Millipore®
Preparation, Separation,
Filtration & Testing Products

Data Sheet

Eshmuno® A Chromatography Media

Introduction

Eshmuno® A media is a rigid, high-capacity, acid and alkaline-resistant Protein A affinity chromatography media for the purification of Fc-containing proteins, including but not limited to monoclonal antibodies.

It exhibits the following key advantages relative to competitive products:

- Acid resistance
- Alkaline resistance
- Aggregate removal

Eshmuno® A media can be cleaned and sanitized under acid and/or alkaline conditions, while maintaining high dynamic binding capacity at high flow rates. In addition, it offers an orthogonal solution for aggregate removal at both the front and tail ends of the elution peak from the Protein A column. This property of the Eshmuno® A media results in reducing the burden of subsequent chromatography steps typically used in the purification of Fc–containing proteins.

Proven Technology

The Eshmuno® A media contains a MilliporeSigma proprietary ligand derived from the C domain of *Staphylococcus aureus* Protein A in a pentameric form. This ligand is recombinantly expressed in *E. coli*. No animal-derived products are used during production.

The Eshmuno® A media is synthesized via immobilization of the aforementioned ligand onto the Eshmuno® base matrix, which is a rigid and hydrophilic polymer based on polyvinylether.

Additional Advantages of Eshmuno® A Media

Productivity

- High Binding Capacity
- Superior Flow Capability
- Reusability
- Low Cost of Goods (COGs)

Product Purity

- Increased Aggregate Removal
- Excellent HCP Reduction
- Low Leached Protein A
- Consistent Viral Clearance
- Reduced Fab Binding

Scalability

- Linear Scale-Up

Reliability

- Batch-to-Batch Consistency
- GMP-like Manufacturing Environment
- Continuous Regulatory Support

High Binding Capacity

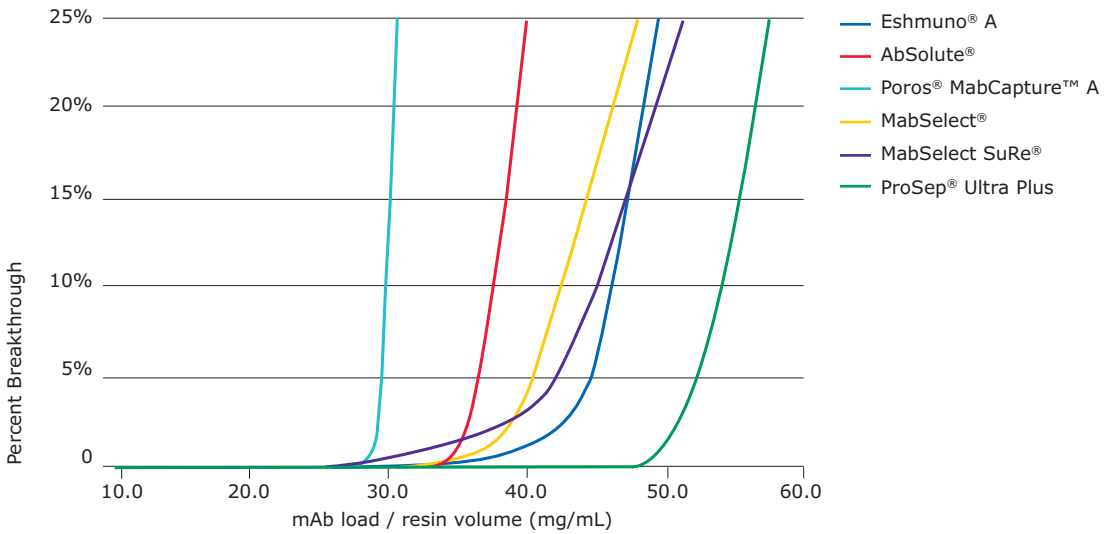
Optimizing the particle and pore size of the Eshmuno® base matrix, along with the immobilization of the MilliporeSigma proprietary ligand, enables a significant increase in dynamic binding capacity.

The breakthrough curves of various commercially available Protein A media at 3 minutes residence time are depicted in Figure 1. As demonstrated, the Eshmuno® A media shows higher dynamic binding capacity than other competitive affinity media.

As a result of the optimized particle and pore size of the Eshmuno® base matrix, the sharp breakthrough curve observed in Figure 1 allows for higher loading of Fc-containing protein to be purified, thereby maximizing column throughput.

Figure 1.

Breakthrough Curve at 3 min Residence Time.



Operational Flexibility

The intrinsic rigidity of Eshmuno® A base matrix ensures a linear relationship between back pressure and flow rates throughout the standard range of operating conditions.

Figure 2.

Pressure vs. Flow Curves.

