

ProSep[®]-vA Ultra Chromatography Media

Designed for cost effective, large-scale purification of today's higher titer therapeutic antibodies

- ▶ High throughput for maximum productivity
- ▶ Lower cost of operation
- ▶ Proven technology
- ▶ Flexible process design
- ▶ Reliable scale-up

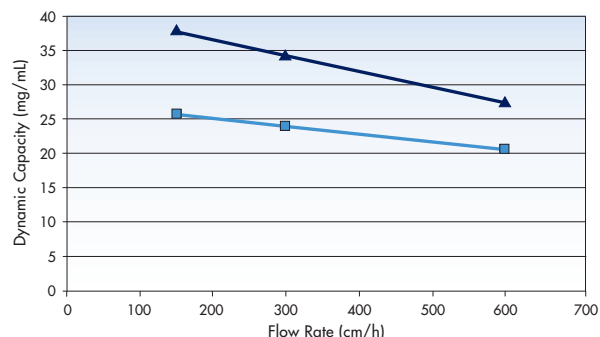
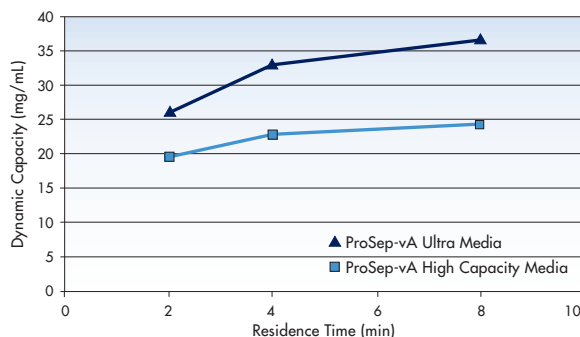
ProSep-vA Ultra chromatography media provides high binding capacity and improved process economics for the capture and purification of monoclonal, polyclonal and engineered antibodies. It has been specifically optimized to address the needs of today's higher titer, large volume fermentation feedstocks. Titrers 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 has been developed from ProSep-A High Capacity media, which is used in the manufacture of a number of approved monoclonal therapeutic antibodies. ProSep-vA Ultra media is the result of extensive investigation into optimizing ProSep media to address the developing needs of the industry.¹

ProSep-vA Ultra media uses the same immobilization chemistry as ProSep-A and ProSep-vA High Capacity media; however 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 similar increase in IgG binding capacity.

Dynamic Binding Capacity



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

Figure 1. Dynamic capacity of ProSep-vA Ultra media

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

The sharp breakthrough curve (see Figure 3) is the result of the uniform open pore structure allowing higher loading percentages to be utilized before risk of premature breakthrough, thereby maximizing column capacity.

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 lengths 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-A 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 also apply to ProSep-vA Ultra media and are illustrated in Figure 2 where productivity (in terms of g IgG processed/hr/unit area of media) is plotted against column bed height and load flow rate. The ability to run at longer bed lengths 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 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.

Reduced Cost of Operation

While dynamic 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 help understand the impact of these different factors, Millipore has 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.

Productivity and Operating Window

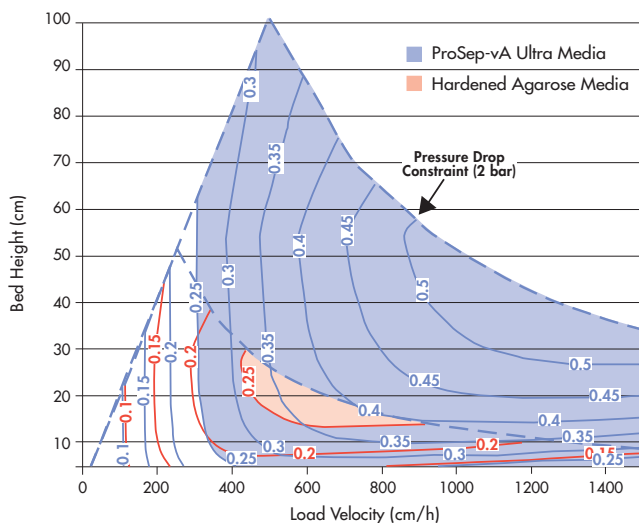


Figure 2. Productivity contours (g IgG processed/hr per unit area) of media for different bed heights and flow rates. A maximum load residence time (bed height/velocity) of 12 minutes and maximum pressure drop of 2 bar were used as the constraints in the plots. Shaded areas (Blue = ProSep-vA Ultra Media;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

