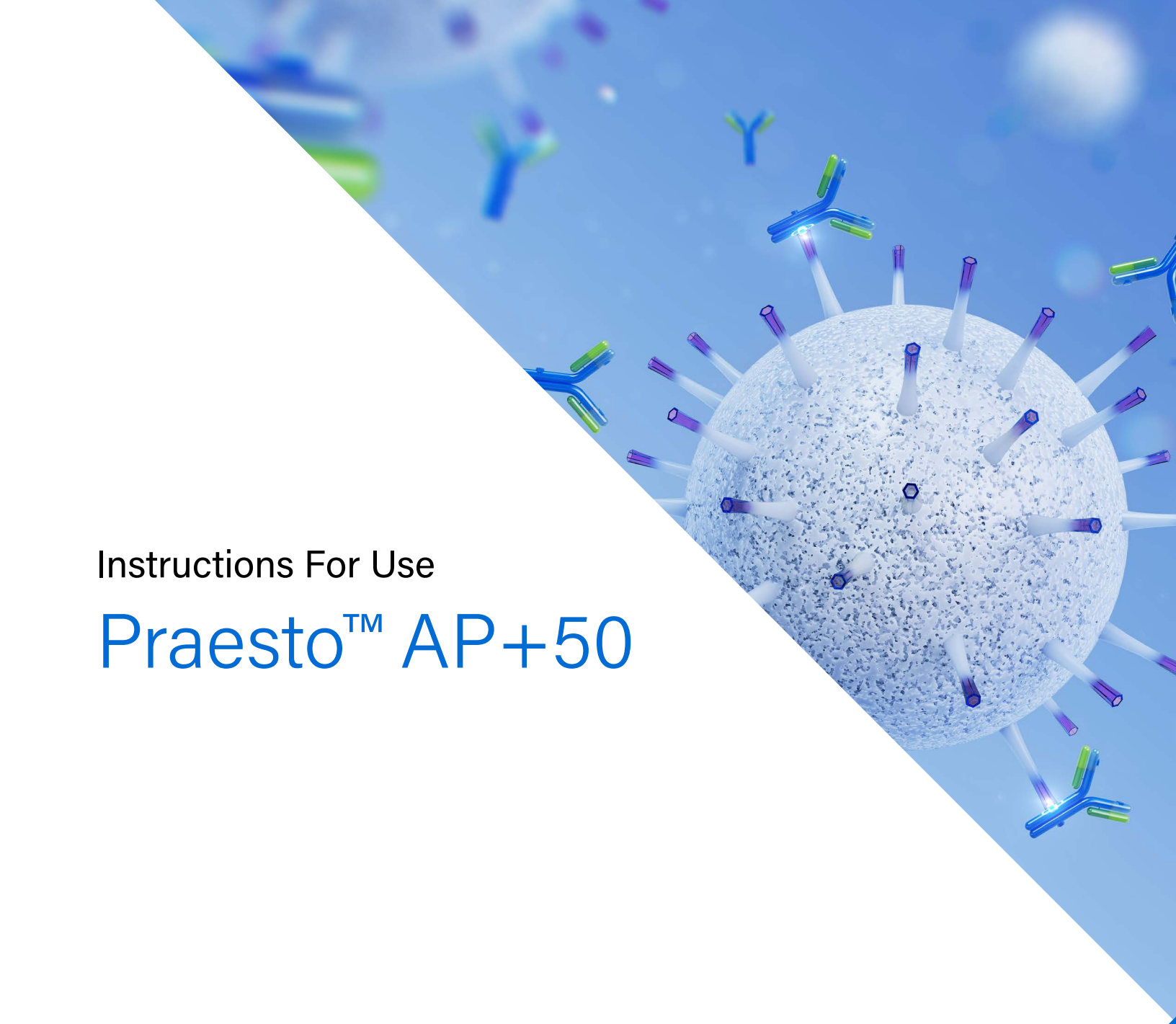


Instructions For Use

Praesto™ AP+50

ECOLAB®

Purolite™ Praesto™ AP+50 Instructions for Use

Contents

Introduction

The first protein A resins were based on the wild type of protein A expressed by a pathogenic *Staphylococcus aureus* strain. Since then, the manufacturing of monoclonal antibodies has grown tremendously, and they are now the most important group of molecules in the biopharmaceutical industry. Along with this development, protein A resins have been significantly improved both with respect to capacity, productivity and alkaline stability, resulting in a dramatic enhancement of process performance. Ligand design, coupled with new bead technologies, has led to the introduction of new resins with vastly improved performance characteristics compared to early resins.