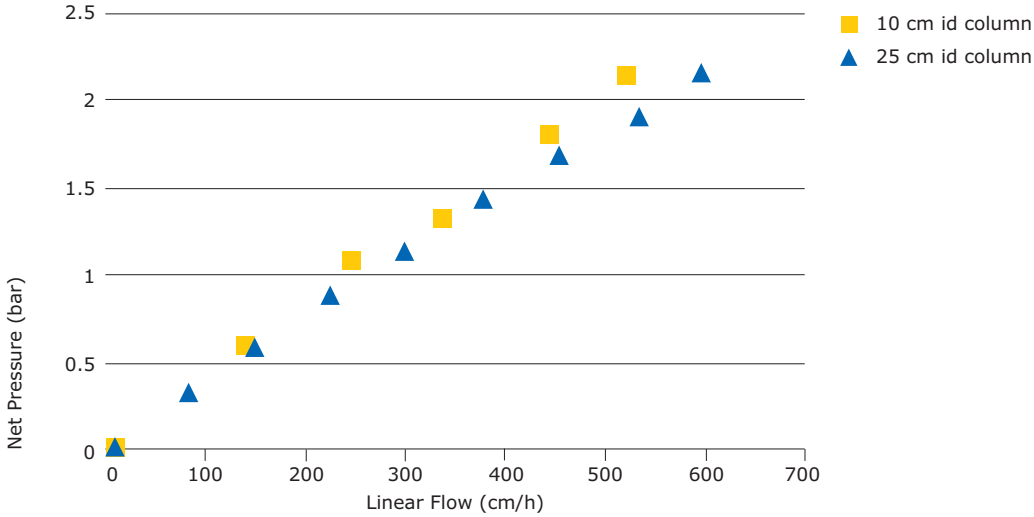
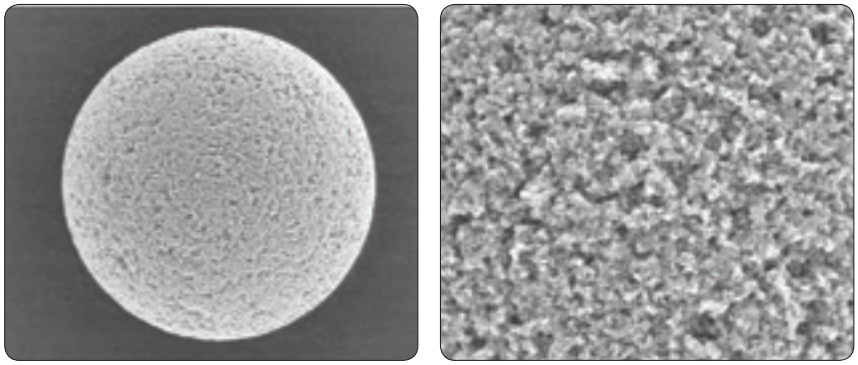


Figure 3.

Scanning electron microscopic images of Eshmuno® A media particle size of 50 µm.



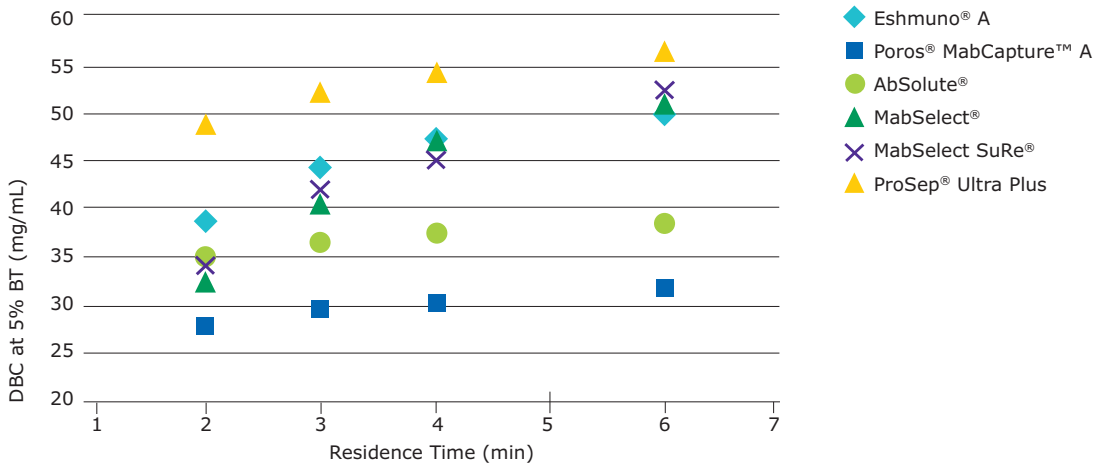
High Productivity

The combination of optimized particle size and high dynamic binding capacity at high flow rate translates into high productivity of Eshmuno® A media.

These benefits are illustrated in Figure 4, where productivity is compared to other competitive media in an exemplary operating scenario.

Figure 4.

Dynamic Binding Capacity (mAb feed).



Cleaning in Place (CIP)

Following recommended handling and cleaning procedures is critical to sustaining column performance. MilliporeSigma recommends routine use of 0.1M NaOH for regeneration and periodic cleaning, or potentially a higher concentration of NaOH may be used. These procedures have proven to be effective in prolonging the usability of the media.

