Millipore®
Preparation, Separation,
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Data Sheet

ProSep®-vA Ultra Chromatography Media

Designed for effective, large-scale purification of high titer therapeutic antibodies

ProSep®-vA Ultra chromatography media provides high binding capacity and improved process economics for the capture and purification of monoclonal, polyclonal and engineered antibodies. It is designed to address the needs of today's higher titer, large volume fermentation feedstocks. Titers in excess of 1g/L and fermenter volumes of 10,000 liters and larger can easily be processed in a single day.

Proven Technology

ProSep®-vA Ultra media is used in the purification of a number of approved monoclonal therapeutic antibodies. While ProSep®-vA Ultra media uses the same immobilization chemistry as ProSep®-vA High Capacity media, the pore size of its controlled pore glass base matrix is smaller. This provides significantly more surface area which, when derivitized at the same protein A surface density, results in a significant increase in IgG binding capacity.

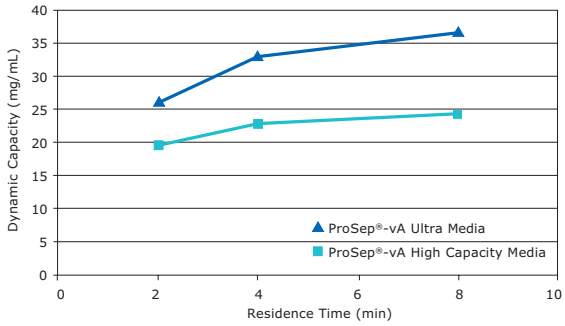
Benefits

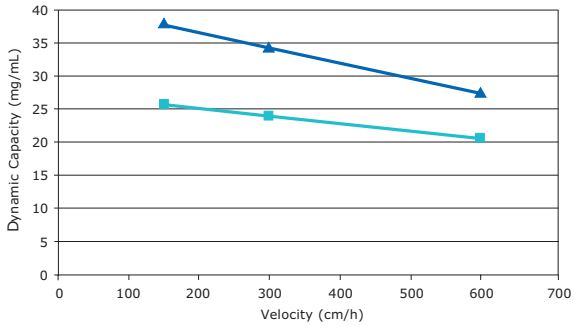
- High throughput and productivity
- Low cost of operation
- Flexible process design
- Reliable scale-up.

Figure 1.

Dynamic Binding Capacity
Dynamic capacity of ProSep®-vA Ultra media

Conditions:
Clarified monoclonal IgG1 feedstock. Column 6 mm I.D. x 200 mm bed height





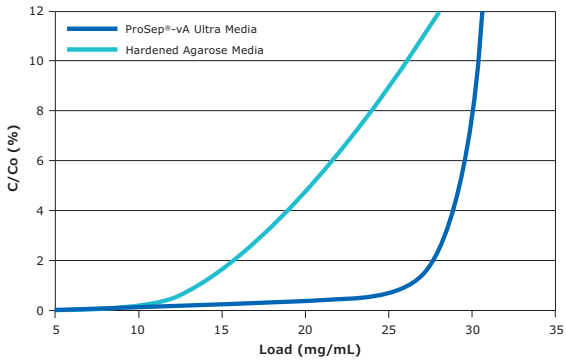
Higher Binding Capacity

Although based on a smaller pore size, the open interconnected pore structure maintains rapid mass transfer resulting in high dynamic binding capacities under a wide range of operating conditions (see Figure 1).

The sharp breakthrough curve of ProSep®-vA Ultra resin (see Figure 2) is the result of the uniform open pore structure of controlled pore glass. This enables higher loading efficiency of antibody without the risk of premature breakthrough. The combination of increased ligand loading per volume and high antibody binding efficiency leads to significantly increased dynamic binding capacity.

Figure 2.

Breakthrough Curve Comparison
Figure shows the sharp breakthrough curves of ProSep®-vA Ultra media compared to hardened agarose media.



Operational Flexibility

The porous glass base matrix is incompressible, leading to a linear relationship between back pressure and flow rate. The response of a ProSep®-vA Ultra packed column to increased flow rate is therefore entirely predictable over different column heights and diameters. The combination of total rigidity and particle size range allows operation at flow rates in excess of 1000 cm/h. This relationship is illustrated in Figure 4.

