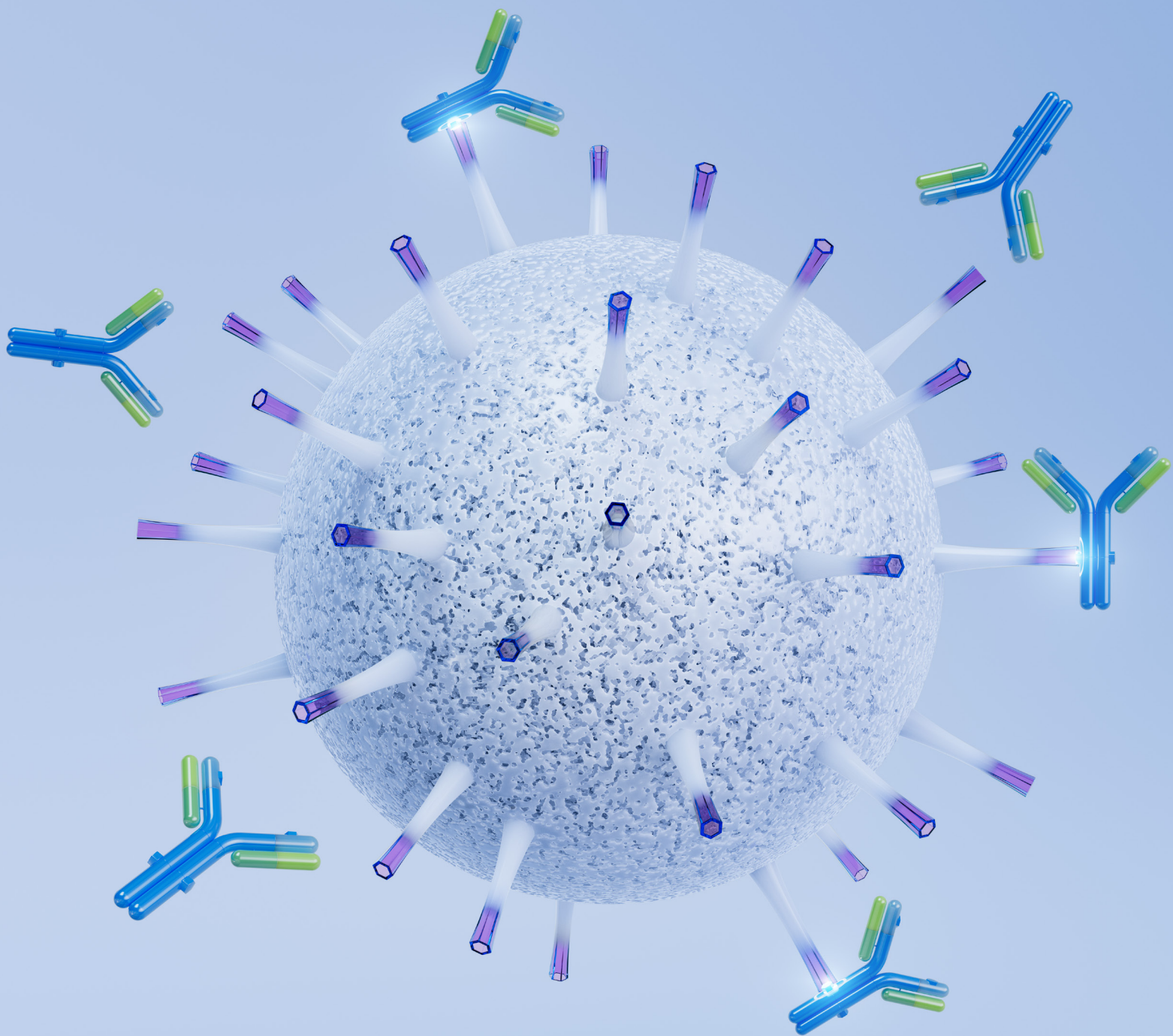




Data File

Praesto™ AP+50



Introduction

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

Praesto AP+50 is an agarose-based Protein A resin which is part of the Purolite bioprocessing resin family based on highly crosslinked agarose. The bead rigidity allows for process and column design flexibility that can be optimized for capacity utilization and economic performance. Bed height, column diameter and flow rate can be varied to accommodate existing equipment with the ability to reduce column size and optimize residence time for maximal capacity and the management of challenging molecules.