Alternatively, 0.15 M H_3PO_4 and equivalent acids can be used for regeneration and periodic cleaning.

Figure 5.

SDS-PAGE of elution fractions with 0, 40, 80, 120, 160 and 200 cleaning cycles using 0.1 M NaOH are compared with the original CHO feed.

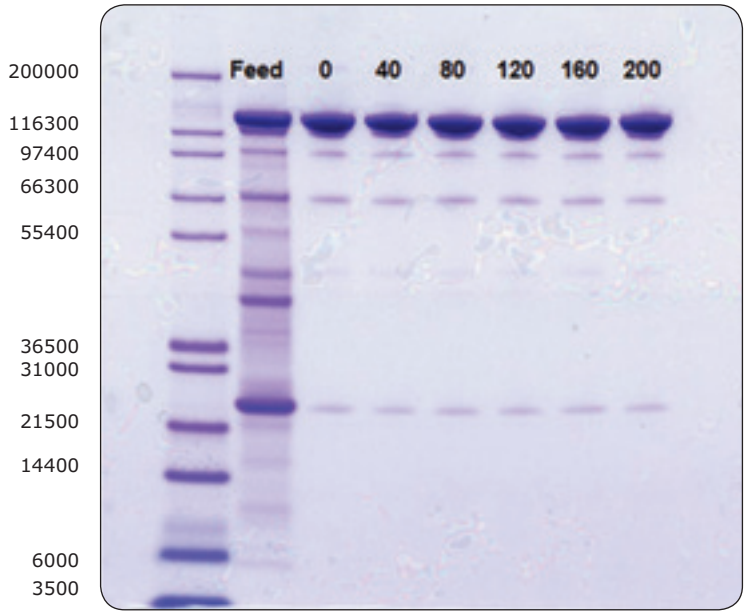
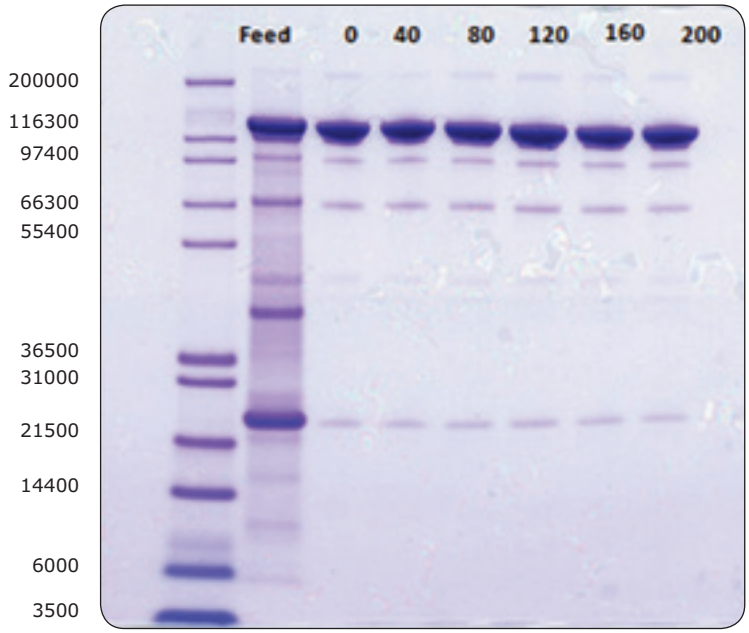


Figure 6.

SDS-PAGE of elution fractions with 0, 40, 80, 120, 160 and 200 cleaning cycles using 0.15 M H_3PO_4 are compared with the original CHO feed.



Reusability

The ability to reuse chromatography media is important for designing cost-effective purification processes. Eshmuno® A media can be used in multiple cycles, employing acid and/or alkaline cleaning without loss of performance.

Two reusability studies of cleaning-in-place over 200 cycles were conducted using a monoclonal antibody feedstock. The regeneration of the media was performed using either 0.1M NaOH or 0.15 M H_3PO_4. The results of these studies are shown in Figures 5 to 8.

Figure 7.

Eshmuno® A media chromatograms over 200 cycles of 0.1 M NaOH cleaning.

