Millipore®
Preparation, Separation,
Filtration & Testing Products

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

MERCK

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, interconnected 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.

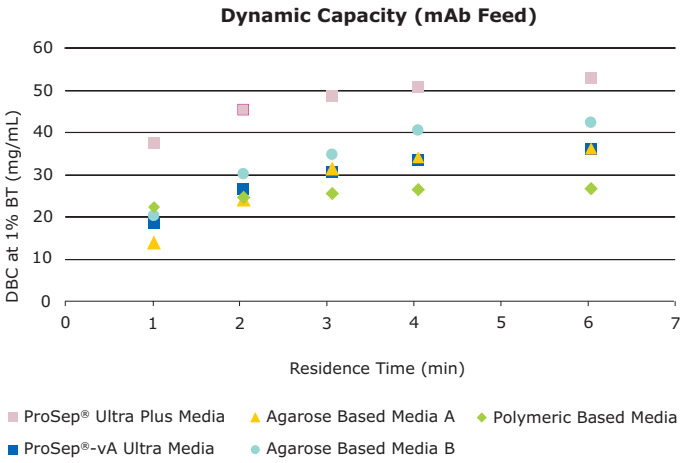


Figure 1.

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

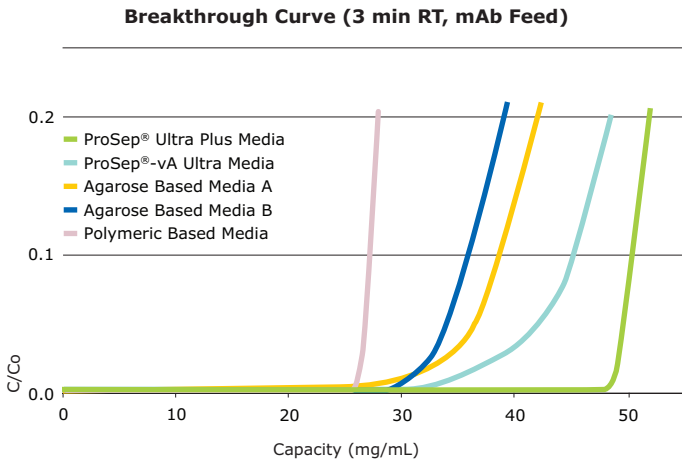


Figure 2.

Breakthrough curves for 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.

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

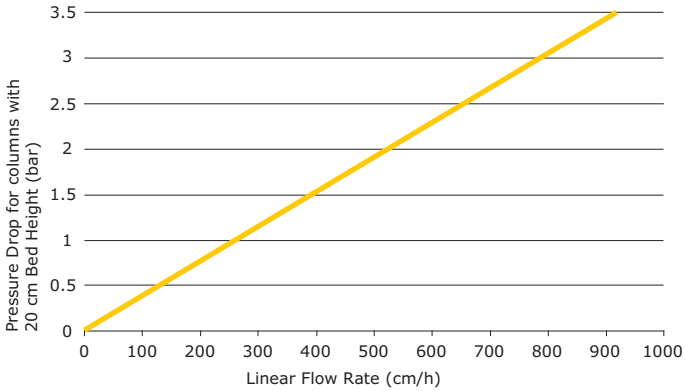


Figure 3.

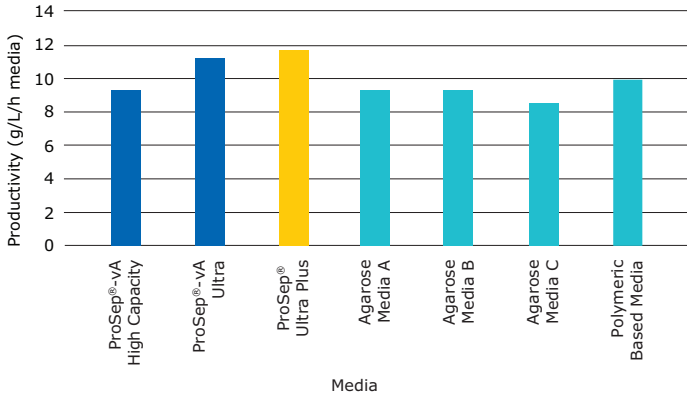
Response of ProSep® Ultra Plus media to increased flow rate.

Highest Productivity

The combination of highest available capacity together with high flow rates translates 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).



