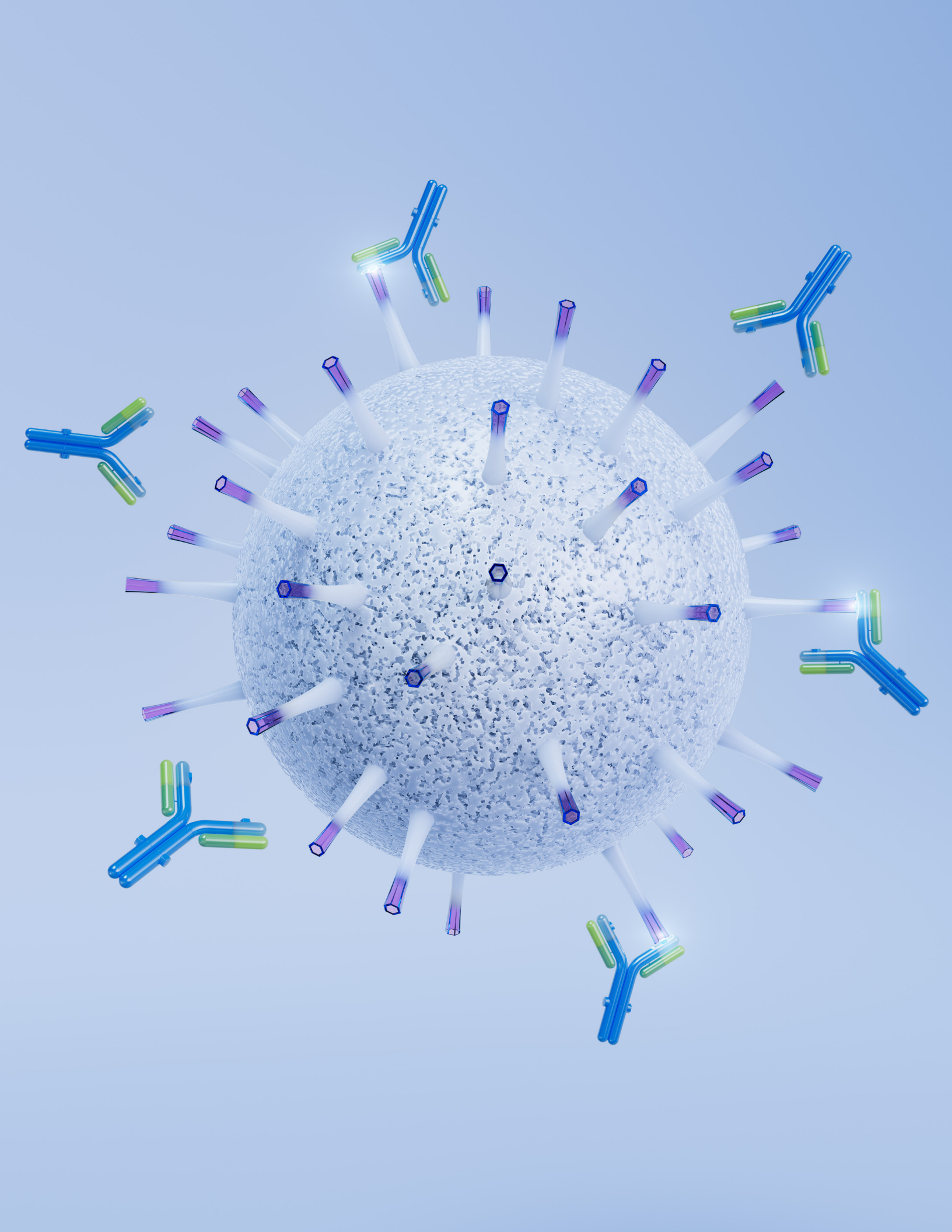
Instructions For Use

Praesto™ AP+80

Purolite™
An Ecolab Company



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Contents

Introduction

Praesto™ AP+80 is a high capacity, high flow, and alkaline stable protein A resin suitable for the purification of a wide range of mAbs and related constructs in the pharmaceutical and CDMO spaces.