Higher Productivity

The combination of low back pressure with the rigidity of ProSep®-vA Ultra media enables operation at high flow rates which has been shown to be advantageous in terms of high throughput operation.[2, 3, 4] It also has the benefit of permitting a wider window of operation in terms of column bed height. These benefits are illustrated in Figure 3 where productivity (in terms of g IgG processed/hr/unit area of media) is plotted against column bed height and load velocity. The ability to run at longer bed heights and higher flow rates compared to more compressible media not only enables higher productivity to be achieved, but also provides more flexibility in process design. The ability to operate the same volume of ProSep®-vA Ultra media in a longer bed height, smaller diameter column makes incorporation into existing facilities easier. Media requiring shorter bed height, larger diameter columns may be difficult or impossible to incorporate into facilities where floor space is already at capacity.

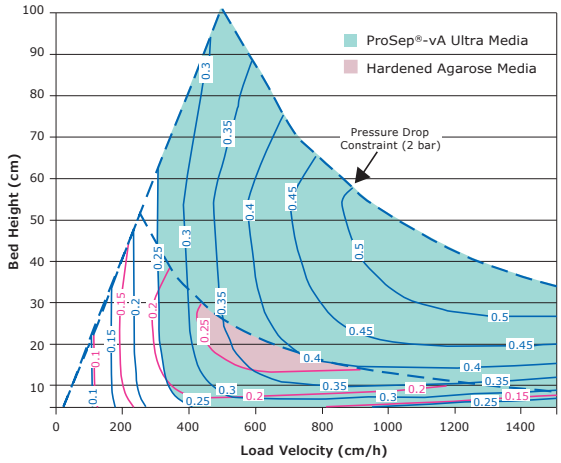


Figure 3.

Productivity and Operating Window
Productivity contours (g IgG processed/ hr per unit area) of media for different bed heights and flow rates. A maximum load residence time (bed height/velocity) of 12 minutes and maximum pressure drop of 2 bar were used as the constraints in the plots. Shaded areas (Blue = ProSep®-vA Ultra Media; Red = Hardened Agarose Media) indicate productivity greater than 0.25 g lgG/hr/unit area.

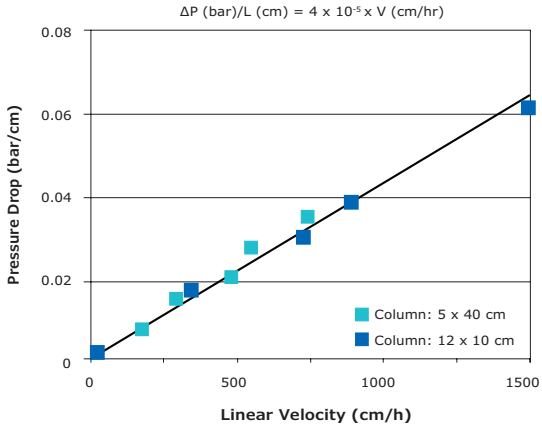


Figure 4.

Flow Rate vs. Pressure Drop
Response of ProSep®-vA Ultra media to increased flow rate. Bed heights in excess of 45 cm are achievable with pressure drops under 2 bar at 1000 cm/hr, in different diameter production columns.

The pressure drop caused by the empty column and fittings was measured separately and the data corrected to show the pressure drop attributable to the matrix alone.

Figure 5.

Cost of Production
Comparison of cost of production, utilizing ProSep®-vA Ultra media versus ProSep®-vA High Capacity media. The cost of operation reflects purification of a 10,000 L fermenter (1.0 g/L mAb) in 8 hours (column height: 20 cm). Media lifetime was assumed to be 300 cycles.

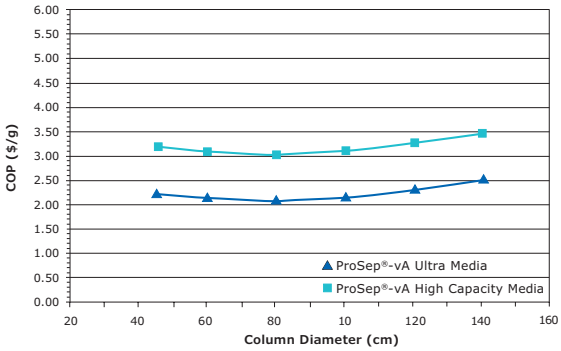


Figure 6.

Media Reuse – Repeat Cycles
Repeat cycle performance data for ProSep®-vA Ultra media.