Praesto AP+50 is a high-capacity protein A resin with uniform particle size distribution manufactured by patented Jetted Technology. Praesto AP+50 is designed for the purification of a wide range of mAbs and related constructs.

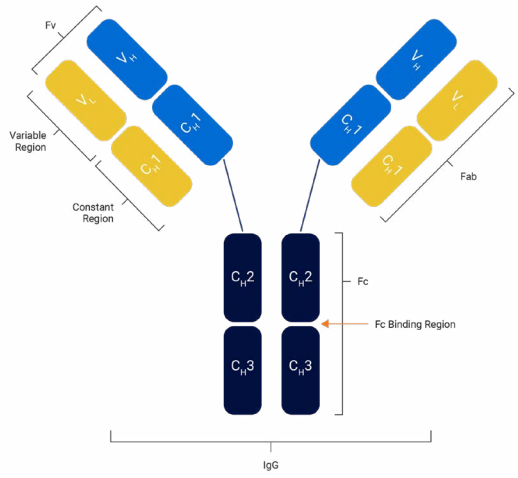
Herein, we describe basic screening and development conditions for protein A chromatography and recommendations for use with Praesto AP+50.

With regards to 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, in general, similar for most published protocols.

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

FIGURE 1

mAb graphic



A typical antibody is Y-shaped (Figure 1) and could be divided into two identical antigen-binding (Fab) regions and one Fc (fragment crystallizable) region. All protein A resins have high affinity to the Fc region of the Immunoglobulin G antibody. However, native protein A and most recombinant protein A ligands can also bind to the VH domain of antibodies that belong to the VH3 family. Praesto AP+50 binds to both the Fc and VH domains as with other protein A resins.

Physical and Chemical Characteristics

TABLE 1 Product specifications

Product Characteristics	Praesto AP+50
Polymer Structure	Highly cross-linked agarose
Dynamic Binding Capacity	Up to 70 mg hIgG/ml resin, 6 minutes RT
Average Particle Size	50 µm
Particle Size Range	95% between 35–90 µm
Pressure/Flow	Up to 200 cm/h (30 x 20 cm)
pH Stability (Working Range)	3–12
pH Stability (CIP)	2–13
Recommended Storage Conditions	2–8 °C in 20% ethanol

Purification Protocol

The protein A step is followed by two (sometimes one) additional chromatography steps to achieve sufficient purity and virus clearance before final formulation.

Table 2 summarizes the suggested buffers and process steps in a "generic" mAb purification protocol. Ideally, the elution buffer should be designed to allow 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, which is why it is recommended to increase the wash and equilibration volumes.

Adsorption and desorption in a bead is mainly a diffusion-driven process. Thus, a high flow rate would have to be compensated with larger buffer volumes to achieve the same contaminant clearance, compared to what would be the result at a lower flow rate. We recommend a flow rate corresponding to a residence time (RT) of 6–8 minutes for 50 µm Praesto beads when using a 20 cm bed height. Higher residence times can be used with shorter bed heights; see the operational flow diagram for more information.