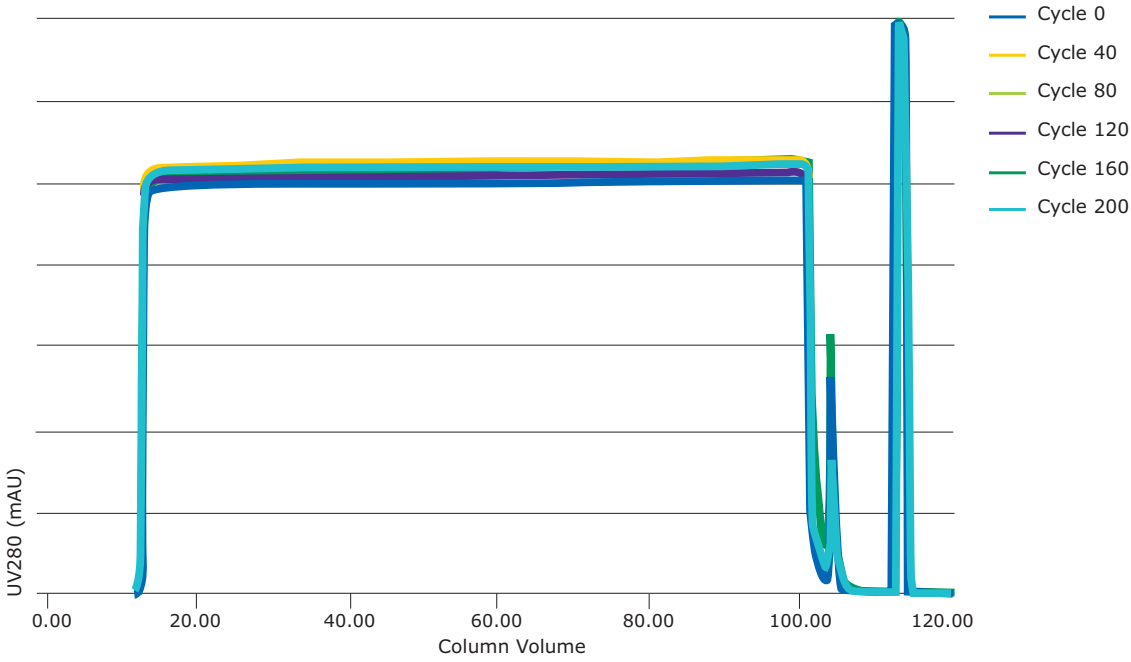
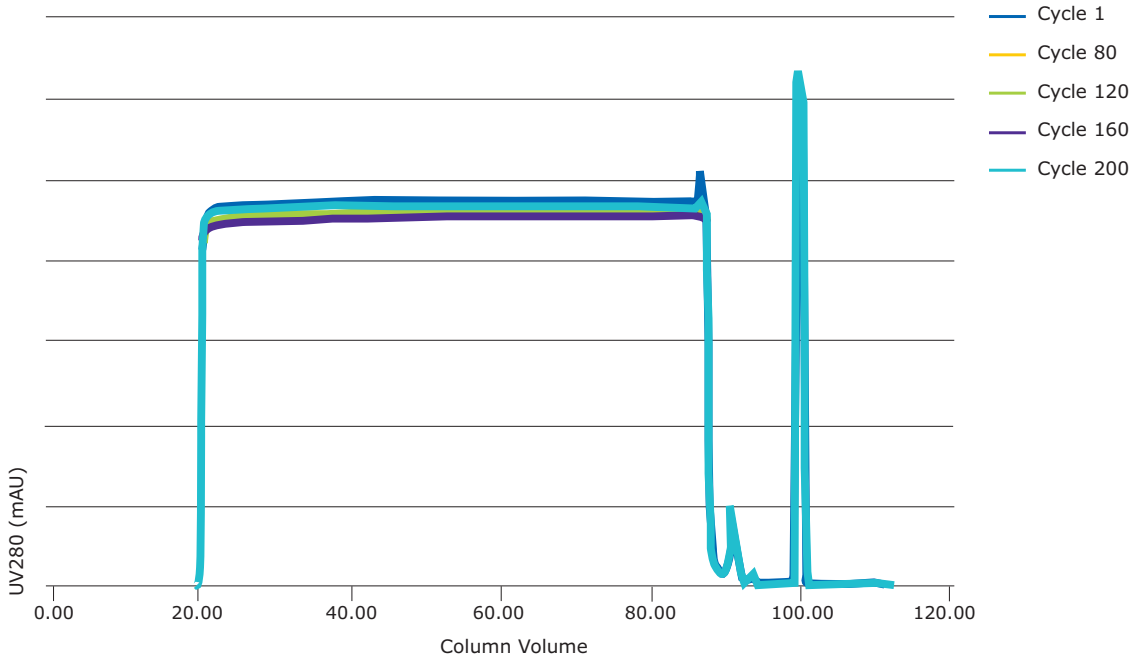


Figure 8.

Eshmuno® A media chromatograms over 200 cycles of 0.15 M H_3PO_4 cleaning.



Product Purity

Product purity is also an important consideration during purification processes. Impurities derived from cell culture media, such as Host Cell Protein (HCP) and DNA, can adversely affect product purity by co-eluting with the product through non-specific binding (NSB). NSB is generally due to either ionic or hydrophobic interactions with the base matrix or immobilization chemistry, and occurs to some degree with all chromatography media.

The hydrophilic nature of Eshmuno® A base matrix ensures low impurity level in the Protein A elution pool, as shown in Figures 9 to 14.

In addition to impurities derived from cell culture media, leached Protein A can also adversely affect product purity. Eshmuno® A media exhibits low levels of leached Protein A, even with the repeated use of the media, under both acid and alkaline cleaning conditions.

