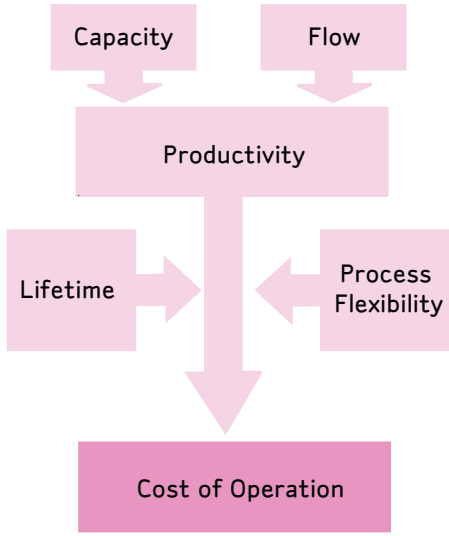
Data Sheet

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. In addition, its rigid base matrix enables predictable scale-up and greater process flexibility, presenting antibody producers with the opportunity to reduce the equipment footprint and purify large volumes of higher titer feedstock in a shorter period of time.



Benefits

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

PROVEN TECHNOLOGY

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

Utilizing a smaller particle size together with refinement of pore size selection and ligand immobilization has enabled a 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).

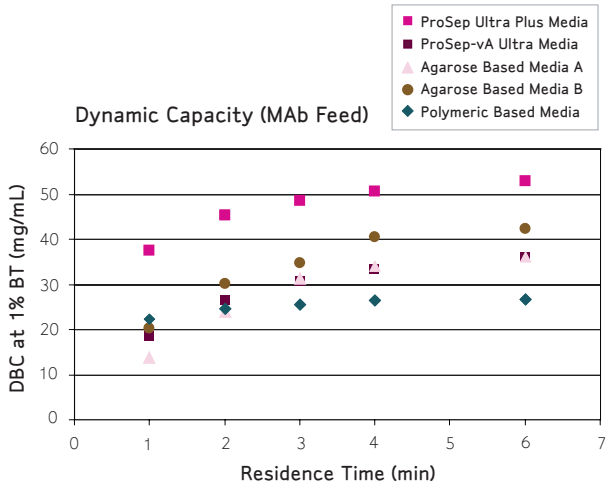


Figure 1. **Dynamic capacity of ProSep Ultra Plus media compared with competitive media**

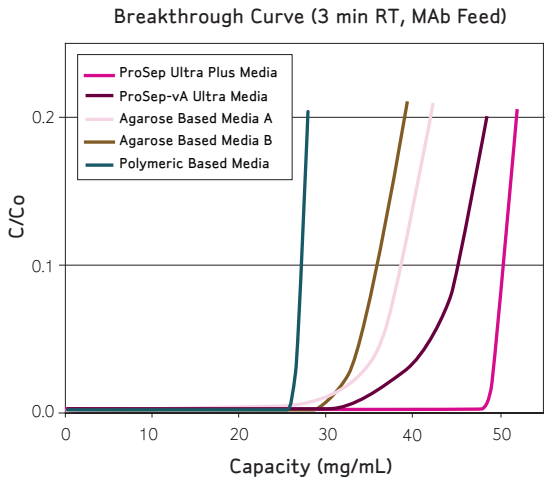


Figure 2. **Breakthrough curves for ProSep Ultra Plus media compared with competitive media**

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.

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 packed column to increased flow rate is therefore entirely predictable over different column lengths and diameters.

PROCESS FLEXIBILITY

An advantage of the flow characteristics of ProSep Ultra Plus media is the ability to run longer bed heights, than would be achievable with more compressible resins. This in turn allows more flexibility in process design, enabling smaller diameter columns to be deployed, taking up less valuable facility floor space.

HIGHEST PRODUCTIVITY

The combination of highest available capacity, together with industry leading flow rates translate into the highest productivity of any available resin. These benefits are illustrated in Figure 5 where productivity (in terms of g IgG processed/hr/unit volume of media) is plotted against column bed height and load flow rate and compared to leading competitive media.

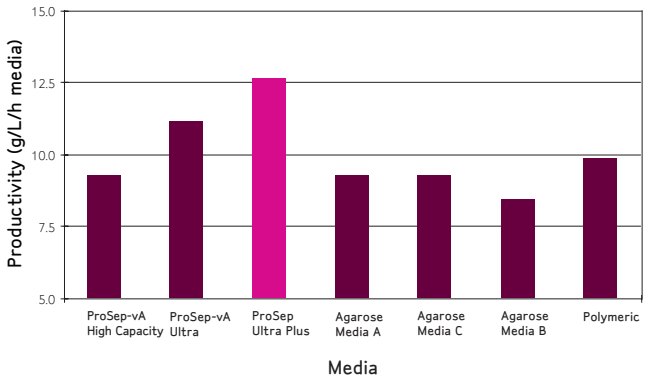


Figure 5. **Comparison of productivity utilizing ProSep Ultra Plus media versus other protein A media. Based on purification of a 10,000 L fermenter (5.0 g/L MAb) in 24 hrs – including start up and cleaning (column height: 20 cm). Media lifetime was assumed to be 200 cycles.**

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, Millipore has developed cost of operation models that allow comparison of different operating scenarios and aids in process and product optimization. For example, Figure 6 illustrates the lower cost of operation utilizing the higher capacity ProSep Ultra Plus media versus other leading commercially available media.