N.B.: 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+50

Step	Buffer	Column Volume**
Equilibration	20 mM sodium phosphate, 0.15 M NaCl, pH 7.0–7.4	3
Sample Load	70–90% of the dynamic binding capacity (DBC10%)	N/A
Intermediate Wash 1	Equilibration buffer	3
Intermediate Wash 2	20 mM sodium phosphate, 1.0 M NaCl, pH 8.0	3
Intermediate Wash 3	20 mM sodium phosphate, pH 6.5	3
Elution	50 mM sodium acetate, pH 3.0-3.5	5
Strip	100 mM acetic acid	5
CIP	0.1 M NaOH	3
Equilibration	20 mM sodium phosphate, 0.15 M NaCl, pH 7.0–7.4	3

**Column volumes for laboratory-scale columns

Loading

In designing loading parameters for the study, it is important to perform a screening process to determine the capacity of the target antibody, and choose the appropriate conditions for optimal process performance, economy, and facility fit.

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

Figure 3 below shows operational flows at a range of bed heights with Praesto AP+50*. The flows have been determined using a column diameter of 30 cm packed by pressure and manual compression. The relationship between residence time and linear velocity detailed here can be used as a guide in conjunction with capacity data to determine the most suitable process conditions.

FIGURE 2

Dynamic binding capacity at 10% breakthrough for polyclonal human immunoglobulin G on Praesto AP+50 at a range of residence times

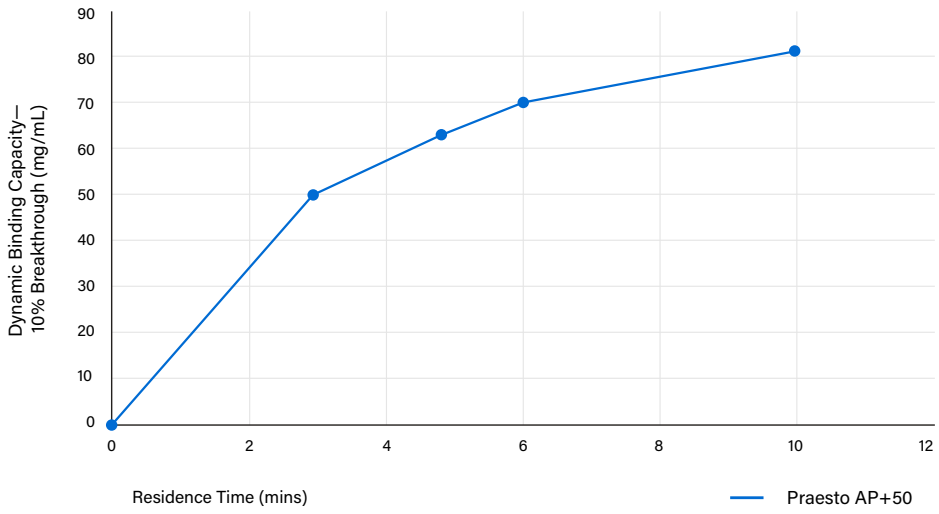


FIGURE 3

Expected operational flow window for Praesto AP+50*, generated at 30 cm inner diameter

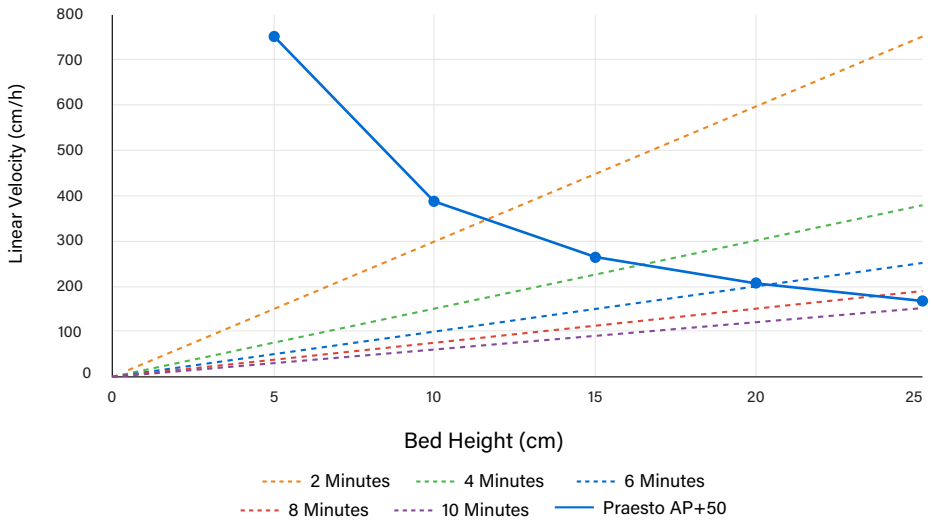


FIGURE 4

Flow expectation on extrapolation of the column diameter at 20 cm bed height in a solution of viscosity of 1 cp