Regarding mAb purification, there are several different buffer and process conditions for protein A capture presented within scientific literature. However, the outcome with respect to yield and purity is generally similar across most published protocols.

It is important to acknowledge that all molecules, whether they be mAbs, Fabs, or multi-specifics, are unique, and will differ both in chemical and physical characteristics. Optimization of the washing and elution steps is important in the development stage.

All Praesto chromatography resins are manufactured using our patented Jetting technology, which produces consistent agarose beads with a uniform particle size distribution. Jetting means supply chain security, reduced lead times, and faster mAb processing, all while contributing towards your sustainability goals.

This guide provides the user with recommended conditions and parameters for the use of Praesto AP+80. For further optimization or troubleshooting support, please contact us via purolite.com or by using the contact details on the back cover of this document.

Physical and Chemical Characteristics

A typical antibody is Y-shaped and could be divided into two identical antigen-binding (Fab) regions and one Fc (fragment crystallizable) region. All protein A resins have a high affinity for the Fc region of human IgG (except subclass 3). However, native protein A resins and most recombinant protein A ligands can also bind to the VH domain of antibodies that belong to the VH3 family. Praesto AP+80 binds predominantly to the Fc region but has demonstrated a partial affinity for the VH domain.

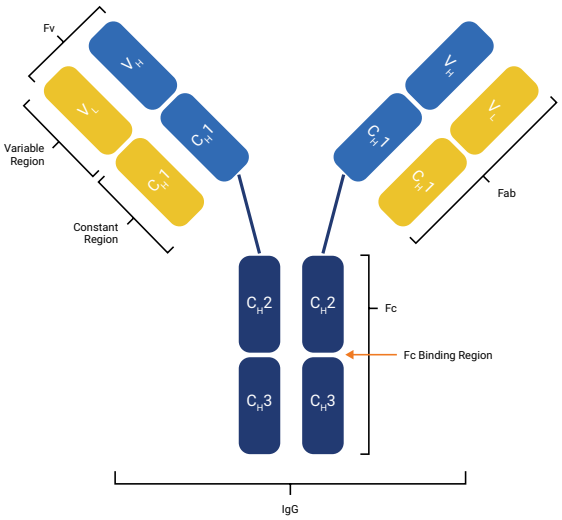


TABLE 1 **Product Specifications**

Product Characteristics	Praesto™ AP+80
Matrix	Highly cross-linked agarose
Ligand	Alkali stabilized recombinant Protein A
Dynamic Binding Capacity	Up to 60 mg mAb/mL resin (6-minute residence time)
Jetted Particle Size	80 µm
Particle Size Range	≥ 95% within range 42–125 µm
Pressure/Flow	Up to 400 cm/h (30 x 25 cm)
pH Stability (Working Range)	3–12
pH Stability (CIP)	2–13
Recommended Storage Conditions	2–8 °C in 20% ethanol

Purification Protocol

The affinity step is most frequently followed by two additional chromatography steps to achieve sufficient purity and virus clearance before the final formulation.

Table 2 summarizes suggested buffers and process steps in a standard mAb purification protocol. Ideally, the elution buffer should be designed to allow for a simple titration to condition the sample for the subsequent step.

The suggested buffer volumes are dimensioned for large columns. In a small-scale lab system, the column-to-system ratio is typically less optimal, so it is recommended to increase the wash and equilibration volumes.

Adsorption and desorption in a bead are mainly diffusion-driven processes. Thus, a high flow rate would have to be compensated with larger buffer volumes to achieve the same contaminant clearance (HCP), compared to what would be the result at a lower flow rate. Purolite recommends a flow rate corresponding to a residence time (RT) of 4–6 minutes for Praesto AP+80 when using a 20 cm bed height, due to the 80-micron bead size.