Figure 9.

Eshmuno® A media retained Dynamic Binding Capacity (DBC) and yield over 200 cycles of NaOH cleaning.

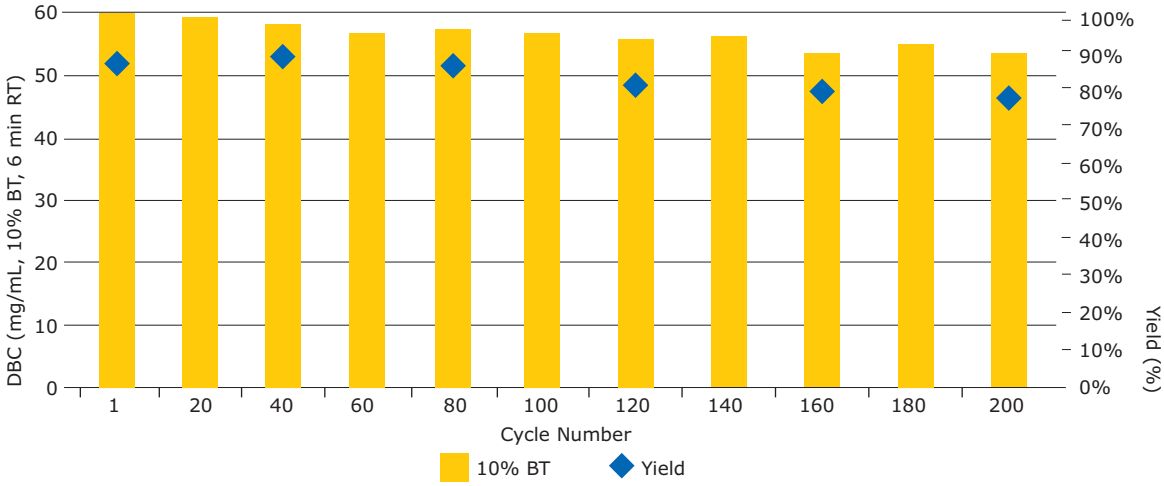


Figure 10.

Eshmuno® A media low leaching Protein A over 200 cycles of NaOH cleaning.

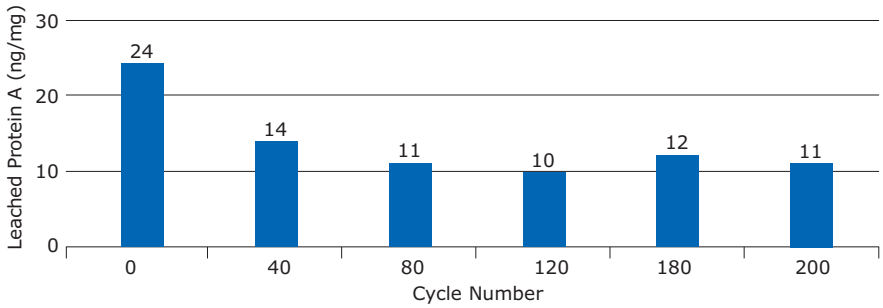


Figure 11.

Eshmuno® A media HCP clearance over 200 cycles of NaOH cleaning.

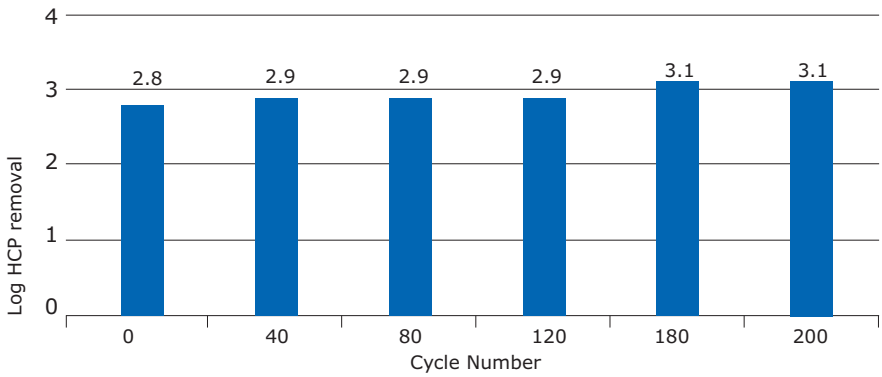


Figure 12.

Eshmuno® A media DBC (5% BT) over 200 cycles of H_3PO_4 cleaning (6 min RT).