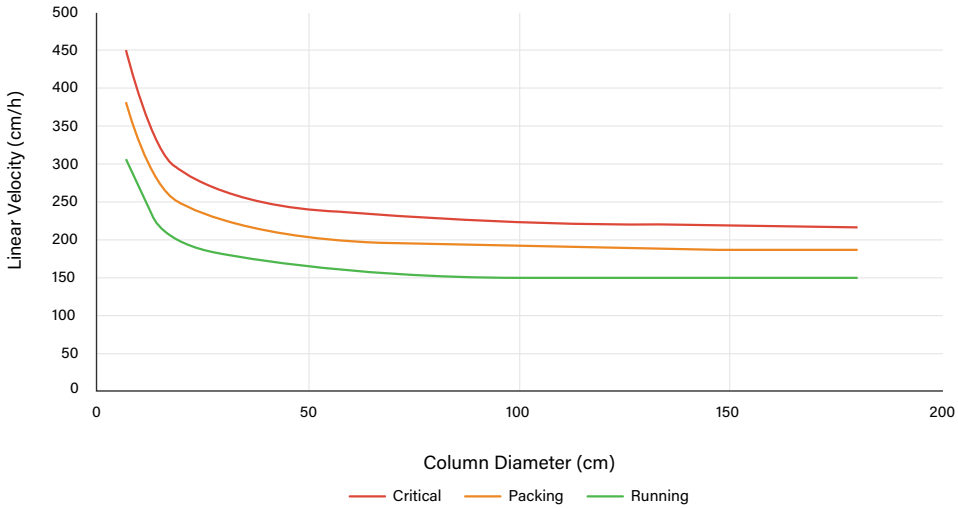


FIGURE 5

Pressure expectation on extrapolation of the column diameter at 20 cm bed height in a solution of viscosity of 1 cp

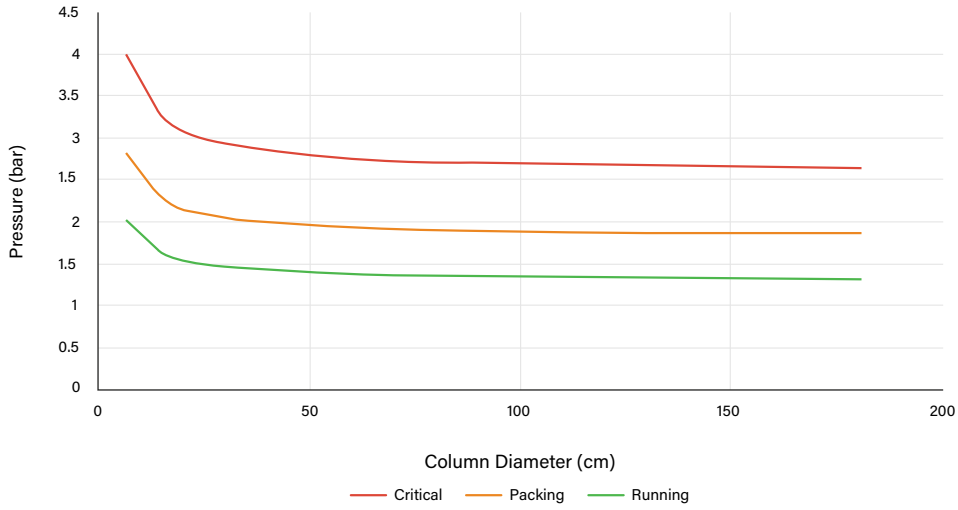


TABLE 3 **Recommended packing factors and compression factors for use at 20 cm bed height with Praesto AP+50***

Column Diameter (cm)	Recommended Packing Factor	Recommended Compression Factor
7	1.20	1.17
14	1.21	1.18
30	1.23	1.20
160	1.25	1.22

FIGURE 6

Pressure and Manual Compression Packing

Pressure versus flow for Praesto AP+50* at 30 cm inner diameter with bed heights of 10, 15, 20 and 25 cm. Packed by pressure and manual compression in a BPG 300 column.