Before cycling a chromatography column and after storage, it is important to run a blank cycle, including CIP, to wash out the storage buffer and minimize the risk of bioburden.

TABLE 2 Generic Purification Protocol for Use with Praesto AP+80

Step	Buffer	Column Volume*
Equilibration	20 mM Na-phosphate, 0.15 M NaCl, pH 7.0–7.4	3
Sample Load	70–90% of the dynamic binding capacity (DBC)	N/A
Intermediate Wash 1	Equilibration buffer	3
Intermediate Wash 2	20 mM Na-phosphate, 1.0 M NaCl, pH 8.0	5
Intermediate Wash 3	20 mM Na-phosphate, pH 7.0	3
Alt. Intermediate Wash 3	50 mM Acetate, pH 6	1
Elution	100 Mm Na-acetate, pH 3.5	3
Regeneration	100 Mm Acetic Acid	3
CIP	0.1 M NaOH	3
Equilibration	20 mM Na-phosphate, 0.15 M NaCl, pH 7.0–7.4	5

**Column volumes for laboratory scale columns*

Loading

In designing loading parameters, it is important to perform a screening process to determine the capacity of the target antibody and — in conjunction with the operational window of the selected resin — select the appropriate conditions for optimal process performance and economics within the selected facility fit.

During process development, it is important to align the resin's pressure flow properties with the process column's dimensions and capabilities of the chromatography system intended for manufacture.

Pressure and Flow

Consideration needs to be taken with respect to flow and pressure when developing a purification process. Choosing the appropriate bed height with respect to capacity and process economics needs to be designed-in at the laboratory bench but achievable at scale.

Figures 3–6 and Table 3 outlines expected operational flows and pressures with recommended compression for Praesto™ AP+80. The relationship between residence time and linear velocity can be used in conjunction with capacity data to determine the most suitable process conditions.

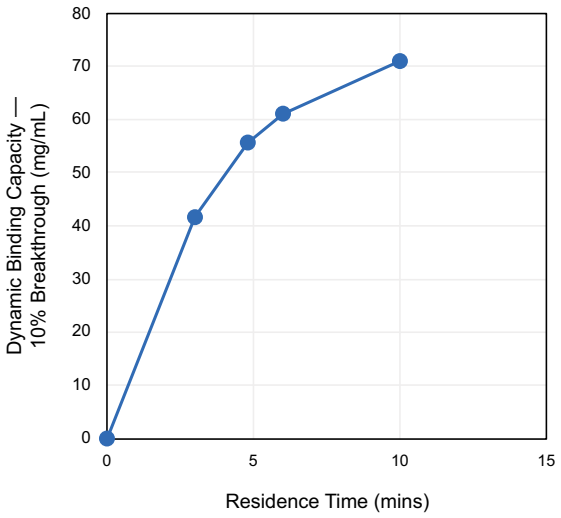
TABLE 3 Recommended Packing Factor and Compression Factor

Column Diameter (cm)	Recommended Packing Factor	Recommended Compression Factor
7	1.17	1.15
14	1.21	1.20
30	1.23	1.22
160	1.25	1.23

For use at a 20 & 25 cm bed height with Praesto AP+80.

FIGURE 2

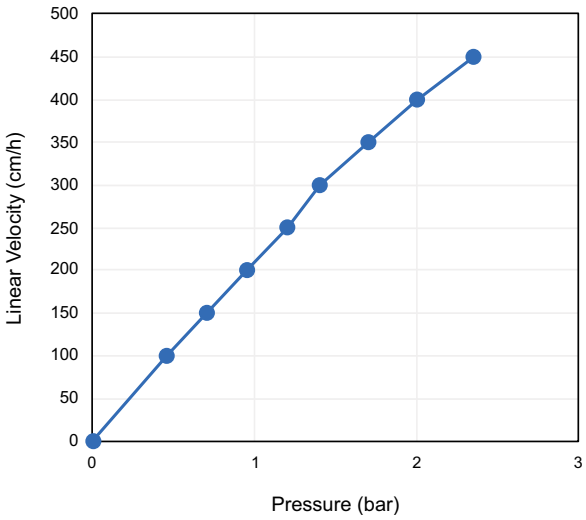
Dynamic Binding Capacity



At 10% breakthrough for polyclonal human Immunglobulin G on Praesto AP+80 over a range of residence times.