Breakthrough Curve Comparison

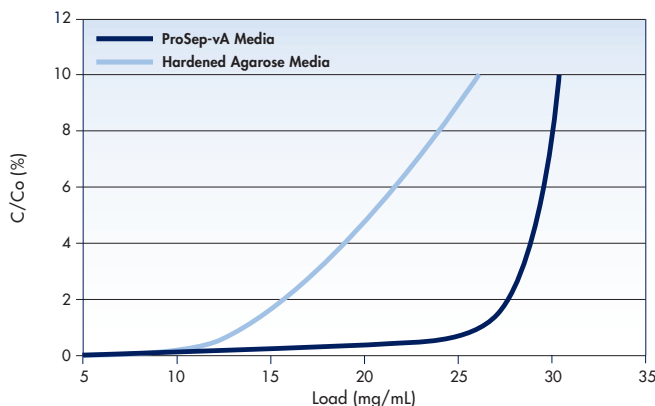


Figure 3. Figure shows the sharp breakthrough curves of ProSep-vA Ultra media compared to hardened agarose media.

Regulatory Acceptability

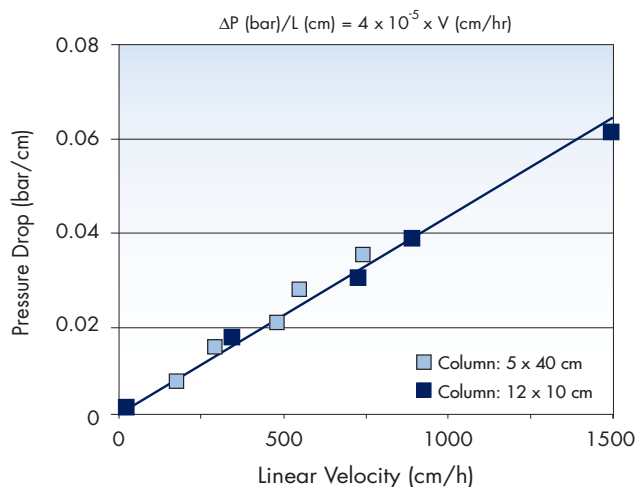
ProSep-vA Ultra media utilizes native protein A derived from *Staphylococcus aureus*, which is the ligand used today to manufacture the majority of approved monoclonal antibody therapeutics. Recognizing the regulatory authorities' desire for the biopharmaceutical industry to remove mammalian derived materials from the manufacturing process, ProSep-vA Ultra media utilizes vProtein A, which uses no animal products in its manufacture. The immobilization to the controlled pore glass base matrix is carried out in clean rooms to cGMP standards.

Low Ligand Leakage

In therapeutic antibody production, protein A must not be allowed to contaminate the final product. Although subsequent chromatography steps, which typically include ion exchange, are effective in achieving this, minimizing leakage from the affinity matrix is still important.

With ProSep-vA Ultra media, the immobilization chemistry employed enables optimum orientation and distribution of the ligand thereby maximizing binding stoichiometry to the antibody. Multipoint, as opposed to single point, attachment is used to minimize protein A leakage and the impact of feedstream protease activity.

Flow Rate vs. Pressure Drop



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 4. Response of ProSep-vA Ultra media to increased flow rate. Bed lengths in excess of 45 cm are achievable with pressure drops under 2 bar at 1000 cm/hr, even in large diameter production columns.

Cost of Operation

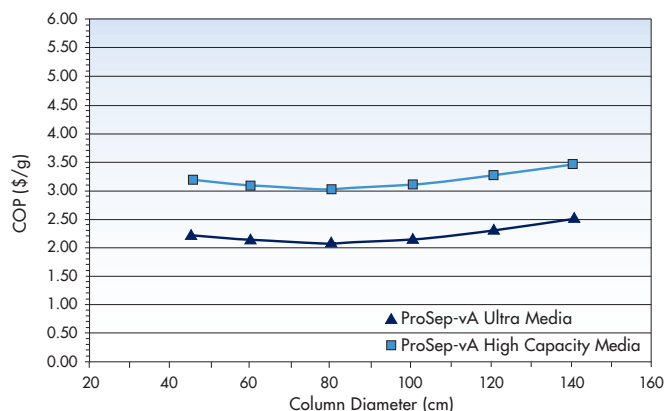


Figure 5. Comparison of cost of operation utilizing ProSep-vA Ultra media versus ProSep-vA High Capacity media. The cost of operation reflects purification of a 10,000 L fermenter (1.0 g/L MAb) in 8 hours (column height: 20 cm). Media lifetime was assumed to be 300 cycles.