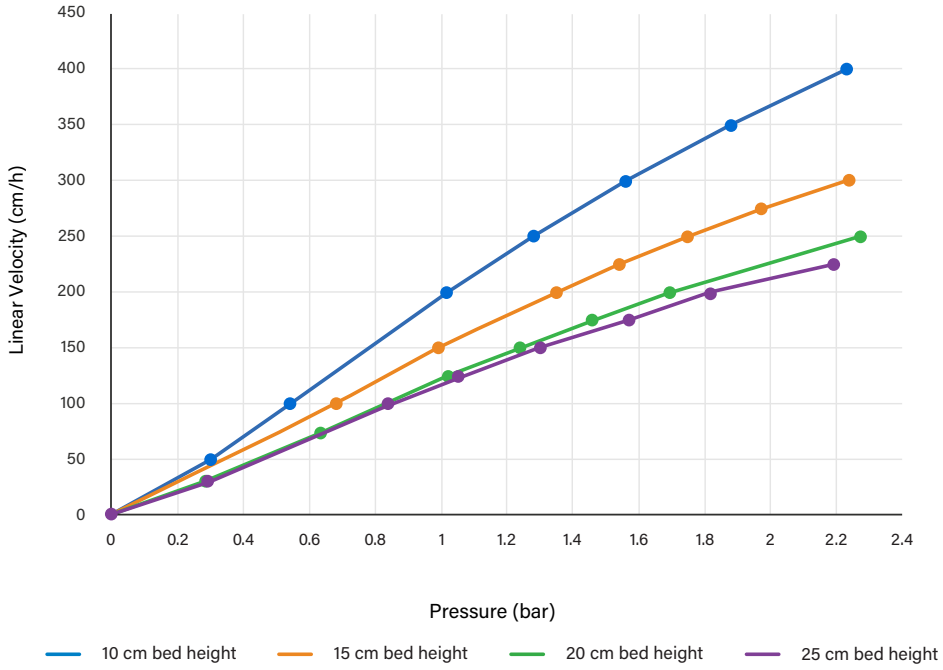


TABLE 4 **Pressure versus flow data for Praesto AP+50* at 30 cm inner diameter with bed heights of 10, 15, 20 and 25 cm; packed by pressure and manual compression in a BPG 300 column**

Bed Height (cm)	Maximum Linear Velocity (cm/h)	Pressure (bar)	Compression Factor
10	340	1.8	1.22
15	250	1.8	1.22
20	200	1.8	1.20
25	180	1.8	1.20

Pressure flow data is generated using pressure packing and manual compression. Axial compression offers the user a 'looser' pack resulting in higher achievable flows.

FIGURE 7

Axial Compression Packing

Pressure versus flow for Praesto AP+50* at 60 cm inner diameter with bed heights of 12, 15 and 20 cm. Packed by axial compression in an AxiChrom 600 column.