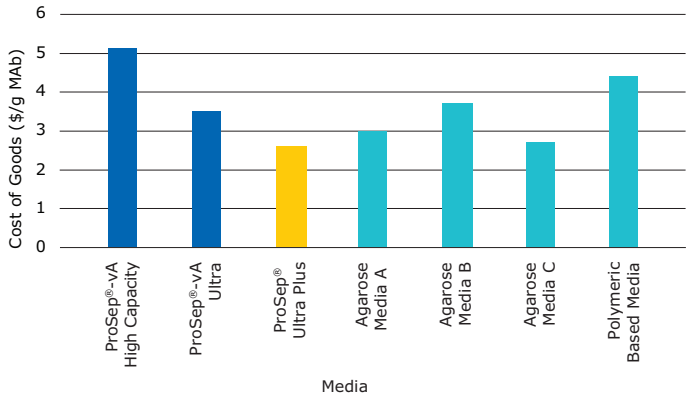
Cost of Operation

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, we have 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.



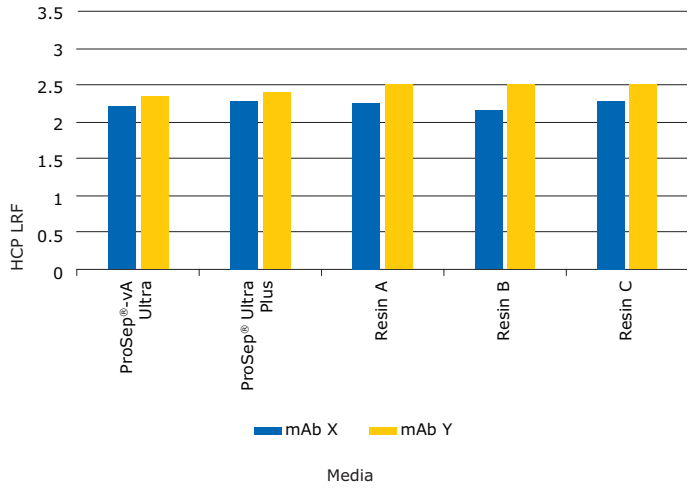
Product Purity

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 dependent. 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.



High Reusability

The ability to reuse chromatography media is important for designing cost-effective purification processes. ProSep® Ultra Plus media builds on the technology of ProSep®-vA High Capacity media 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 used in multiple cycles without loss of performance.

A 200-cycle study was conducted using a mAb feedstock; regeneration of the resin was done on each cycle using phosphoric acid pH 1.5. The performance was consistent throughout the study, as illustrated in Figures 7-9.

Recently, the use of buffer combinations comprising 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 we have 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 7.

Consistent yield over 200 cycles.

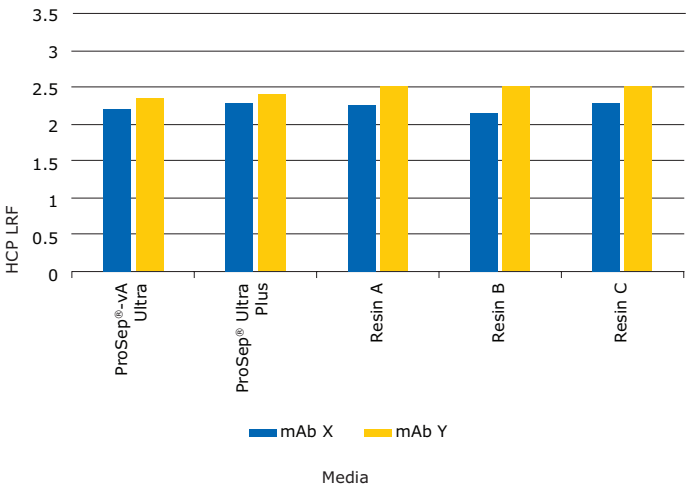


Figure 8.

Consistent chromatographic performance, as demonstrated by the overlay of chromatograms.

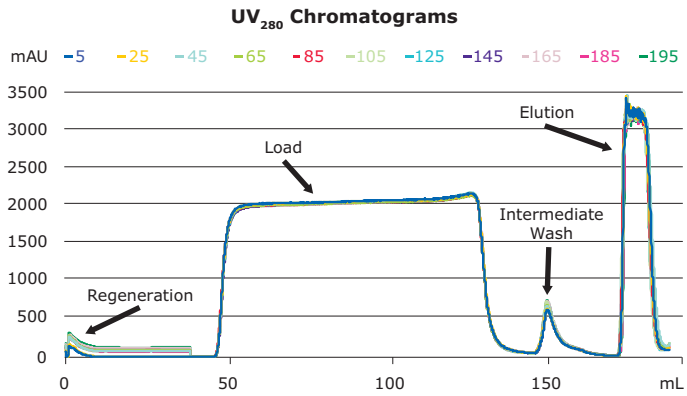
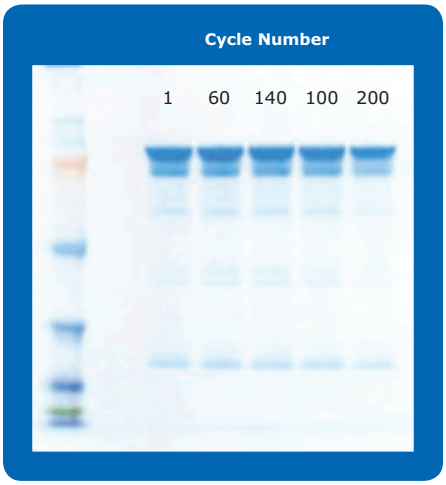


Figure 9.

