Data Sheet

ProSep® Ultra Plus Chromatography Media

The highest dynamic binding capacity protein A affinity chromatography media, designed for cost effective, large-scale purification of today's higher titer therapeutic antibodies.

ProSep® Ultra Plus media is a protein A based affinity resin with the highest dynamic binding capacity and flow rate capability of any comparable resin on the market. Based on the proven technology of ProSep® media, ProSep® Ultra Plus media provides increased capacity and productivity compared to competing resin based technologies.

Benefits

- Highest capacity
- Proven technology
- High throughput for maximum productivity
- Reliable scale-up
- Lower cost of operation

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

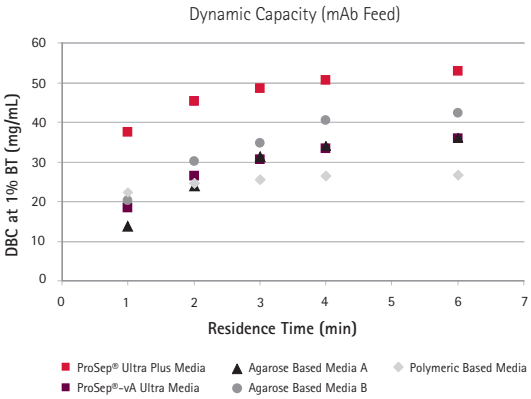
Proven Technology

ProSep® Ultra Plus media has been developed from ProSep®-vA media, which is used extensively in the manufacture of today's approved therapeutic monoclonal antibodies. ProSep® Ultra Plus media is the result of extensive investigation into optimizing ProSep® media to address the developing needs of the industry.

Utilizing smaller CPG particles together with refinement of pore size selection and ligand immobilization has enabled the significant increase in dynamic capacity. The open inter-connected pore structure maintains rapid mass transfer, resulting in these higher dynamic capacities being achievable over a wide range of flow rates or residence times (see Figure 1).

As a result of the open pore structure and outstanding mass transfer characteristics (see Figure 2), the sharp breakthrough curves permit higher loading percentages to be utilized before risk of premature breakthrough, thereby maximizing column capacity.

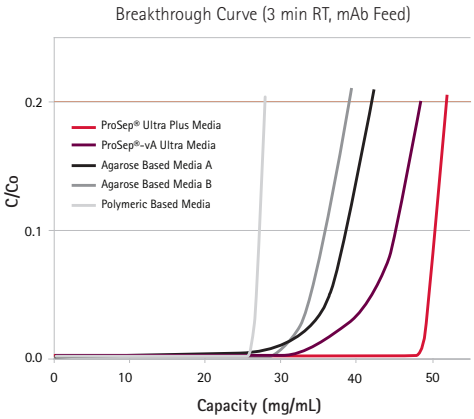
Figure 1.
Dynamic capacity of ProSep® Ultra Plus media compared with competitive media.



Operational Flexibility

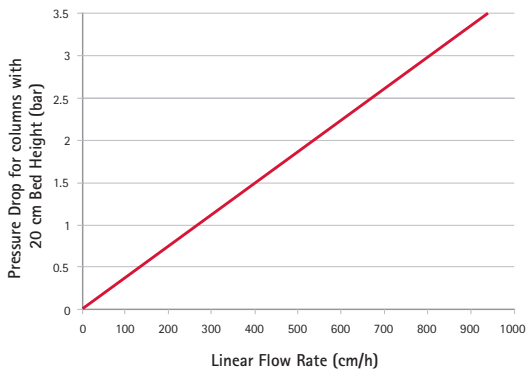
The porous glass base matrix is fully incompressible, leading to a linear relationship between back pressure and flow rate. The response of a ProSep® Ultra Plus media packed column to increased flow rate is therefore entirely predictable over different column lengths and diameters. Although a smaller particle size is utilized in ProSep® Ultra Plus media, the combination of total rigidity and particle size still allows operation at flow rates of 500 cm/h if desired. This relationship is illustrated in Figure 3 with data generated using columns of different diameters.

Figure 2.
Breakthrough curves for ProSep® Ultra Plus media compared with competitive media.