Jetted Technology

All bioprocess resins are manufactured using our patented Jetted Technology. Unlike resins synthesized by older emulsification processes, which result in a wide range of particle sizes requiring extensive sieving, our novel Jetting process creates more uniform particle distribution size monodisperse beads without using harsh chemicals, resulting in higher yields with less waste.

Key Benefits

- High dynamic binding capacity allowing for increased processing per gram mAb per cycle
- Good pressure flow properties for scalability
- Maintains capacity under intense standard cleaning in place (CIP) protocols, such as 0.1 – 0.5 M, with 0.1 M NaOH recommended

TABLE 1 Praesto AP+50 Features

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

High Dynamic Binding Capacity

Praesto AP+50 offers high dynamic binding capacity for polyclonal and monoclonal antibodies when compared to the highest selling market resin and the market leading alkaline stable resin, offering excellent process performance in terms of grams of antibody captured per cycle.

FIGURE 1

Dynamic binding capacity of human polyclonal immunoglobulin G at multiple residence times. Loading using PBS at pH 7.2 in a column of 0.66 inner diameter and 10 cm bed height.

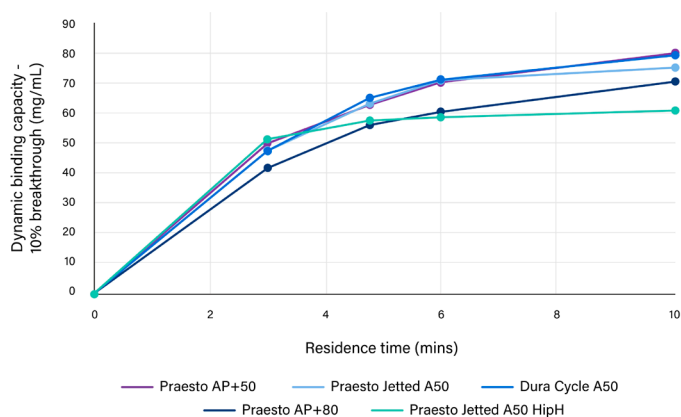
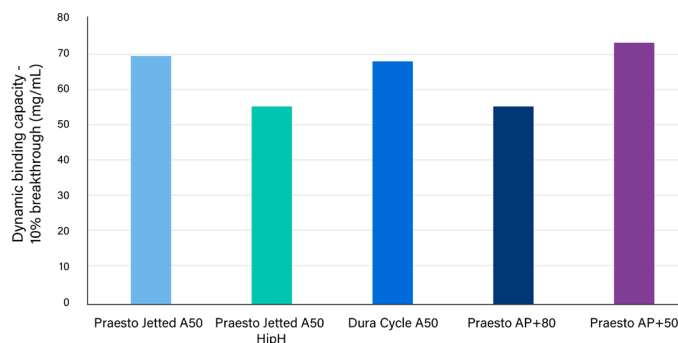


FIGURE 2

Dynamic binding capacity of a human monoclonal antibody (mAb), IgG1 subclass, at a 6 minute residence time. Loading using PBS at pH 7.2 in a column of 0.66 inner diameter and 10 cm bed height.