FIGURE 3

Packed Bed Pressure Flow



For Praesto AP+80 (30 cm ID) at a 25 cm bed height water at 20 °C.

FIGURE 4

Flow Expectation

For Praesto AP+80 on extrapolation of column diameter at a 25 cm bed height in a solution with a viscosity of 1 cp.

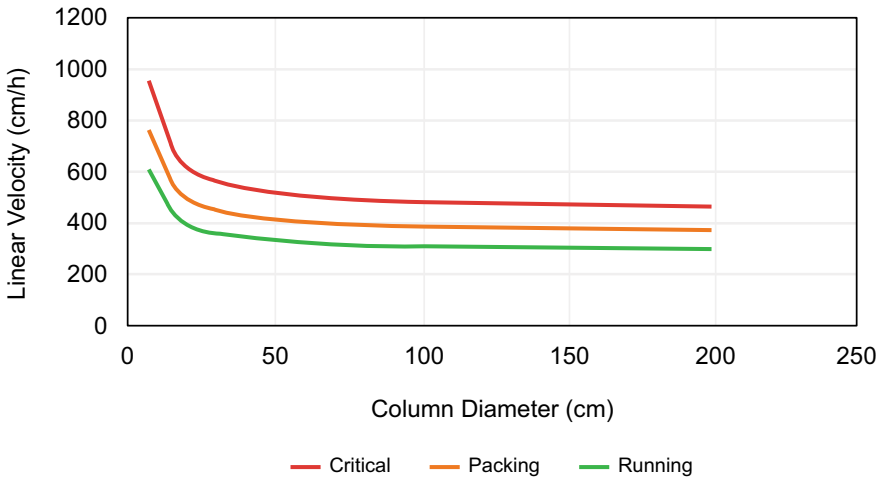


FIGURE 5

Pressure Expectation

For Praesto AP+80 on extrapolation of column diameter at a 20 cm bed height in a solution with a viscosity of 1 cp.

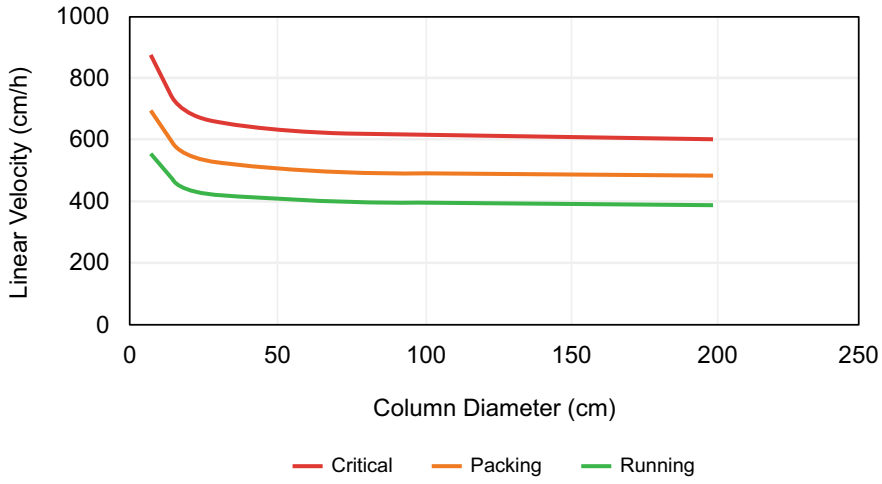
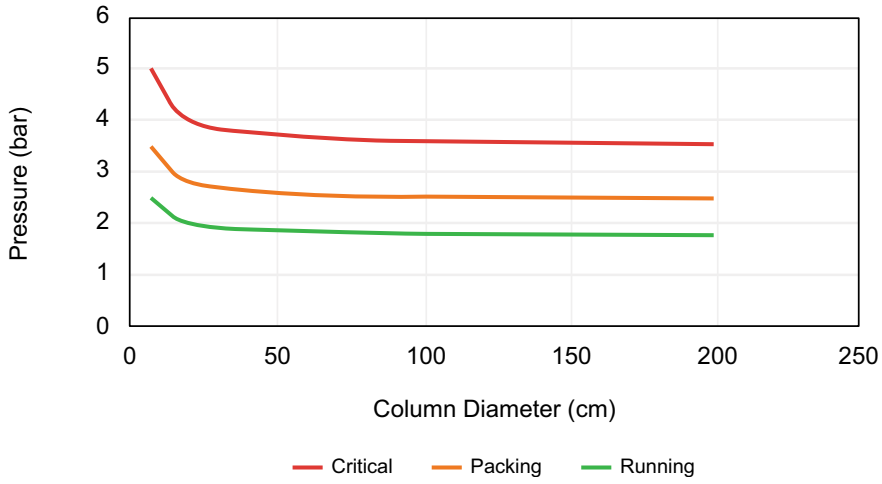


