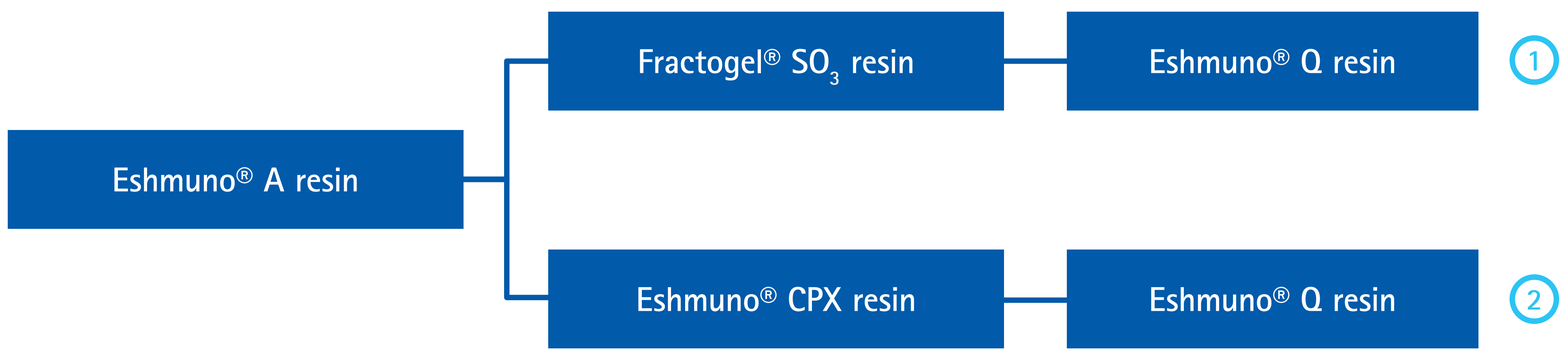
A Case Study: 3-Step Process for Efficient mAb Purification Using Different Commercially Available Chromatography Resins

Melissa Holstein*, Kristen Cotoni, Nanying Bian, *EMD Millipore Corporation, 80 Ashby Road, Bedford, MA 01730*

Summary

This study showcases a portfolio of commercially available biopharmaceutical chromatography resins designed for the efficient purification of monoclonal antibodies. A 3-step purification process has been implemented which showed effective removal of the main contaminants, low ligand leakage, and high yields over the entire process. Eshmuno® A affinity chromatography resin was evaluated as the first step in the process. The Protein A elution pool was further purified using cation exchange chromatography. Two cation exchange resins with different selectivities were compared. The final purification step consisted of anion exchange chromatography. The product yield, HCP removal, leached Protein A removal, and aggregate content were evaluated at each step in the process. Final process yields ranged from 74 to 81 percent. This work highlights the comparable purification capabilities of a panel of EMD Millipore resins designed for efficient mAb purification.

3-Step process schematic showing two different process trains



STEP 1: Protein A

Eshmuno® A resin: High capacity, acid and alkaline resistant Protein A affinity chromatography resin for purification of Fc-containing proteins, including but not limited to monoclonal antibodies

STEP 2: Cation Exchange

Eshmuno® CPX resin: Strong cation exchange resin based on tentacle technology; combines high aggregate removal efficiency with high dynamic binding capacity

Fractogel® SO_3 (M) resin: Strong cation exchange resin based on tentacle technology

STEP 3: Anion Exchange

Eshmuno® Q resin: Strong anion exchange resin combining tentacle structure with a hydrophilic polyvinyl ether base matrix

Experimental Conditions

An in-house CHO-S mAb feed clarified by depth filtration (Millistak+® DOHC and XOHC) and filtered (Opticap® XL 150 Millipore Express® SHC) is used for this study. The clarified feed was sterilized using 0.22 µm Stericup® filters immediately prior to use.