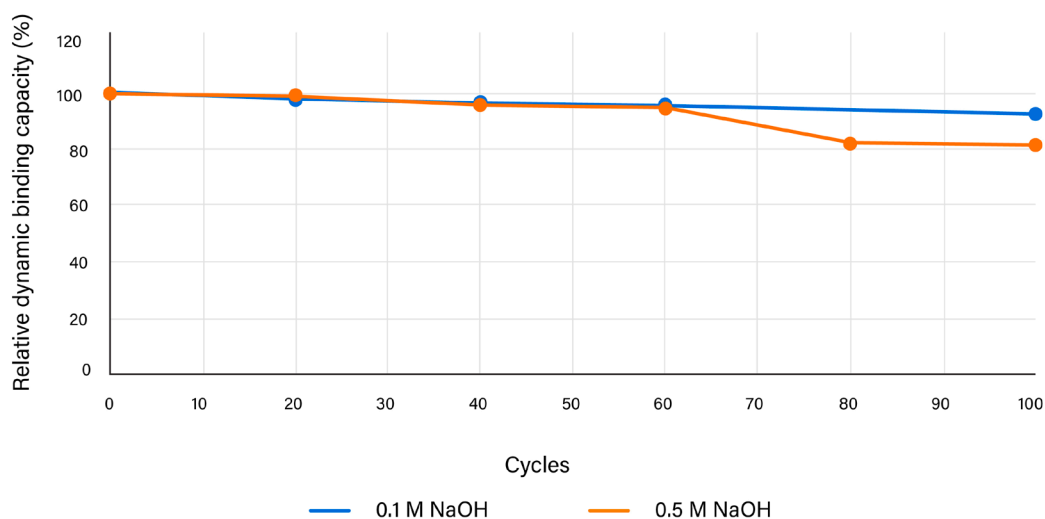
Alkaline Stability

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 3

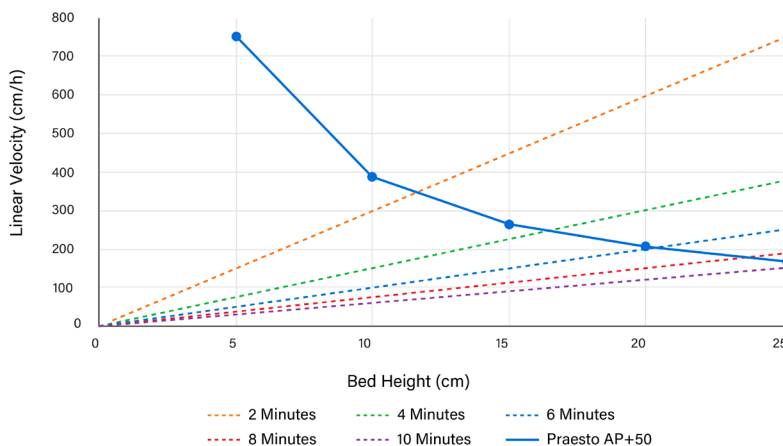
Relative dynamic binding capacity at 6 minutes residence for Praesto AP+50 after blank cycling using 0.1 and 0.5 M NaOH CIP, with hIgG capacity testing at 20 cycle intervals.



Pressure Flow

FIGURE 4

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



The figure above shows the modeled operational flows at a range of bed heights with Praesto AP+50. The flows have been modeled using a column diameter of 30 cm packed with pressure and manual compression (BPG). The relationship between residence time and linear velocity is represented in the figure above; it can be used as a guide in conjunction with capacity data to determine the most suitable process conditions.

FIGURE 5

Flow extrapolation modeling of column diameter at a 20 cm bed height in a solution of viscosity of 1 cp.

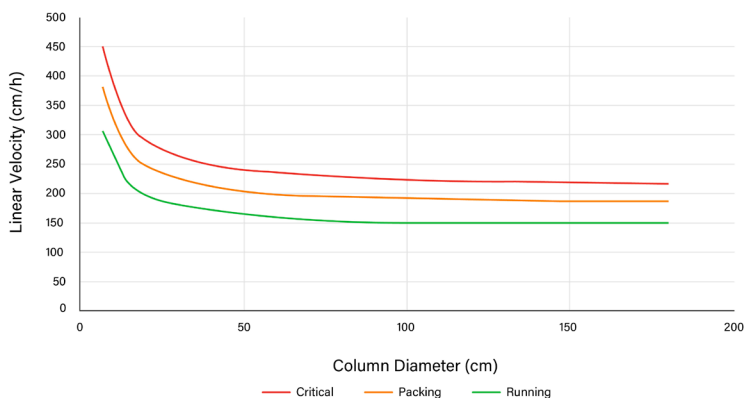


FIGURE 6

Pressure extrapolation modeling of column diameter at a 20 cm bed height in a solution of viscosity of 1 cp.