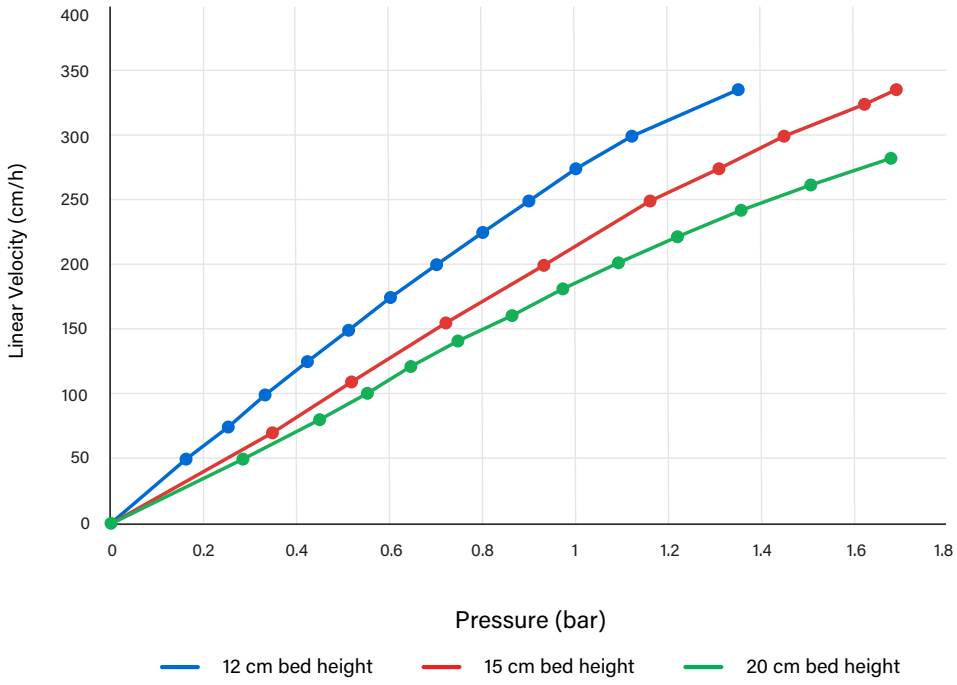


TABLE 5 Pressure versus flow data for Praesto AP+50* at 60 cm inner diameter with bed heights of 12, 15 and 20 cm; packed by axial compression in an AxiChrom 600 column

Bed Height (cm)	Maximum Linear Velocity (cm/h)	Pressure (bar)	Compression Factor
12	340	1.3***	1.22
15	325	1.6	1.22
20	275	1.6	1.20

***Reached maximum pump speed during test

It is recommended to pack Praesto AP+50* above 30 cm inner diameter in axial compression columns for optimal flow properties.

Elution

There is no clear rationale for choosing between acetate, citrate or glycine buffers. Acetate and citrate are the most 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, which 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 (but be aware that the preceding buffer and the elution pool volume will impact the pH of the eluate pool). Typical concentrations used are 10–100 mM.

When optimizing elution, it is important to understand the highest pH possible to desorb the target antibody by loading a small amount of the antibody under neutral conditions and performing an elution gradient over 10–20 CV at a residence time greater than 6 minutes. Buffers containing A: 50 mM citrate, pH 7.0 and B: 50 mM citrate, pH 3.0 can be used for gradient evaluation. If running a gradient is not possible, a pH of 3.0–3.5 is a good starting point for screening.

TABLE 6 Suggested protocol for determining maximum pH elution for the target molecule

Step	Buffer	Column Volume
Equilibration	20 mM sodium 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 sodium citrate, pH 6.0	5
Elution	100 mM sodium 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 sodium phosphate, 0.15 M NaCl, pH 7.0–7.4	3

Storage

It is recommended that Praesto AP+50 should be stored between 2–8 °C in 20% ethanol. After storage, you should equilibrate your resin with the starting buffer and perform a blank run, including CIP. This will ensure that there is no contamination on your resin.

Leached Protein A

The protein ligand from Praesto AP+50 can be analyzed using the commercially available ELISA kit for the Detection of Native and Recombinant Protein A – 9000-1.

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.