Protein A: (6 min RT for load and elution, 3 min RT for other steps)
- EQ: 50 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.2
- Load to 35 mg/mL
- Wash: 0.1 M citric acid, pH 5
- Intermediate Wash: 50 mM Tris, 1 M Arginine, pH 8.5
- EQ Wash: 50 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.2
- Elution: 0.1 M acetic acid, pH 3

Neutralized the Protein A elution pool to pH 5 using 2 M Tris

Cation Exchange (Bind and elute mode):
- EQ, Load, and Wash: 50 mM sodium acetate, pH 5
- Gradient elution over 10 CV: 50 sodium acetate, 0.5 M NaCl, pH 5

Pooled cation exchange fractions, then neutralized and diluted

Anion Exchange (Flow-through mode):
- Eshmuno® Q resin: 25 mM Tris, pH 7.3

Results

mAb purity was evaluated based on Host Cell Proteins (HCP) and leached Protein A after each purification step. The cation exchange elution fractions were pooled in order to maximize purity while maintaining at least 80% step yield.

- Yield: Based on UV absorbance at 280 nm
- HCP: Cygnus Technologies F550 CHO HCP ELISA Kit 3G Immunoenzymetric Assay
- mAb Feed HCP: 281,190 ppm
- Leached Protein A: Repligen Protein A Kit 9000-1

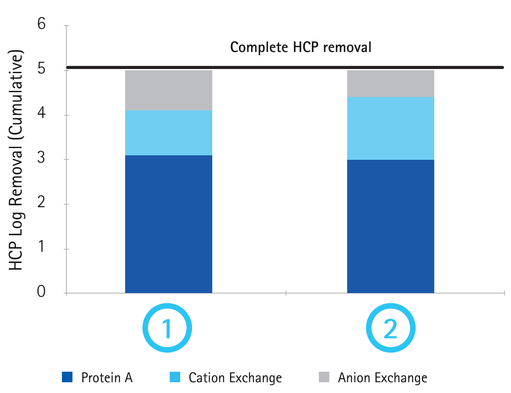
Purity and Yield for the Two Process Trains

1	HCP (ppm)	HCP log removal (cumulative)	Protein A (ppm)	Protein A log removal (cumulative)	Step Yield (%)
PrA - Eshmuno® A	212	3.1	35	N/A	97.8
CEX - Fractogel® SO_3	81	4.1	7.8	0.65	86.1*
AEX - Eshmuno® Q	ND	> 5	8.8	0.60	87.3
Overall					**73.5**

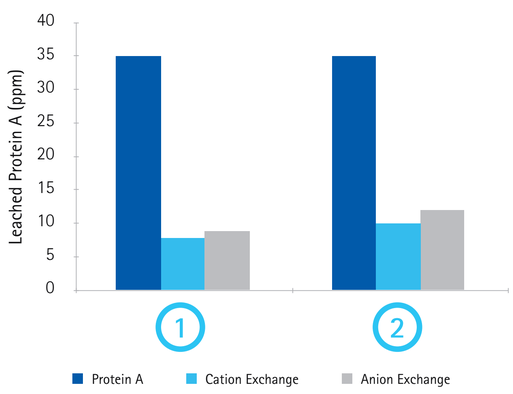
2	HCP (ppm)	HCP log removal (cumulative)	Protein A (ppm)	Protein A log removal (cumulative)	Step Yield (%)
PrA - Eshmuno® A	283	3.0	35	N/A	97.8
CEX - Eshmuno® CPX	12	4.4	10	0.55	89.0*
AEX - Eshmuno® Q	2	> 5	12	0.62	93.0
Overall					**81.0**

ND = Not Detectable (<1 ppm); * After pooling fractions

Comparison of HCP Removal



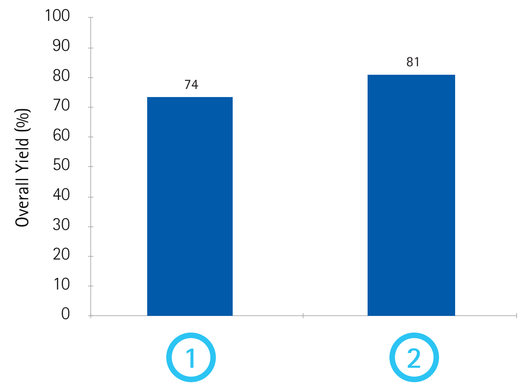
Comparison of Leached Protein A Levels



Conclusion

A 3-step purification process has been implemented which showed effective removal of the main contaminants, low residual leached Protein A, and high yields over the entire process.

The two different combinations of commercially available biopharmaceutical resins from EMD Millipore used in this case study gave comparable yields and final mAb impurity profiles.



Corresponding Author: melissa.holstein@emdmillipore.com