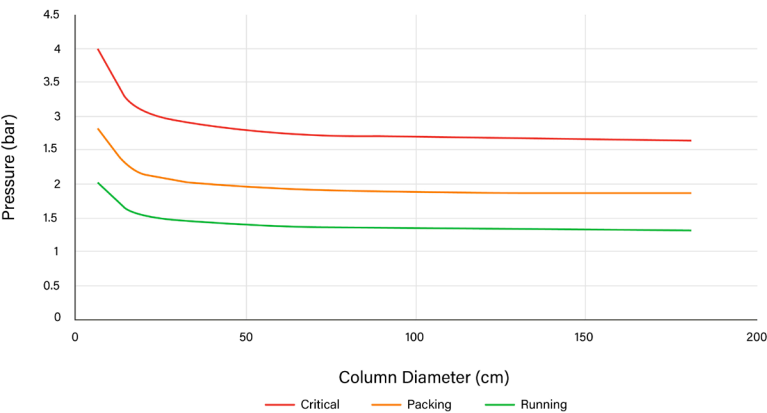


FIGURE 7

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

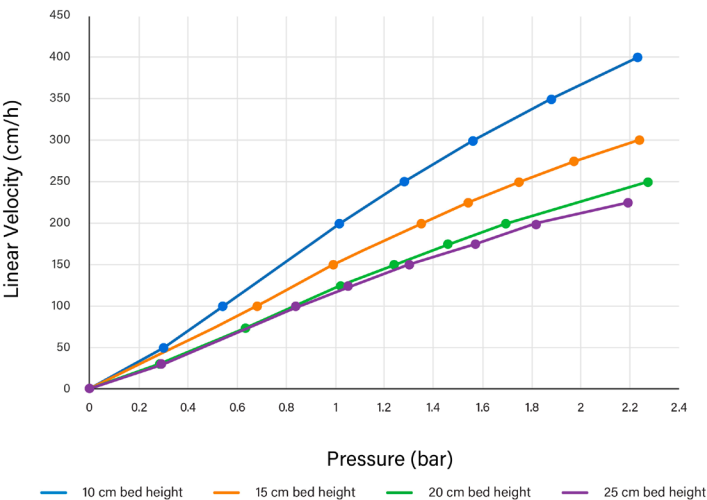
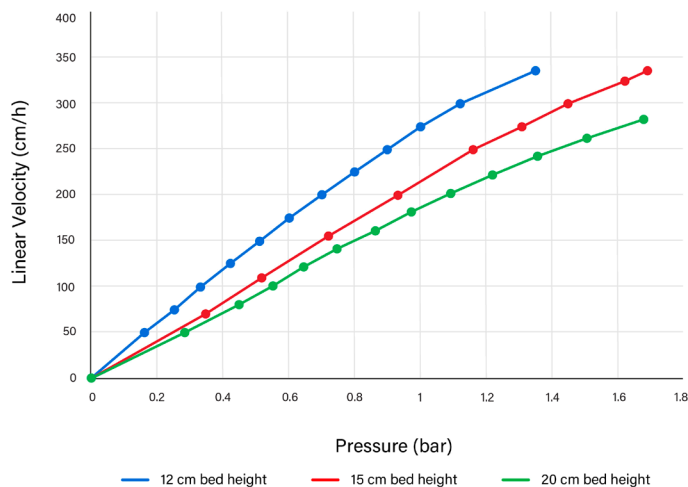


FIGURE 8

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



The uniform particle size and highly cross-linked agarose base bead of Praesto AP+50 elucidates the benefit of increased surface area of a smaller bead size without severely impacting the pressure flow properties. Praesto AP+50 has good flow properties, with flows of up to 200 cm/h achievable in a standard mAb platform process. This has been verified in process scale columns.

The data shown is for columns with ID of ≥ 30 cm where the effects of wall support on the resin start to become negligible, therefore similar pressure flow can be expected in larger diameter columns. If the resin is used in narrower columns, it is possible to achieve higher flow rates at an equivalent pressure, however the intended final scale or application (SMART cycling) should determine the bed height and max flow used for initial process development. Axial compression offers the user a 'looser' pack. It is recommended to pack Praesto AP+50 above 30 cm diameter in axial compression columns for optimal flow properties.

Purification Performance

Elution pH

It is important to screen the optimum elution for the target molecule with regards to optimal elution CV, purity and process related impurity removal. Please refer to the Praesto AP+50 instructions for use for recommended screening conditions.

All protein A resins bind to the Fc region of human immunoglobulin G antibodies. Praesto AP+50, Praesto AP+80, DurA Cycle A50 and Praesto Jetted A50 have an increased affinity to the VH3 region. However, this is only one aspect that dictates elution performance. Herein, we demonstrate the purification performance of Ecolab protein A resins at a range of elution pHs against two commercial agarose protein A resins.

