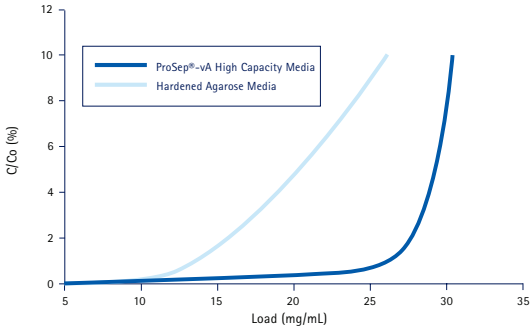


Figure 2. Breakthrough Curve Comparison

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

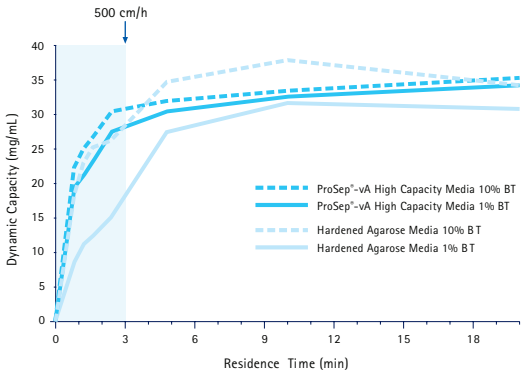


Figure 3. Dynamic Capacity vs. Residence Time

ProSep®-vA High Capacity 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®-vA 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 or 4-6M urea. These are detailed in the User Instruction Manual that accompanies the product.

Storage and Handling

ProSep®-vA High Capacity media is supplied in 0.1M acetate buffer, pH 5.2 and 1% benzyl alcohol as a preservative.

During use, it is recommended to store ProSep®-vA High Capacity media in 0.1M acetate buffer, pH 5.2 containing 1% or 2% benzyl alcohol as a preservative. Alternatively, 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.

Table 1. Antibody Capacity

Capacity re-use data of ProSep®-vA High Capacity media over 100 cycles.

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

Elution Profile

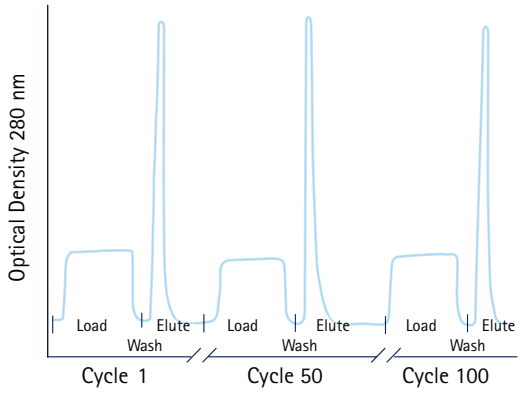


Figure 4. Consistent Performance

Chromatograms over 100 cycles of re-use.

Table 2. ProSep®-vA High Capacity Characteristics

Base Matrix:	Controlled Pore Glass
Particle size:	74 - 125 µm
Binding Capacity (Static):	Typically >/= 40 mg/mL
Binding Capacity (Dynamic):	Typically >20mg/mL (1% breakthrough at 3-6 min residence time)
Recommended Long Term Storage:	2-8 °C plus bacteriostat

Viral and DNA Clearance

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

Viral Clearance

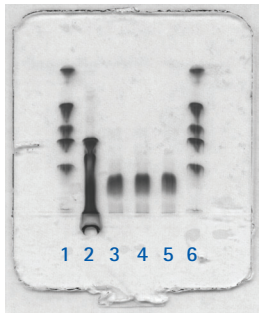
Three model viruses were used in the study: Herpes Simplex (HSV), an enveloped, double stranded DNA virus; Murine Leukemia (MuLV), an enveloped, single stranded RNA virus, and Polio virus, a non-enveloped, single stranded RNA virus.

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®-vA 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®-vA High Capacity media showed 2.35 logs clearance and Protein A agarose showed 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 Profile

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

Table 3.

Viral clearance results.

Virus	ProSep®-vA 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

Manufacturing Standards and Quality Assurance

EMD Millipore recognizes the importance of providing regulatory support and meeting industry quality standards. No Mammalian derived materials are used in the manufacture of ProSep®-vA High Capacity media. 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	Qty	Catalogue No.
ProSep®-vA High Capacity	2 mL	113 115 822
ProSep®-vA High Capacity	10 mL	113 115 824
ProSep®-vA High Capacity	100 mL	113 115 827
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

Column Cleaning & Storage of ProSep® Resins

Product	Order No.
Acetic acid 1 mol/L suitable for biopharmaceutical production EMPROVE® bio	137035
Acetic acid 30% suitable for biopharmaceutical production EMPROVE® bio Ph Helv	137047
L-Arginine suitable for use as excipient EMPROVE® exp Ph Eur, USP	101587
Benzyl alcohol suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, NF, ACS	137043
Ortho-Phosphoric acid 75% suitable for biopharmaceutical production	100250

Chromatography Columns and Systems

Chromatography columns and systems are critical factors to the successful separation of your valuable molecule. EMD Millipore provides standard and custom columns and systems from labscale to pilot and process scale. From screening to large-scale production, our columns, systems and single-use solutions are designed to provide robust, consistent performance while providing you with the processing flexibility required in today's changing production environment.

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