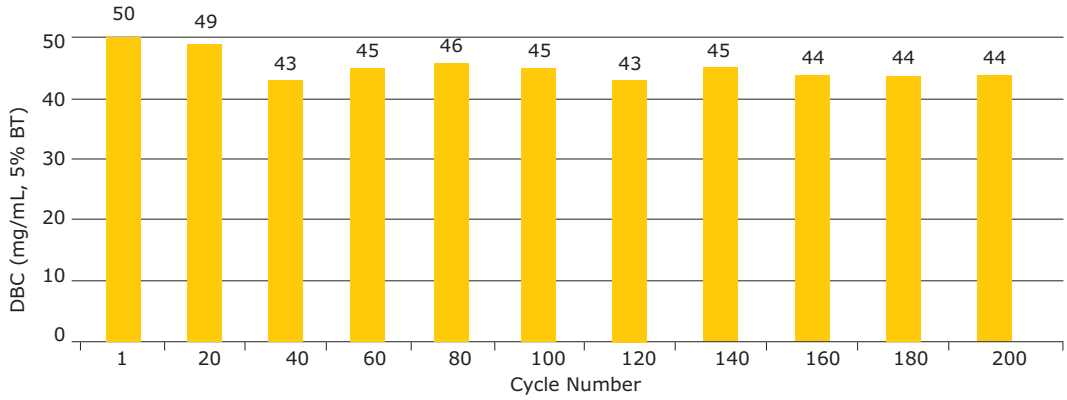


Figure 13.

Eshmuno® A media low leaching protein A over 200 cycles of H_3PO_4 cleaning.

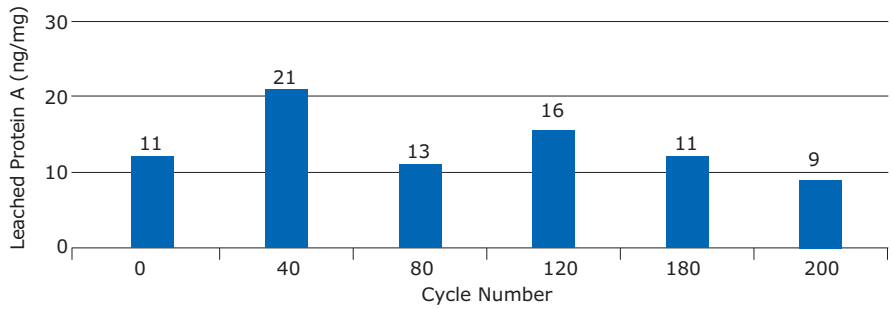
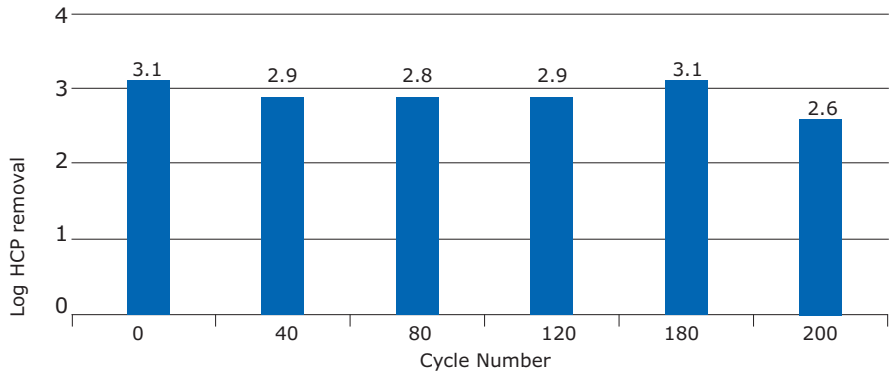


Figure 14.

Eshmuno® A media HCP clearance over 200 cycles of H_3PO_4 cleaning.



Aggregate Removal

Aggregates are typically eluted at the tail end of the Protein A elution peak. However, when using the Eshmuno® A media, a significant population of the aggregates are eluted at the front end of the elution peak, as well as at the tail end. This in turn leads to higher product purity due to the increased aggregate removal, using either step or gradient pH elution conditions.

Figure 15.

Aggregate removal.