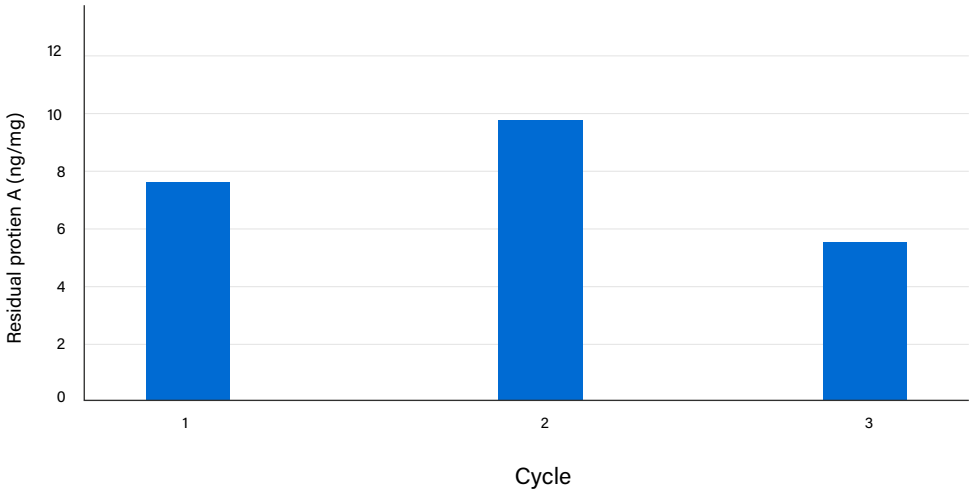
Representative cycling was performed on Praesto AP+50, with the analyzed protein A levels after the captured step presented.

TABLE 7 Purification protocol for an IgG1 HCCF capture step using Praesto AP+50

Phase	Buffer	Column Volume	Residence Time (mins)
Equilibration	Phosphate buffered saline, pH 7.4	5	6
Load	HCCF – IgG1 mAb at 8.5 g/L	Loaded to 80% DBC 10%	6
Wash 1	20 mM sodium phosphate, pH 7 + 500 mM NaCl	5	6
Wash 2	50 mM sodium acetate, pH 6.0	5	6
Elution	50 mM sodium acetate, pH 3.5	5	6
Strip	100 mM acetic acid, pH 2.9	5	6
CIP	0.1 M NaOH	3	6
Equilibration	Phosphate buffered saline, pH 7.4	3	6

FIGURE 8

Protein A levels after capture using Praesto AP+50



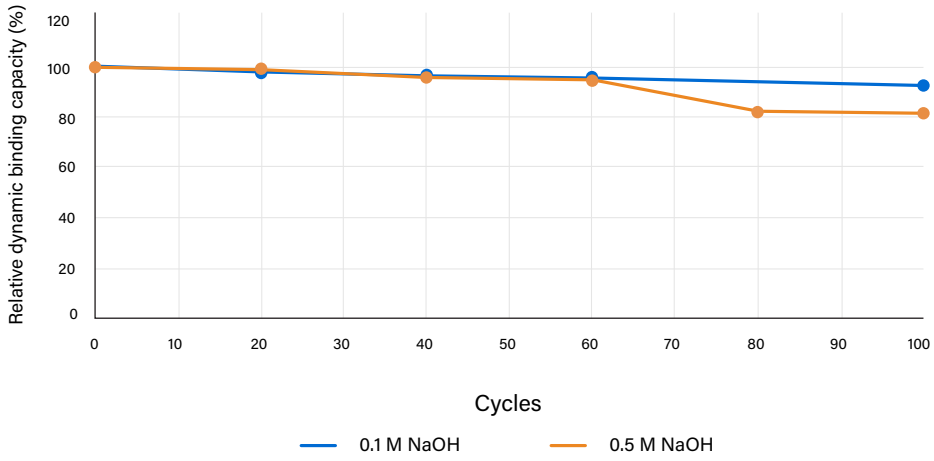
Cleaning In Place (CIP)

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 CIP. 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.

To maximize column lifetime, use the recommended CIP protocol of 0.1 M NaOH with a 15 min contact time. For more aggressive feeds consider the use of 0.1 M NaOH every cycle with a higher NaOH (0.2–0.5 M) concentration every tenth cycle and between campaigns. Exposure to NaOH is cumulative and will impact the overall lifetime of the resin and the maximum number of cycles.

FIGURE 9

Relative dynamic binding capacity at 6 minutes residence for Praesto AP+50 after blank cycling using 0.1 and 0.5 M NaOH CIP, with hIgG capacity testing at 20 cycle intervals



Regeneration

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

Intermediate Wash (Wash 2)

While the mAb is bound to the protein A resin, it is common practice 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. It is recommended to be working within the pH range of 6 to 10 for any intermediary washes.