Below, we demonstrate the expected performance of Praesto AP+50 in regards to recovery and host cell protein clearance ligand leakage at a range of elution pHs.

As demonstrated, Praesto AP+50 is a traditional protein A resin in terms of elution pH, with consistently high recovery and clearance under standard elution conditions of 3 – 3.5.

TABLE 2 Purification protocol for IgG1 HCCF capture with Ecolab protein A resins

Phase	Buffer	Column Volumes (CV)	Residence Time (mins)
Equilibration	100 mM PBS, pH 7.2	5	6
Sample Load	IgG1 mAb	Loaded to 80% of DBC10%	6
Chase	100 mM PBS, pH 7.2	5	6
Wash 1	20 mM Tris, 1 M NaCl, pH 8.0	5	6
Wash 2	100 mM PBS, pH 7.2	5	6
Elution	100 mM sodium acetate (pH 3.0, 3.5, 4.0, 4.2 & 4.5)	5	6
Strip	100 mM acetic acid, pH 3.0	5	6
CIP	100 mM sodium hydroxide	3 + 15 mins static	6
Equilibration	100 mM PBS, pH 7.2	5	6

FIGURE 9

Recovery/yield (%) from protein A capture of an IgG1 mAb with elution pH 3, 3.5, 4, 4.2 and 4.5.

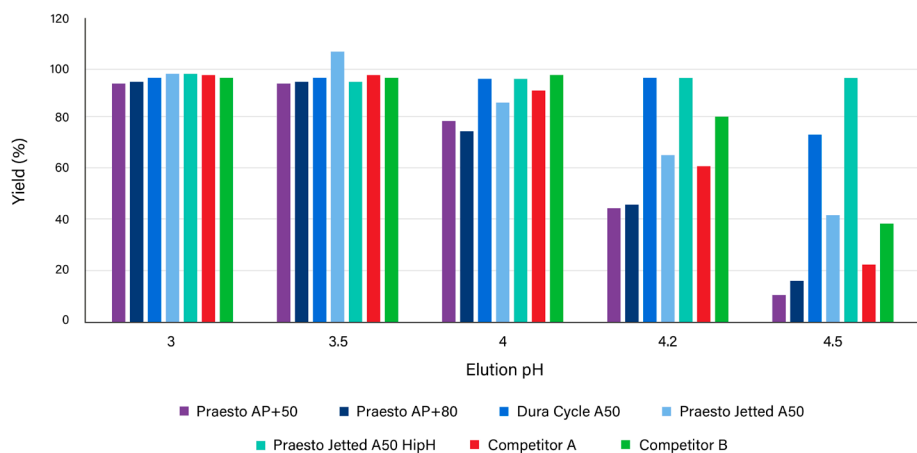


FIGURE 10

Elution volume from protein A capture of an IgG1 mAb with elution pH 3, 3.5, 4, 4.2 and 4.5 (Note: maximum elution CV for phase set at 5 CV).

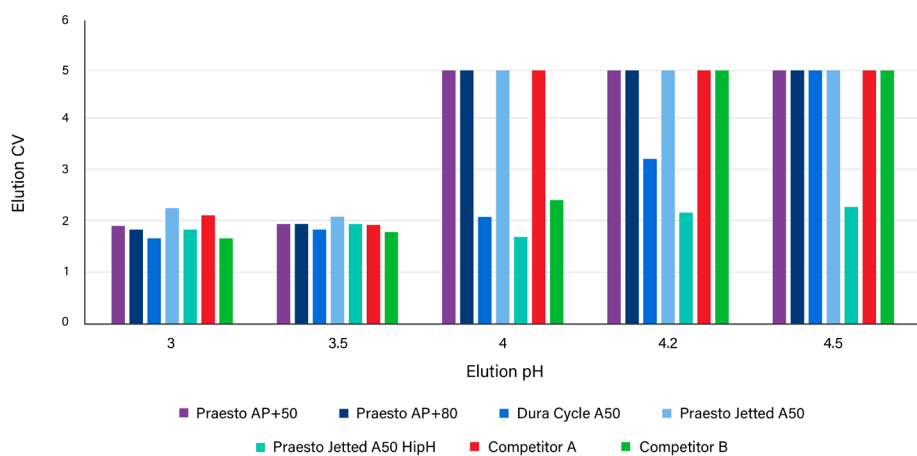


FIGURE 11

Leached protein A from protein A capture of an IgG1 mAb with elution pH 3, 3.5, 4, 4.2 and 4.5 (Note: analysis not performed on samples with less than 70% yield).