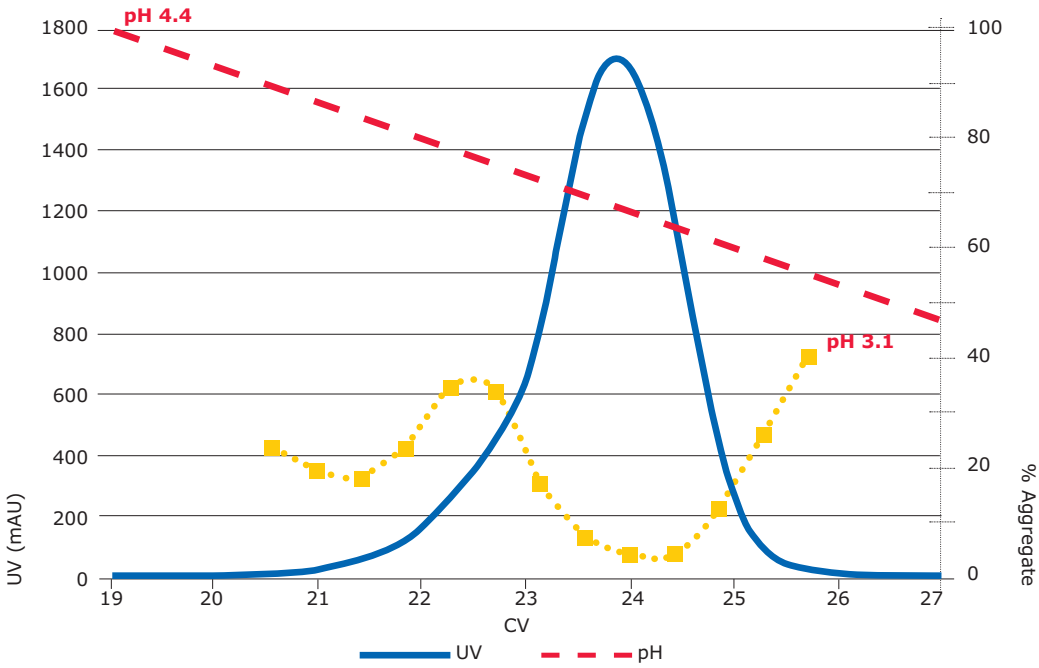
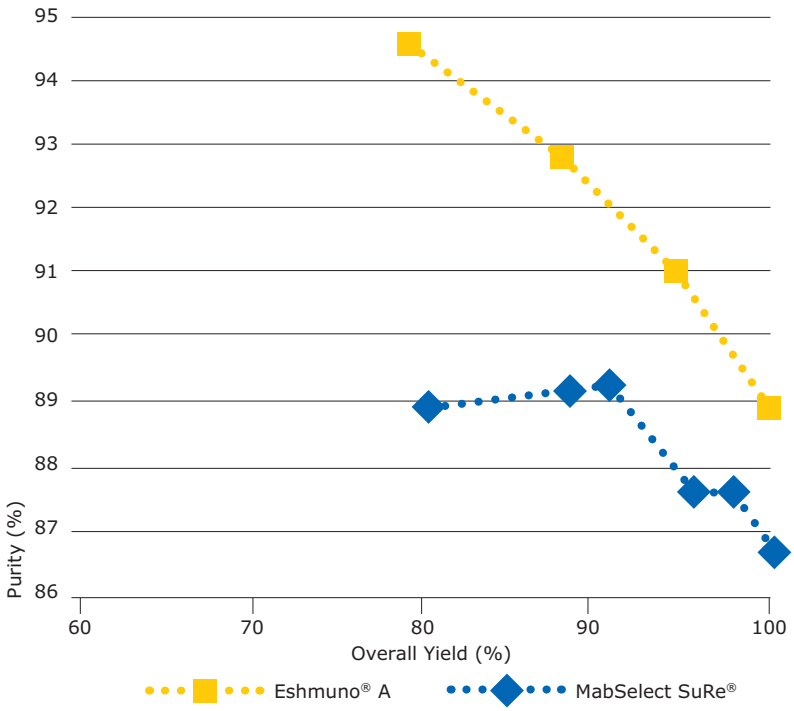


Figure 16.

Purity vs. yield for optimal aggregate removal of mAb 1.



Sanitization

MilliporeSigma recommends a PAB sanitization solution (120 mM phosphoric acid, 167 mM acetic acid, 2.2% (v/v) benzyl alcohol), as it offers adequate sanitization without sacrificing resin binding capacity. Acidification of benzyl alcohol significantly improves the microbial kill kinetics, thereby enabling effective sanitization in less than 3 hours even with spore-forming organisms. Alternatively, Eshmuno® A media may be sanitized using higher NaOH concentration.