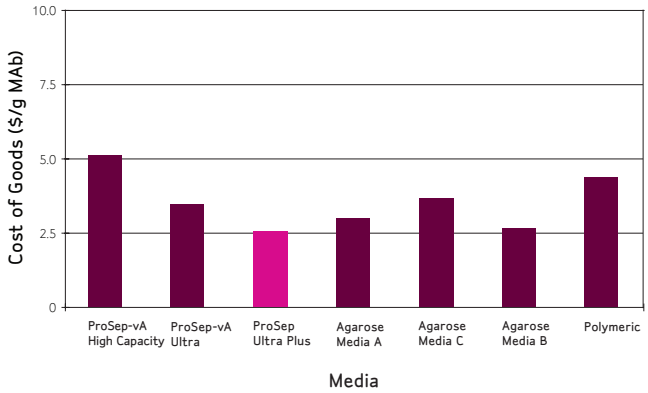


Figure 6. **Comparison of Cost of Operation (COP) utilizing ProSep Ultra Plus media versus other protein A media. Based on purification of a 10,000 L fermenter (5.0 g/L MAb) in 24 hrs – including start up and cleaning (column height: 20 cm). Media lifetime was assumed to be 200 cycles.**

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 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. This can be addressed 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 intermediate between that of the loading and the elution buffers, and/or the inclusion of salt, detergents or amino acids (i.e. arginine).

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 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.

Host Cell Protein (HCP) reduction levels are comparable to the other competitive media although specific values have been shown to be feedstock dependent.

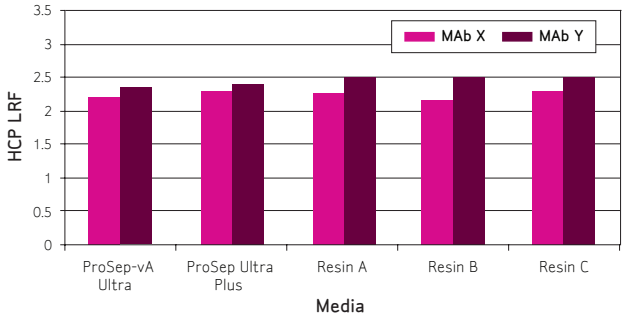


Figure 7. **Host cell protein log reduction for MAb X and Y**

HIGH REUSABILITY

The ability to reuse chromatography media is an important decision for designing cost effective purification processes. ProSep Ultra Plus media builds on the technology of ProSep-vA High Capacity and ProSep-vA Ultra media which have an established record of demonstrating extended lifetime capability under manufacturing conditions.[1,2] ProSep Ultra Plus media can be expected to be used in multiple cycles without loss of performance.

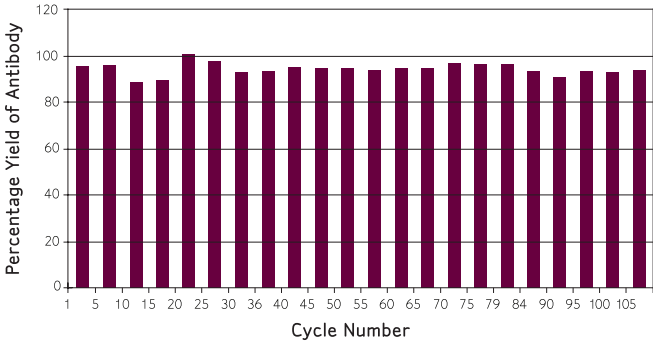


Figure 8. **Multi-cycle performance data for ProSep Ultra Plus media (ongoing study)**

ESTABLISHED CLEANING

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 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.