FIGURE 6

Pressure Expectation

For Praesto AP+80 on extrapolation of column diameter at a 20 & 25 cm bed height in a solution with a viscosity of 1 cp.



Elution

There is no clear rationale in choosing between acetate, citrate, or glycine buffers. Acetate and citrate are the most commonly used buffers.

Acetic acid has little buffer capacity at the elution pH and is easy to titrate without significantly increasing conductivity for the following step that commonly is cation exchange.

Citrate has buffering capacity over a wider pH range (3–7) and is preferable if it is important to have a very specific elution buffer pH. However, users should be aware that the preceding buffer and the elution pool volume will impact the pH of the eluate pool. Typical concentrations used are between 10–100 mM.

When optimizing elution, it is important to understand the highest pH possible to desorb the target antibody, which can be determined by loading a small amount of antibody under neutral conditions and performing an elution gradient over 10 CV at a residence time greater than 6 minutes.

Buffers containing A: 50mM Citrate, pH 6.0; and B: 50mM Citrate, pH 3.0 can be used for gradient evaluation. If running a gradient is not possible, pH 3–3.5 is a suitable starting point for screening.

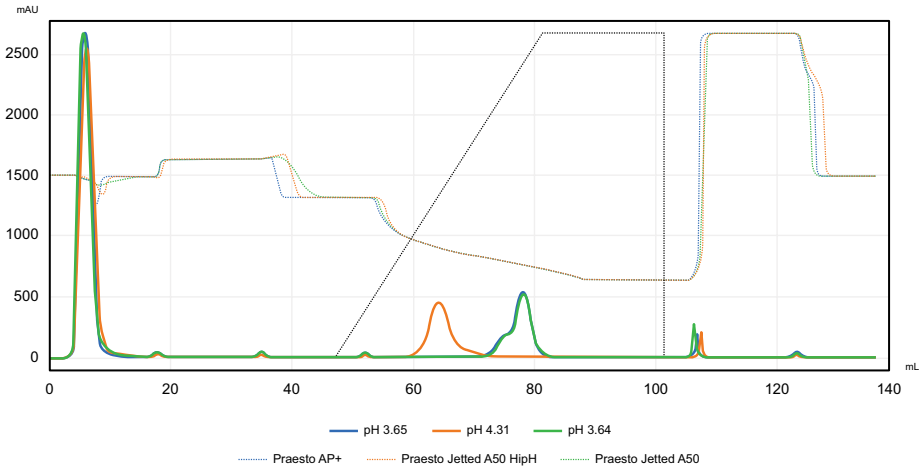
TABLE 4 Suggested Protocol for Determining Maximum pH Elution