Media Reuse – Repeat Cycles

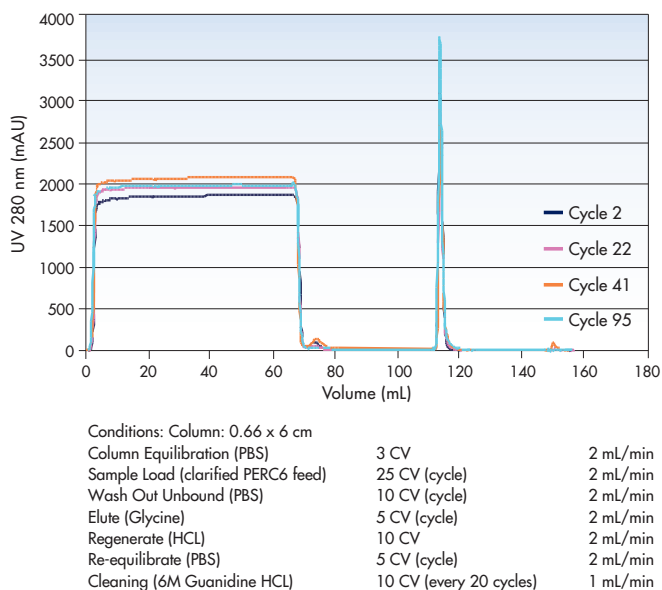


Figure 6. Repeat cycle performance data for ProSep-vA Ultra media.

High Reusability

Reuse of chromatography media is an important factor in designing cost effective purification processes. By using similar controlled pore glass base matrix and immobilization chemistry as Prosep-A and 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).

Established Cleaning and Sanitization

Following recommended handling and cleaning procedures is critical to sustaining column performance. Millipore recommends routine use of a low pH regeneration (e.g. Phosphoric acid pH 1.5) and periodic cleaning as required (e.g. 6M guanidine hydrochloride). Phosphoric acid and guanidine hydrochloride are also effective sanitants. Refer to the user instructions for more detailed recommendations.

Media Reuse – Impact on Dynamic Binding Capacity

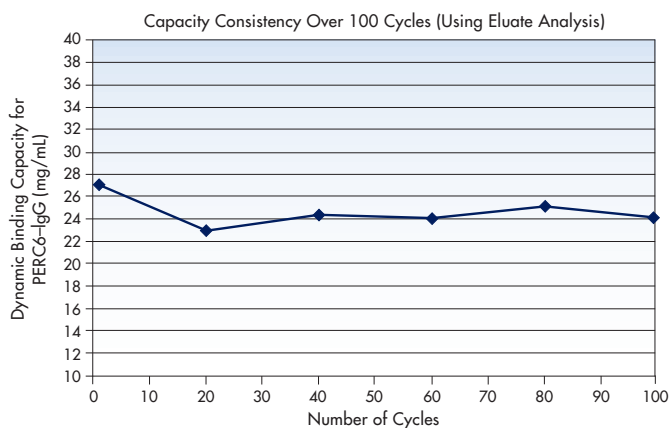


Figure 7. Dynamic binding capacity was determined every 20 cycles using a residence time of 2.5 minutes.

Storage and Handling

ProSep-vA Ultra media is supplied in 0.1 M acetate buffer, pH 5.2 and 1% benzyl alcohol as a preservative. During use, ProSep-vA Ultra media may be stored in phosphate buffered saline (PBS) or other suitable buffer containing a preservative. The acceptable environmental storage temperature for ProSep-vA Ultra media is between 2 – 8 °C.

MAB Purification Example

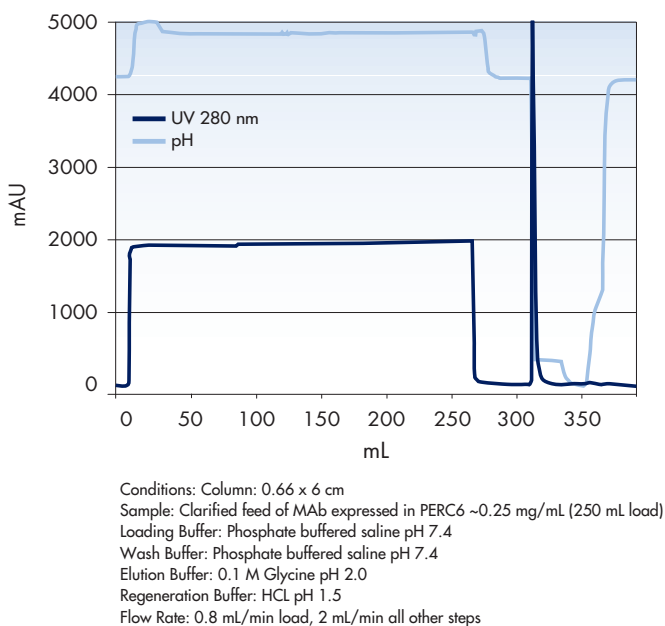


Figure 8. Purification of a monoclonal antibody using ProSep-vA Ultra media.