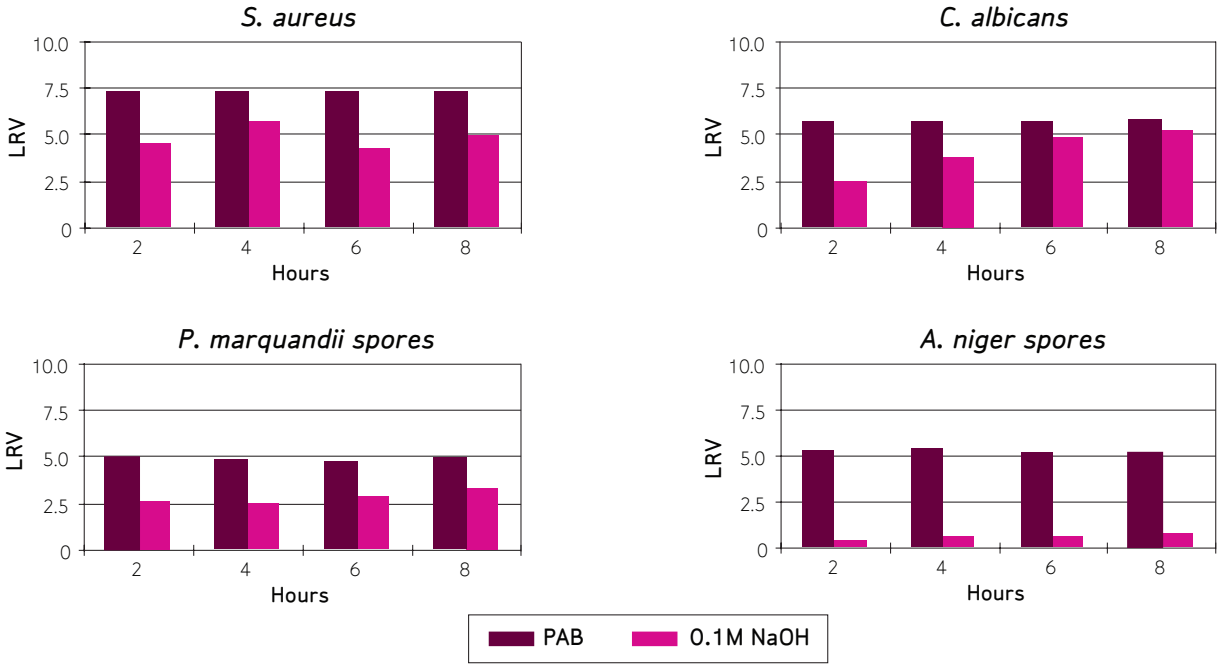


Figure 9. **Comparison of Microbial Kill (Log Reduction Values) for PAB and 0.1M NaOH for vegetative and spore forming organisms at 15 °C**

SANITIZATION

ProSep Ultra Plus media can easily be both sanitized and stored for prolonged periods in 0.1M NaAcetate 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, Millipore has developed an 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 9 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 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.

STREAMLINED SCOUTING AND PROCESS DEVELOPMENT

ProSep Ultra Plus affinity media is also available in prepacked, ready-to-use, disposable 1.0, 2.5 and 5.0 mL columns. These small-scale columns are the ideal tool for performing initial media screening, scaling and optimization studies. These easy-to-use, economical columns can be used with any chromatography system.

PROSEP ULTRA PLUS MEDIA CHARACTERISTICS

Base Matrix	Controlled Pore Glass
Particle Size	60 µm
Ligand	Recombinant native protein A
Ligand Immobilization	Multipoint
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 600 cm/h
pH range	1.5 – 8.5
Recommended long-term storage	2 – 8 °C, plus bacteriostat

PROSEP ULTRA PLUS PREPACKED COLUMN SPECIFICATIONS

Components:	**Column -** Polypropylene (PP) **Bed Supports -** 17 µm Polypropylene/Polyethylene (PP/PE)
Connections	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 mL 8 mm (i.d.) x 50 mm 2.5 mL 8 mm (i.d.) x 100 mm 5 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

MANUFACTURING STANDARDS AND QUALITY ASSURANCE

Millipore recognizes the importance of providing regulatory support and meeting industry quality standards. ProSep Ultra Plus utilizes recombinant native protein A derived from *E. coli*. No mammalian derived materials are used to manufacture ProSep Ultra Plus and its components. All ProSep products are manufactured in a facility certified to internationally recognized standard BS EN ISO® 9001 and subjected to routine independent surveillance audits.

PROSEP ULTRA PLUS PREPACKED COLUMNS

ProSep Ultra Plus Prepacked Columns are ready-to-use and available in 1, 2.5 and 5 mL bed volume in the following geometries:

Column Dimensions	Column Bed Volume
8 mm (i.d.) x 20 mm (bed length)	**1 mL**
8 mm (i.d.) x 50 mm (bed length)	**2.5 mL**
8 mm (i.d.) x 100 mm (bed length)	**5 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.

ORDERING INFORMATION

ProSep Ultra Plus Media in a Prepacked Column	Catalogue No.
1 mL	175118521
2.5 mL	175118522
5 mL	175118523

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

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

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