Step	Buffer	Column Volume*
Equilibration	20 mM Na-phosphate, 0.15 M NaCl, pH 7.0–7.4	5
Sample Load	70–90% of the dynamic binding capacity (DBC)	N/A
Chase	Equilibration buffer	5
Column Wash	100 mM Na-citrate, pH 6.0	5
Elution	100 Mm Na-citrate, pH 3.0	0–100% 10 CV 100% B 5 CV
Regeneration	100 Mm Acetic Acid	3
CIP	0.1 M NaOH	3
Equilibration	20 mM Na-phosphate, 0.15 M NaCl, pH 7.0–7.4	3

FIGURE 7

Elution Chromatogram

For an IgG_1 subclass monoclonal antibody captured on Praesto AP+80, Praesto Jetted A50 HipH, and Praesto Jetted A50.



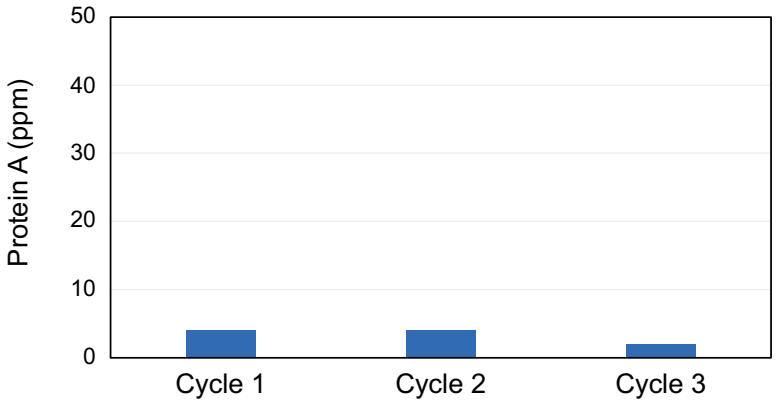
Leached Protein A

It is important to use the appropriate kit when determining leached protein A levels, use of alternative ligand calibration standards can lead to anomalous results. The protein ligand from Praesto AP+80 can be analyzed using the commercially available kit: ELISA Kit for the Detection of Native and Recombinant Protein A — 9000-1.

FIGURE 8

Expected Protein A Levels

Eluted with IgG1 mAb when captured using Praesto AP+80.



Regeneration

0.1 M acetic acid or low pH (3.0) for 2–3 column volumes post-elution is sufficient for the regeneration of the resin.

Intermediate Wash

While the mAb is bound to the Protein A resin, it is common to introduce an intermediate wash step. There are different strategies, but in principle, any shift from the sample loading conditions with respect to conductivity and pH will lower HCP levels in the elution pool.

There are published methods, including solvents or detergents. However, such additives must be assayed to show the removal, which can be difficult. Intermediate wash buffers with a pH range of pH 6–10 with 0.5–1.0 M NaCl are commonly used.

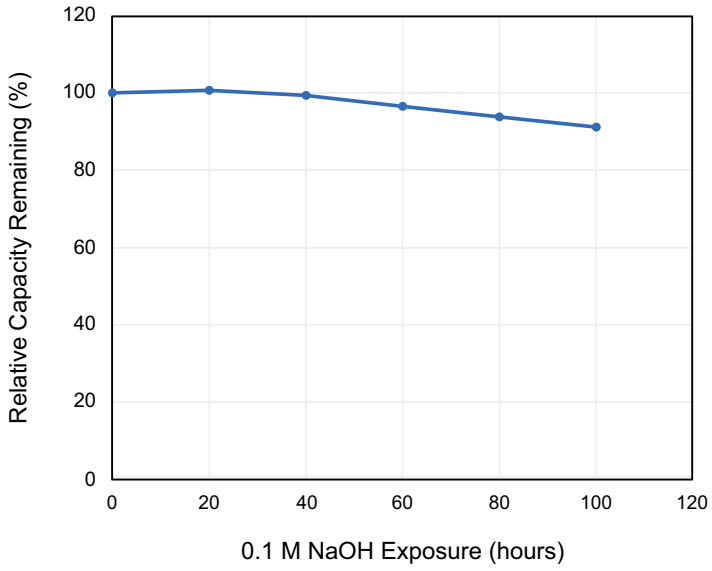
Cleaning In Place (CIP)