Large scale column (1.6 m) packed with ProSep® media

Figure 3.
Response of ProSep® Ultra Plus media to increased flow rate.



Highest Productivity

The combination of highest available capacity, together with high flow rates translate into high productivity. These benefits are illustrated in Figure 4 where productivity (in terms of g IgG processed/hr/unit volume of media) compared to leading competitive media.

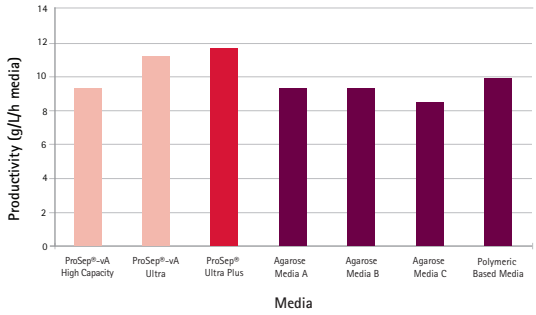


Figure 4.
Comparison of productivity utilizing ProSep® Ultra Plus media versus other protein A media. Based on purification of a 10,000 L fermenter (5.0 g/L mAb) in 24 hrs – including start up and cleaning (column height: 20 cm).

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 will also incorporate costs of buffers as well as capital equipment depreciation costs.

To help understand the impact of these various factors, EMD Millipore has developed cost of operation models that allow comparison of different operating scenarios and aids in process and product optimization. For example, Figure 5 illustrates the lower cost of operation utilizing the higher capacity ProSep® Ultra Plus media versus other leading commercially available media.

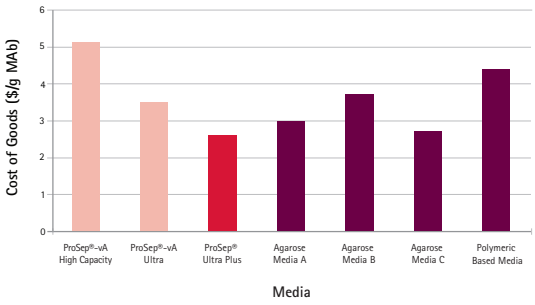


Figure 5.
Comparison of Cost of Operation (COP) utilizing ProSep® Ultra Plus media versus other protein A media. Based on purification of a 10,000 L fermenter (5.0 g/L mAb) in 24 hrs – including start up and cleaning (column height: 20 cm). Media lifetime was assumed to be 200 cycles.

Product Purity

Product purity is also an important consideration. Purity of the mAb post protein A can be reduced if non-specifically bound (NSB) material co-elutes with the antibody. NSB material is generally due to either ionic or hydrophobic interaction with the base matrix or immobilization chemistry and occurs to some degree with all chromatography resins. As shown in Figure 6, Host Cell Protein (HCP) reduction levels are comparable to other competitive media, although specific values have been shown to be feedstock dependant. Further reduction of NSB material, if required, can be achieved by modifying the post-load wash buffer in such a way as to disrupt these interactions, thus eluting the non-specifically bound contaminants without prematurely eluting the mAb.

Several approaches have proven to be effective. These include selecting a pH for the intermediate wash buffer between that of the loading and the elution buffers, and/or the inclusion of salt, detergents or amino acids (i.e. arginine).

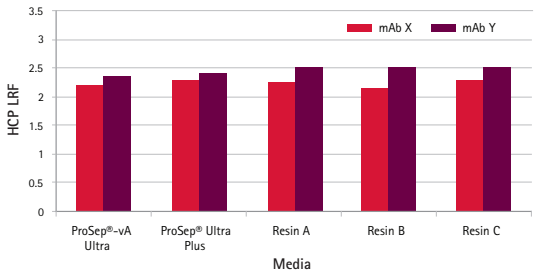
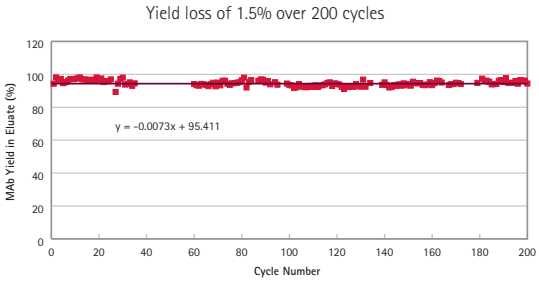


Figure 6.
Host cell protein log reduction for mAb X and Y.