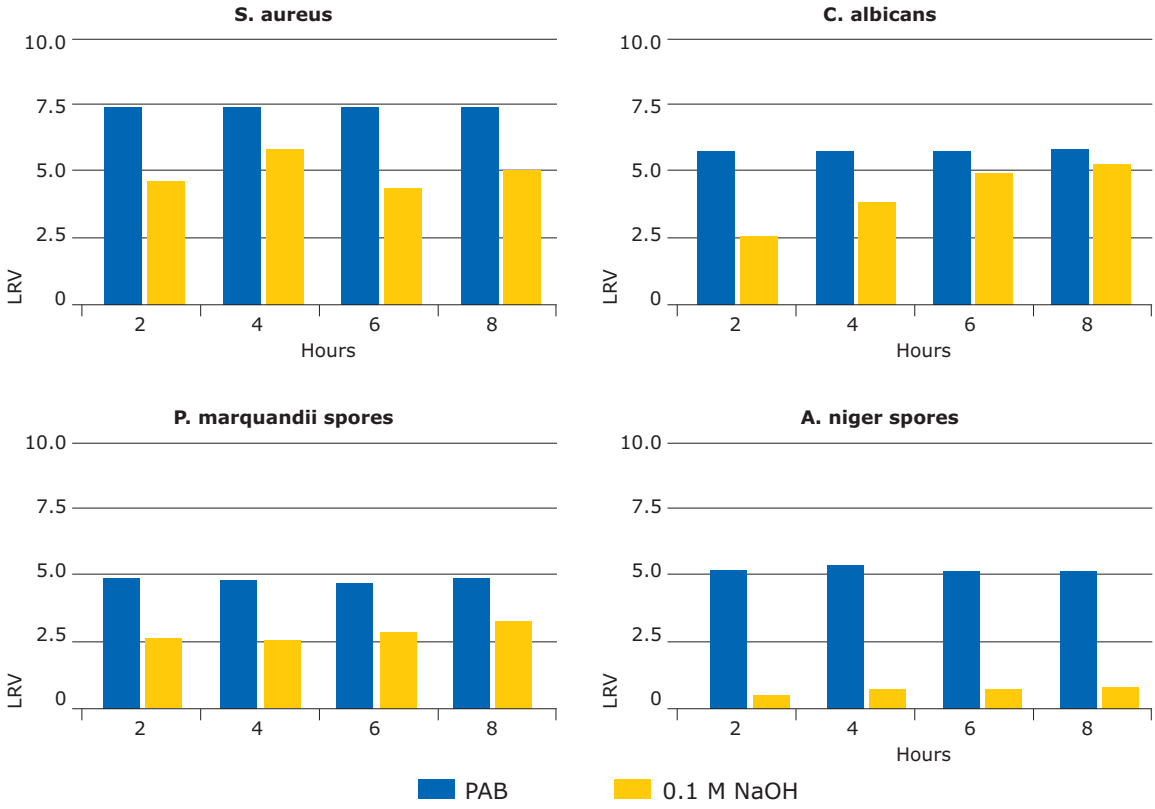


Figure 17.

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

Viral Clearance

Protein A affinity chromatography is a key orthogonal viral clearance step in downstream purification. Reliable viral clearance has been achieved using Eshmuno® A media.

Two common model viruses (Table 1) were used to investigate viral clearance:
A model retrovirus, Xenotropic Murine Leukemia Virus (XMuLV), 80 – 110 nm; and a model parvovirus, Mouse Minute Virus (MVM) 18 – 25 nm, which is more difficult to inactivate or remove by filtration.

Cell culture supernatants containing an antibody were spiked with either virus and applied to chromatography columns containing the Eshmuno® A media. Virus concentrations were measured in the flow-through, wash fractions and elution pools from each column.

	Eshmuno® A (1)	Eshmuno® A (2)
MVM	2.93	3.36
XMuLV (TCID50/mL)	> 4.9	> 4.9
XMuLV (qPCR)	3.71	3.82

Table 1.

Viral clearance data of Eshmuno® A media in duplicate.

Media Characteristics Overview

Type	affinity media
Base Material (or Matrix)	hydrophilic polyvinylether
Functional Group	rec. Protein A produced in *E. coli,* derived from C domain of native Protein A
Mean Particle Diameter	~ 50 µm
Dynamic Binding Capacity	40 – 55 mg/mL at 3-6 min RT and 5% breakthrough for mAbs.
Cleaning pH Stability	1.5 – 13.5
Operating pH Range	2.5 – 8.0
Mechanical Stability	8 bar
Linear Flow Rate	>500 cm/hr (20 cm bed height at 2 bar)
Shipping Solution	20% Ethanol, 150 mM NaCl

Ordering Information

Product	Order No.	Size
Eshmuno® A media	**1.20089.0010**	10 mL
	1.20089.0100	100 mL
	1.20089.0500	500 mL
	1.20089.5000	5 L
	1.20089.9010	10 L
MiniChrom™ Column	**1.25160.0001**	1 mL
	1.25161.0001	5 mL
RoboColumn®	**1.25162.0001**	0.2 mL
	1.25163.0001	0.6 mL

Order information for commonly used buffer and cleaning/sanitization solutions from MilliporeSigma.

Buffer Preparation

Product	Order No.
Potassium dihydrogen phosphate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, NF	**137039**
Dipotassium 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 Eshmuno® Resins

Product	Order No.
Ethanol 20% for cleaning of biochromatography resins	**480910**
Ethanol 20% v/v with 150 mol/L sodium chloride solution for storage of chromatography resins	**480940**
Guanidinium hydrochloride suitable for biopharmaceutical production EMPROVE® bio Ph Eur	**137037**
Sodium hydroxide solution 0,1 mol/L suitable for biopharmaceutical production EMPROVE® bio	**137058**
Sodium hydroxide solution 0,5 mol/L suitable for biopharmaceutical production EMPROVE® bio	**137060**

Column Cleaning & Storage of Affinity Resins

Product	Order No.
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**

Recommended ELISA Kit

Product	Order No.
Protein A ELISA kit purchased directly from Cygnus Technologies, Inc.	**F400**

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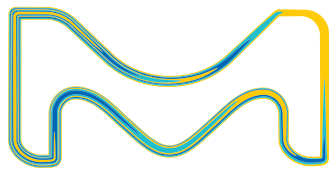

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