Application Example

An example of a monoclonal antibody purification using ProSep-vA Ultra media is shown in Figure 8. Clarified supernatant from a PERC6 cell culture was loaded directly onto the column with a column loading of 250 mg/L IgG/mL of column volume. The recovery was 99% of highly purified antibody. SDS PAGE analysis of results is shown in Figure 9.

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 the internationally recognized standard BS EN ISO 9001: 2000 and subjected to routine independent surveillance audits.

ProSep-vA Ultra Media Versus ProSep-vA High Capacity Media

For most applications purifying complete monoclonal antibodies or smaller entities, such as fusion proteins, ProSep-vA Ultra media will provide the best combination of dynamic capacity versus flow rate and therefore throughput and productivity. When purifying larger fusion proteins or antibody conjugates where the effective molecular weight is larger than IgG (i.e. >150K Daltons), ProSep-vA High Capacity media may be more appropriate. Although ProSep-vA High Capacity media has a lower surface area than ProSep-vA Ultra media and less protein A immobilized, the larger pore diameter provides less diffusional resistance to larger molecules so that in practice the achieved dynamic capacity may in fact be higher. For additional information, refer to the ProSep-vA High Capacity media data sheet.

SDS-PAGE Analysis of Purified MAB

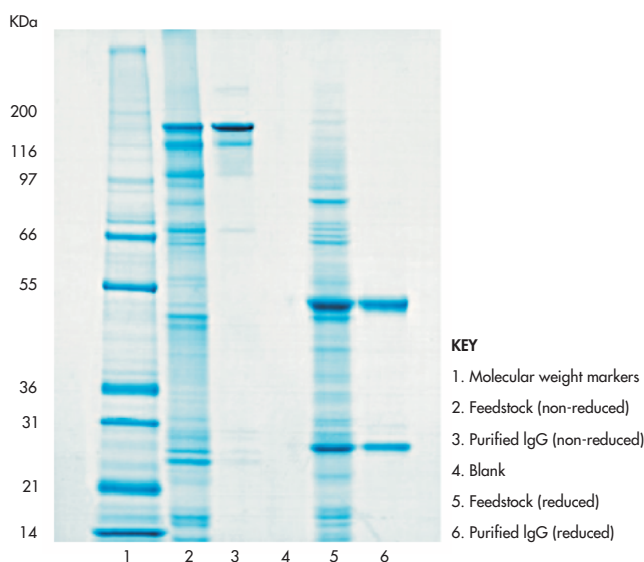


Figure 9. SDS-PAGE Coomassie™ Blue stained analysis of purification of a monoclonal antibody on ProSep-vA Ultra media.

Characteristics

Base Matrix
Controlled pore glass

Particle Size
75 – 125 µm

Density
1.3 g/cm³

Ligand
Native vProtein A

Coupling Chemistry
Multipoint

Binding Capacity – Static
Greater than or equal to 56 mg/mL (hlgG)

Binding Capacity – Dynamic
Typically 35 mg/mL (10% breakthrough 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

References

1. McCue, J.T., Kemp, G., Low, D., Quinones-Garcia, I., (2003) *Evaluation of Protein-A Chromatography Media*, J. Chromatogr. A, 989, 139 – 153

Ordering Information

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

Supplied as 50% slurry in 0.1M acetate buffer, pH 5.2, 1% benzyl alcohol

2. Fahrner, R. Whitney, D.H., Vanderlaan, M., Blank, G.S., (1999) *Performance Comparison of Protein A Affinity Chromatography Sorbents for Purifying Recombinant Monoclonal Antibodies*. Biotechnol. Appl. Biochem. 30 121–128
3. Fahrner, R.L., Knudsen, H.L., Basey, C.D., Galan, W., Feuerhelm, D., Vanderlaan, M., and Blank, G. (2001) *Industrial Purification of Pharmaceutical Antibodies: Development, Operation and Validation of Chromatography*
4. Iyer, H., Henderson, F., Cunningham, E., Webb, J., Hanson, J., Bork, C., Conley, L., (2002) *Considerations During Development of a Protein A Based Antibody Purification Process* BioPharm 15 No 1, 14 – 20
5. O’Leary, R.M., Feuerhelm, D., Peers, D., Xu, Y., Blank, G.S., (2001) *Determining the Useful Lifetime of Chromatography Resins* BioPharm Vol 14 No 9, 10 – 18

Processes. Biotechnology and Genetic Engineering Reviews 18, 301 – 327

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