Figure 7.
Consistent yield over 200 cycles.



Recently, the use of buffer combinations comprising of salts and detergents, salts and solvents, salts and polymers, as well as high Tris buffer concentrations have shown to also be effective. These latter methods are the subject of US Patent 6,870,034 to which EMD Millipore has obtained a license, allowing it to grant a sub-license to ProSep® A users. This permits users to utilize these buffer combinations, if required in their process, royalty free.

Figure 8.
Consistent chromatographic performance as demonstrated by the overlay of chromatograms.

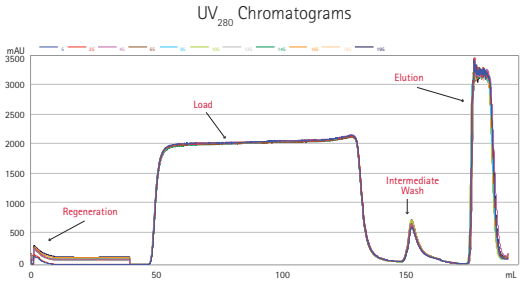
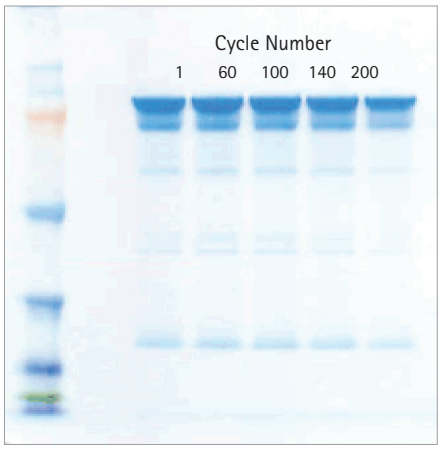


Figure 9.
SDS PAGE (non-reduced) profiles of elution fractions over 200 cycles.



Established Cleaning

Following recommended handling and cleaning procedures is critical to sustaining column performance. EMD Millipore recommends routine use of a low pH regeneration (e.g. Phosphoric acid pH 1.5) and periodic cleaning if required (e.g. 6M Urea). These procedures have proven to be effective in prolonging the lifetime of the media. Refer to user instructions for detailed recommendations.

Sanitization

ProSep® Ultra Plus 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. For more rapid sanitization, for instance if columns need to be turned around more quickly in order to process the next bioreactor harvest, EMD Millipore has developed a more rapid sanitant solution PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2% (v/v) benzyl alcohol). Acidification of the benzyl alcohol significantly improves the microbial kill kinetics enabling effective sanitization times of less than 3 hours even with spore forming organisms. While significantly reducing the sanitization step time, it also has the advantage of not introducing novel chemical species into the process. Figure 10 demonstrates PAB to be more effective than 0.1M NaOH.