A regular cleaning in place (CIP) procedure is recommended to be performed after each cycle. Sodium hydroxide (NaOH) is commonly used in bioprocessing as an industry standard for cleaning in place. Sodium hydroxide exhibits high efficiency in removing bound proteins, nucleic acids, and lipids from bioprocess resins, alleviating the risk of fouling on heavily burdened Protein A columns.

A basic protocol for CIP is 15 minutes, 0.1 M NaOH (adjust the flow rate to match 15 minutes of contact time). However, depending on the intended number of cycles needed, contact time and concentration of NaOH could be varied.

FIGURE 9

Alkaline stability determined by polyclonal hIgG dynamic binding capacity at 10% breakthrough after static hold exposure to 0.1 M NaOH.



Conclusion

This instruction provides a starting point for protein A purification using Praesto AP+80. For subsequent polishing steps, Purolite provides a wide range of high-performance agarose-based ion exchange resins.

TABLE 7 Ordering Information

Product Description	Product Code
Praesto AP+80 (100mL)	PR00370-164
Praesto AP+80 (100mL in BnOH)	PR00370-164-BA
Praesto AP+80 (500mL)	PR00370-165
Praesto AP+80 (500mL in BnOH)	PR00370-165-BA
Praesto AP+80 (25mL)	PR00370-166
Praesto AP+80 (25mL in BnOH)	PR00370-166-BA
Praesto AP+80 (200µl) Robocolumn	PR00370-174
Praesto AP+80 (1mL) MiniChrom	PR00370-175
Praesto AP+80 (5mL) MiniChrom	PR00370-176
Praesto AP+80 (1mL) HT Column	PR00370-275
Praesto AP+80 (5mL) HT Column	PR00370-276
Praesto AP+80 (600µl) Robocolumn	PR00370-279
Praesto AP+80 (1L)	PR00370-310
Praesto AP+80 (1L in BnOH)	PR00370-310-BA
Praesto AP+80 (5L)	PR00370-311
Praesto AP+80 (5L in BnOH)	PR00370-311-BA
Praesto AP+80 (10L)	PR00370-312
Praesto AP+80 (10L in BnOH)	PR00370-312-BA
Praesto AP+80	PR00370-36
FC927320	PR00370
FC927330	PR00370-BA

Innovative Solutions for Bioprocessing

In partnership with Repligen, Purolite™ develops and supplies innovative solutions for the bioprocessing industry, working with many of the top pharmaceutical companies to deliver the next-generation of healthcare. Our resins are used across the globe to deliver lifesaving medicines.

Global Support Network

No matter the location, our expert field application team members are positioned to help you solve your technical and downstream purification challenges, together. We provide the guidance necessary to develop robust, scalable, high productivity purification processes for mAbs and recombinant processes using Praesto™ Jetted chromatography resins. For wherever you are in your biomanufacturing journey, we are here to help.

Purolite Affinity Resin Toolbox

Purolite's diverse toolbox offers Protein A resins, Praesto Jetted A50 and Praesto AP+80, designed for high performance and increased sustainability, as well as novel resins, Praesto Jetted A50 HipH and Praesto 70 CH1, designed to enable cost-effective and reliable purification of bispecifics and Fc fusion proteins.

Purolite Ion Exchange Toolbox

Purolite's ion exchange toolbox consists of Praesto SP and Praesto Q resins in four particle sizes to ensure predictable selectivity across particle sizes, allowing for rapid performance screening.

Notes

Purolite, an Ecolab company, is a leading manufacturer of quality ion exchange, catalyst, adsorbent and specialty high-performance resins with global sales support.

www.purolite.com

We're ready to solve your process challenges.

For further information on Purolite products and services,
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