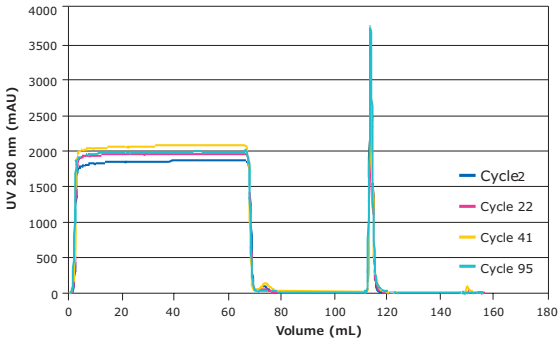
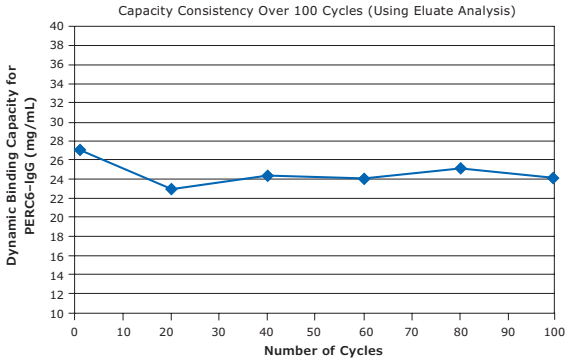


Figure 7.

Media Reuse – Impact on Dynamic Binding Capacity
Dynamic binding capacity was determined every 20 cycles using a residence time of 2.5 minutes.



Reduced Cost of Operation

While dynamic binding capacity is an important criterion in media selection, it is only one contributing factor in determining overall cost of operation. Throughput, productivity and lifetime are also major contributors to media usage costs. Overall cost of operation also includes buffer costs as well as capital equipment depreciation.

To better understand the impact of these different factors, we have developed cost of operation models. Such models allow users to compare different scenarios to determine optimal media usage. For instance, Figure 5 illustrates the reduction in cost of operation utilizing the higher capacity ProSep®-vA Ultra media versus standard ProSep®-vA High Capacity media.

Low Ligand Leakage

In therapeutic antibody production, protein A must not contaminate the final product. Although subsequent chromatography steps, which typically include ion exchange, are effective in reducing leached protein A levels, minimizing leakage from the affinity matrix is highly desirable.

With ProSep®-vA Ultra media, the immobilization chemistry enables multipoint covalent bond formation between the protein A ligand and base matrix. As opposed to single point attachment, this minimizes protein A leakage upon exposure to different buffer pH and protease from the feedstream.

High Reusability

Reuse of chromatography media is an important factor in designing cost effective purification processes. By using a similar controlled pore glass base matrix and immobilization chemistry as ProSep®-vA High Capacity media, which is proven to show extended lifetime capability[3,5], ProSep®-vA Ultra media can be used in multiple cycles without loss of performance (Figure 6 and 7).

Sanitization

ProSep®-vA Ultra media can easily be both sanitized and stored for prolonged periods in 0.1M Na Acetate pH 5.2 ± 0.5 with 2% Benzyl Alcohol. While this solution is an effective sanitant, it may require 24 hours to achieve the desired microbial kill with spore forming organisms.

Use santization agents and conditions that are suitable for the process requirements. Santization and preservative agents must comply with applicable local regulations.

Storage and Handling

ProSep®-vA Ultra media is supplied in 0.1M acetate buffer, pH 5.2 and 1% benzyl alcohol.

During use, it is recommended to store ProSep®-vA Ultra media in 0.1M acetate buffer, pH 5.2 containing 1% or 2% benzyl alcohol. Alternatively, ProSep®-vA Ultra media may be stored in other suitable storage solutions. The recommended storage temperature for ProSep®-vA Ultra media is 2–8°C.

Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Application Example

An example of a monoclonal antibody purification using ProSep®-vA Ultra media is shown in Figure 9. Clarified supernatant from a PERC6 cell culture was loaded directly onto the column with a loading of 250 mg/L of IgG per mL of column volume. The recovery was 99% of highly purified antibody. SDS PAGE analysis of the results is shown in Figure 10.