Storage and Handling

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

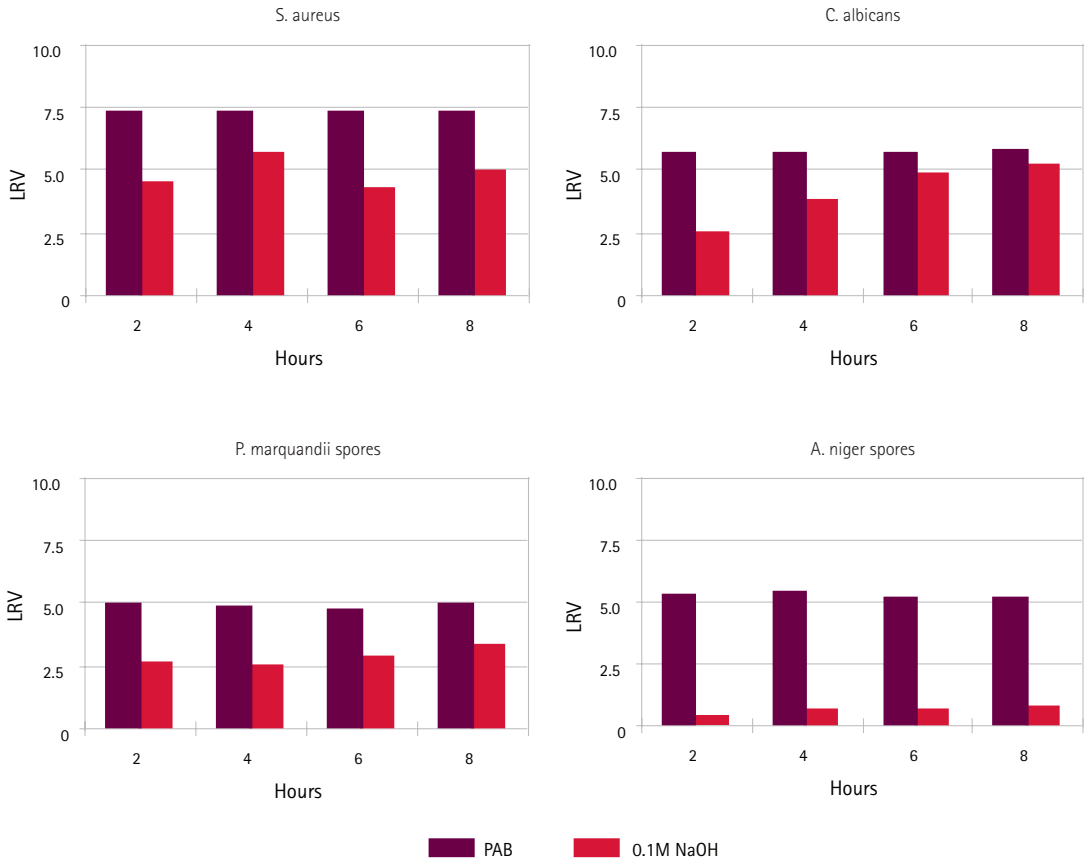


Figure 10.
Comparison of Microbial Kill (Log Reduction Values) for PAB and 0.1M NaOH for vegetative and spore forming organisms at 15 °C.

Viral Clearance

Viral clearance studies were conducted to compare the clearance of viruses using ProSep® Ultra Plus media with other commercially available protein A based agarose media.

Two model viruses were used in the study: A model retrovirus, Xenotropic Murine Leukemia Virus (X-MuLv), 80 – 110 nm; a model parvovirus, Mouse Minute Virus (MMV) 18 – 25 nm, which is more difficult to inactivate or remove by filtration.

Cell culture supernatants were spiked with virus and applied to the chromatography columns containing each of the media. 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.

As shown in Table A, the overall MMV log virus removal (LRV) was greater for all ProSep® media (LRV of 4.3) than protein A based agarose media (LRV of 3.2).

In the wash step, 5.5 logs of MMV were removed from ProSep® media compared with 4.0 logs from protein A agarose media, suggesting some MMV may have been held up in the agarose based matrix. There was no differentiation in viral clearance of X-MuLv as the acidic elution buffer was very effective at inactivating the virus as demonstrated in Table B.

Table A. MMV Clearance

Media	LRV
ProSep® Ultra Plus	≥4.36
ProSep®-vA Ultra	≥4.22
ProSep®-vA High Capacity	≥4.10

Table B. X-MuLv Clearance

Media	LRV
ProSep® Ultra Plus	≥3.25
ProSep®-vA Ultra	≥3.25
ProSep®-vA High Capacity	≥3.29

ProSep® Ultra Plus Prepacked Columns

ProSep® Ultra Plus media is available in prepacked, ready-to-use, disposable columns for research and lab development scale. The MiniChrom columns and RoboColumns® are the ideal tools for performing initial media screening, scaling and optimization studies. The easy-to-use, economical small scale columns can be used with any chromatography system.