**Performance data at scale was executed with an uncoupled, 50 µm base-bead to demonstrate packing, pressure and flow parameters for the following resins: Praesto Jetted A50, Praesto Jetted A50 HipH, Praesto AP+50 and DurA Cycle A50.*

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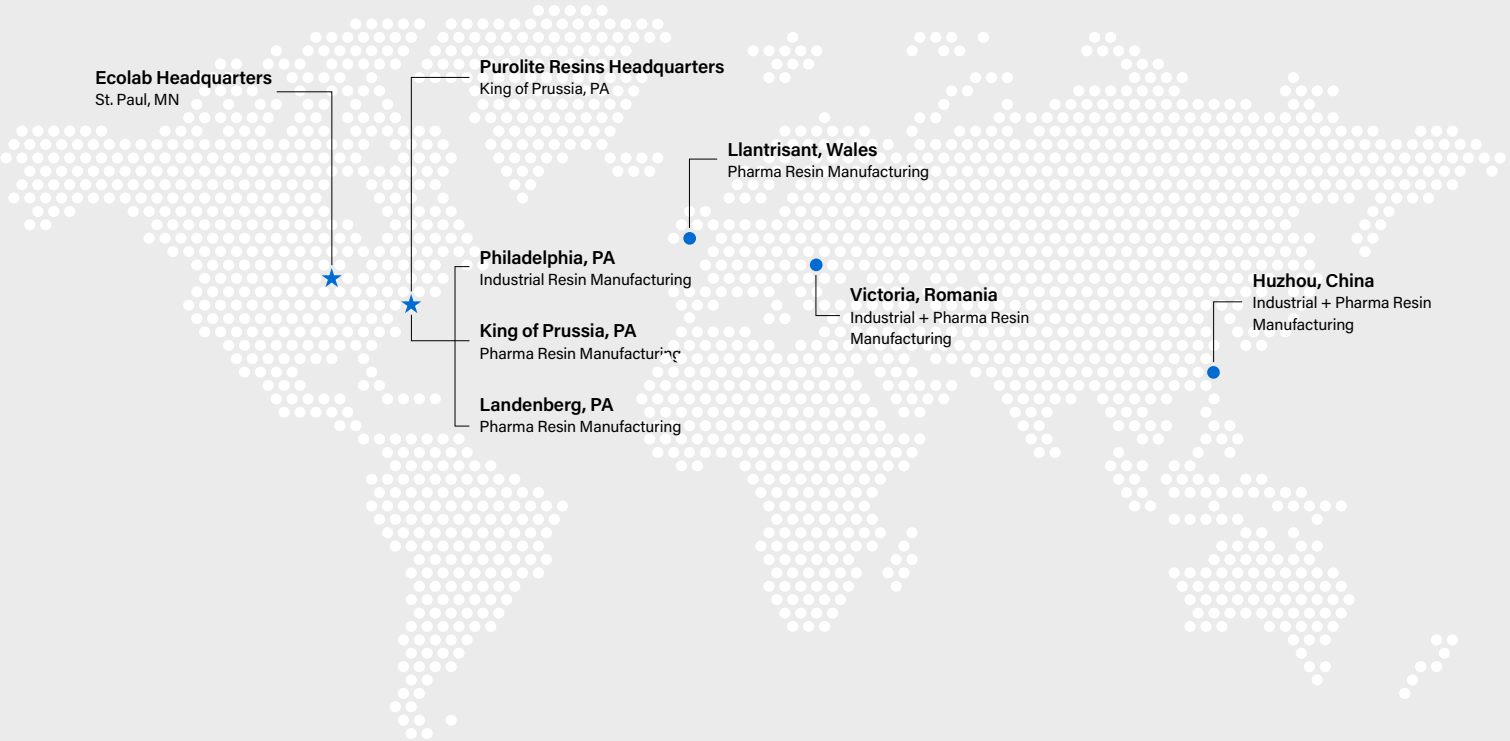

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