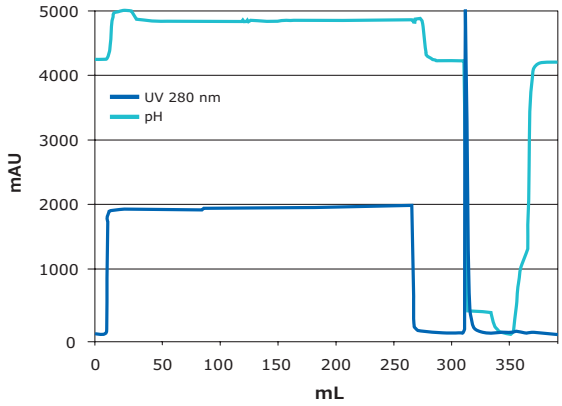


Figure 9.

mAb Purification Example
Purification of a monoclonal antibody using ProSep®-vA Ultra media.

Conditions:
Column: 0.66 x 6 cm
Sample: Clarified feed of mAb expressed in PERC6 ~0.25 mg/mL (250 mL load)
Loading Buffer: Phosphate buffered saline pH 7.4
Wash Buffer: Phosphate buffered saline pH 7.4
Elution Buffer: 0.1 M Glycine pH 2.0
Regeneration Buffer: HCL pH 1.5
Flow Rate: 0.8 mL/min load, 2 mL/min all other steps

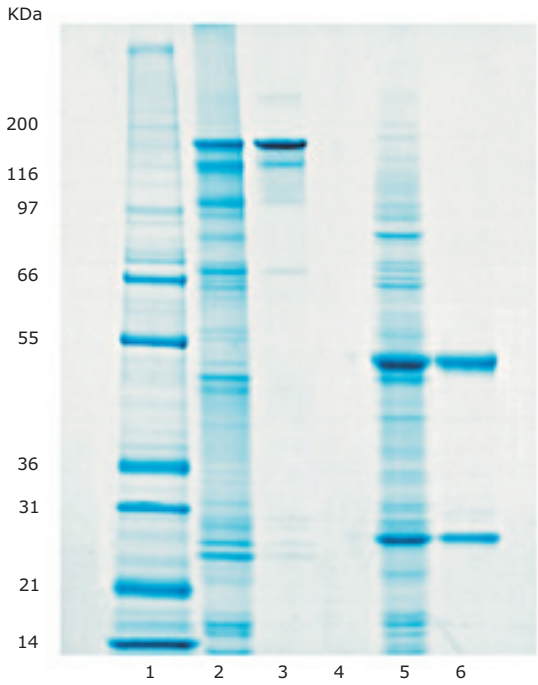


Figure 10.

SDS-PAGE Analysis of Purified mAb
SDS-PAGE Coomassie™ Blue stained analysis of purification of a monoclonal antibody on ProSep®-vA Ultra media.

Key:
1. Molecular weight markers
2. Feedstock (non-reduced)
3. Purified IgG (non-reduced)
4. Blank
5. Feedstock (reduced)
6. Purified IgG (reduced)

Manufacturing Standards and Quality Assurance

We recognize the importance of providing regulatory support and meeting industry quality standards. ProSep®-vA Ultra media utilizes native protein A derived from Staph. aureus. No mammalian derived materials are used to manufacture ProSep®-vA Ultra media and its components. All ProSep® media products are manufactured in a facility certified to internationally recognized standard ISO® 9001 and subjected to routine independent surveillance audits.

ProSep®-vA Ultra media characteristics

Base Matrix	Controlled pore glass
Particle Size	75 – 125 µm
Density	1.3 g/cm^3
Ligand	Native vProtein A
Coupling Chemistry	Multipoint
Binding Capacity – Static	≥ 56 mg/mL (HlgG)
Binding Capacity – Dynamic	Typically 35 mg/mL (10% break-through at 2.4 min residence time)
Recommended Mobile Phase Velocity	Up to 1,000 cm/h
Recommended Bed Height	Up to 45 cm
pH Range	1 – 9
Recommended Long Term Storage	2 – 8 °C, plus bacteriostat

Chromatography Columns and Systems

Chromatography columns and systems are critical factors to the successful separation of your valuable molecule. We provide 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.

Ordering Information

ProSep®-vA Ultra Media	Catalogue No.
10 mL	**115 115 824**
100 mL	**115 115 827**
1 L	**115 115 830**
5 L	**115 115 833**
10 L	**115 115 835**

Column Cleaning & Storage of ProSep® Resins

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

References

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