ProSep® Ultra Plus prepacked columns are available in 1 and 5 mL MiniChrom columns and 0.2 and 0.6 mL RoboColumns®:

Column Dimensions	Column Bed Volume
MiniChrom Columns	
8 mm (i.d.) x 20 mm (bed length)	1 mL
8 mm (i.d.) x 100 mm (bed length)	5 mL
RoboColumns®	
5 mm (i.d.) x 10 mm (bed length)	0.2 mL
5 mm (i.d.) x 30 mm (bed length)	0.6 mL

The 8 mm diameter columns allow for scale up from 1 to 5 mL column volume with a constant internal diameter. These columns are compatible with any HPLC, FPLC™ or AKTA® system.

ProSep® Ultra Plus Prepacked Column Specifications

Components	**Column** - Polypropylene (PP) **Bed Supports** - 17 µm Polypropylene/Polyethylene (PP/PE)
Connections of the MiniChrom columns	10 – 32 UNF 1/16 in. fingertight, PEEK or PTFE Capillaries 1/16 in. (o.d.) with 0.5 – 0.8 mm (i.d).
Column Geometries/Volumes	8 mm (i.d.) x 20 mm 1.0 mL 8 mm (i.d.) x 100 mm 5.0 mL
Maximum Back Pressure	20 bar
Chemical Stability	Columns are tolerant to aqueous buffers and salt solutions, 8M urea, 6M guanidine hydrochloride, organic solvents and detergents.
Temperature Range	4 – 30 °C
Storage	2 - 8°C

ProSep® Ultra Plus Media Characteristics

Base Matrix	Controlled Pore Glass
Particle Size	60 µm
Ligand	Recombinant native protein A
Binding Capacity – Static	Typically ≥ 67 mg/mL (hIgG)
Binding Capacity – Dynamic	Typically >50 mg/mL (10% breakthrough at 3 – 6 min residence time)
Recommended Mobile Phase Velocity	Up to 500 cm/hr
Recommended Bed Height	20 cm
Recommended long-term storage	2 – 8 °C, plus bacteriostat

Manufacturing Standards and Quality Assurance

EMD Millipore recognizes the importance of providing regulatory support and meeting industry quality standards. ProSep® Ultra Plus media utilizes recombinant native protein A derived from *E. coli*. No mammalian derived materials are used to manufacture ProSep® Ultra Plus 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.

Ordering Information

Media	Qty/Pk	Catalogue No.
ProSep® Ultra Plus	2 mL	175118822
ProSep® Ultra Plus	10 mL	175118824
ProSep® Ultra Plus	100 mL	175118827
ProSep® Ultra Plus	1 L	175118830
ProSep® Ultra Plus	5 L	175118833
ProSep® Ultra Plus	10 L	175118835
ProSep® Ultra Plus	25 L	175118834

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

Column	Qty/Pk	Catalogue No.
MiniChrom Column	1 mL	1.25067.0001
MiniChrom Column	5 mL	1.25076.0001
RoboColumn®	0.2mL	1.25135.0001
RoboColumn®	0.6mL	1.25143.0001

Supplied in 0.1M acetate buffer, pH 5.2, 1% benzyl alcohol.

Ordering Information

Description	Catalogue No.
Buffer Preparation	
Phosphoric acid 75% suitable for biopharmaceutical production	100250
di-Potassium hydrogen phosphate anhydrous suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP	137010
Sodium chloride suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, USP	137017
Sodium dihydrogen phosphate dehydrate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JPE	137018
Sodium hydroxide pellets suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, NF, ACS	137020
Sodium hydroxide solution 1 mol/L suitable for biopharmaceutical production EMPROVE® bio	137031
Tris(hydroxymethyl)aminomethane (Trometamol) TRIS suitable for use as excipient EMPROVE® exp Ph Eur, BP, USP	108386
Tris(hydroxymethyl)aminomethane (Trometamol) TRIS high purity suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JPC, USP, ACS	108307
Tris(hydroxymethyl)aminomethane hydrochloride TRIS-HCl suitable for biopharmaceutical production EMPROVE® bio	108219
Column Cleaning & Storage of ProSep® Resins	
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
PAB	480949

References

1. 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 Processes.* Biotechnology and Genetic Engineering Reviews 18, 301- 327
2. 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, 10-17
3. Millipore Corporation. ProSep®-vA High Capacity Media Datasheet. 2004 May. Billerica, MA. Millipore Product Lit. No. DS1013EN00, Rev A.

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