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. We recommend 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 — we have 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.

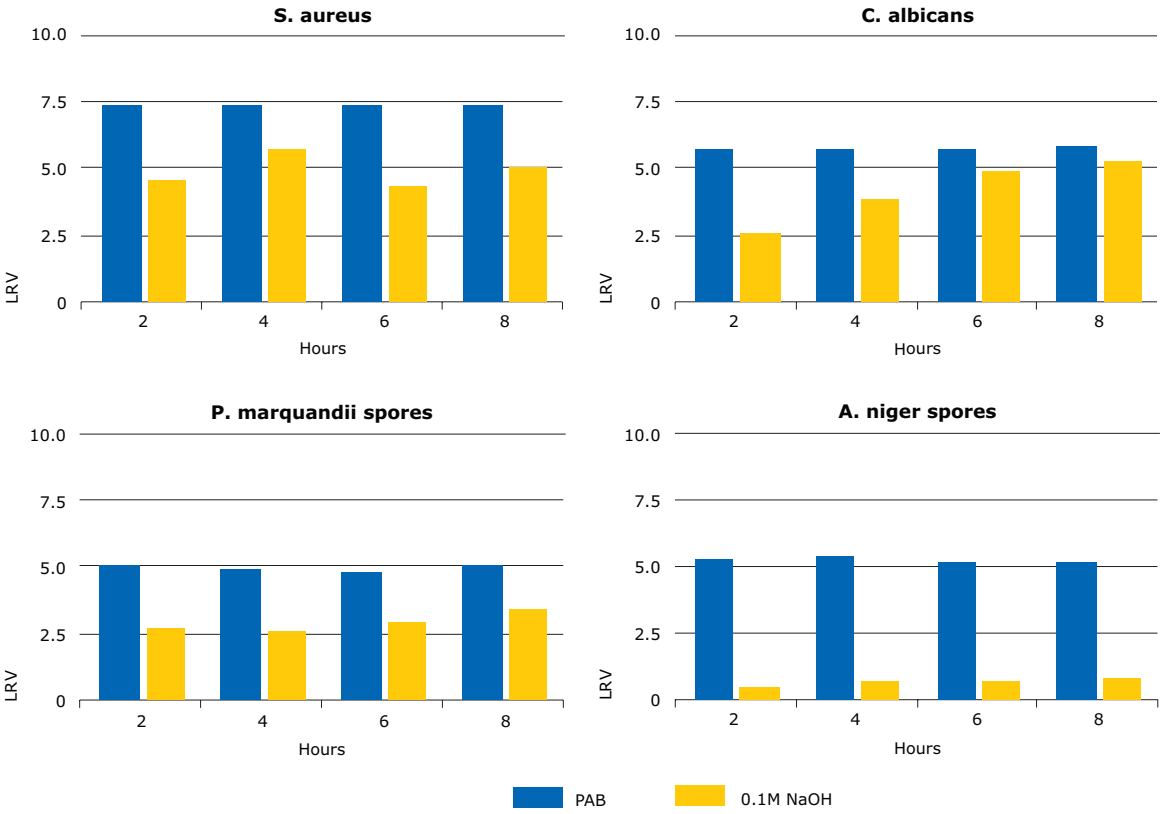


Figure 10.

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

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.

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

Table B. X-MuLv Clearance

Media	LRV
ProSep® Ultra Plus Media	≥3.25
ProSep®-vA Ultra Media	≥3.25
ProSep®-vA High Capacity Media	≥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 RoboColumn® columns 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 RoboColumn® columns.

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
RoboColumn® Columns	
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

We recognize 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.

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. Merck Corporation. ProSep®-vA High Capacity Media Datasheet. 2004 May. Darmstadt, Germany. Merck Product Lit. No. DS1013EN00, Rev A.

Ordering Information

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

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

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

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

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**

Description	Catalogue No.
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**

Recommended ELISA Kit	Catalogue No.
ELISA Kit for the Detection of Native and Recombinant Protein A*	**9000-1**

*This product can be purchased directly from Repligen Corp.

To Place an Order or Receive Technical Assistance

Please visit
MerckMillipore.com/contactPS

For additional information, please visit
MerckMillipore.com

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

DS2234EN00 Ver. 6.0
2016 - 00203
1/2018