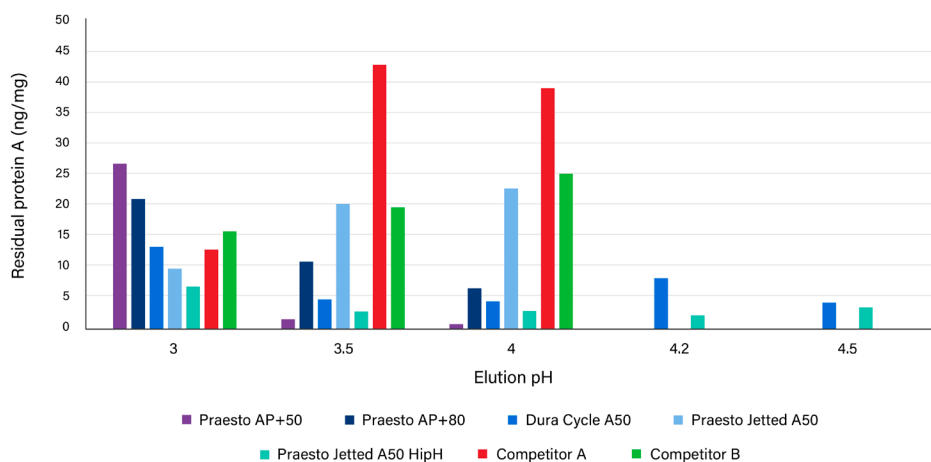
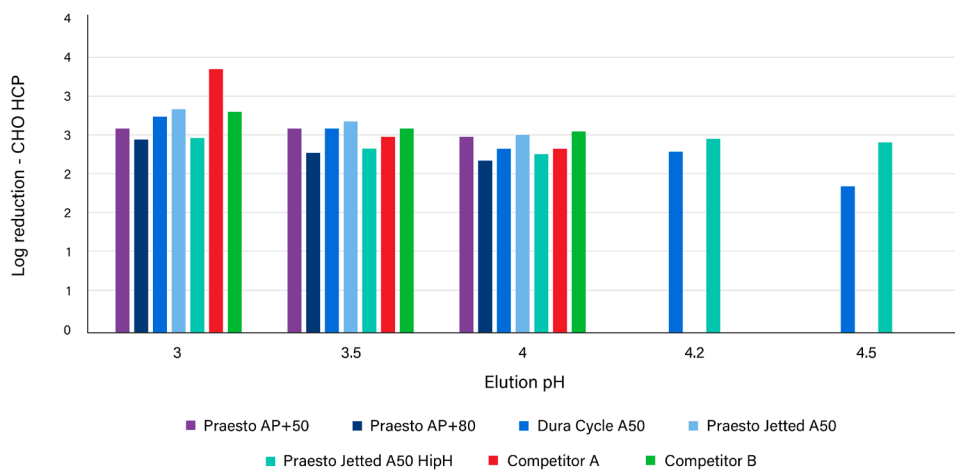


FIGURE 12

Log reduction HCP from protein A capture of an IgG1 mAb with elution pH 3, 3.5, 4, 4.2 and 4.5 (Note: analysis not performed on samples with less than 70% yield).



Consistent Performance

Praesto AP+50 is designed to fit into common purification processes with consistent performance. Herein, we demonstrate performance of Praesto AP+50 using a widely adopted purification protocol. Praesto AP+50 demonstrates equivalent performance in terms of recovery and host cell protein clearance with low levels of ligand leakage.

TABLE 3 Purification protocol for IgG1 HCCF capture with Ecolab protein A resins

Phase	Buffer	Column Volumes (CV)	Residence Time (mins)
Equilibration	Phosphate buffered saline, pH 7.4	5	6
Load	HCCF – IgG1 mAb at 8.5 g/L	Loaded to 80% DBC10%	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 acetic acid, 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 13

Recovery/yield (%) from protein A capture of an IgG1 mAb.

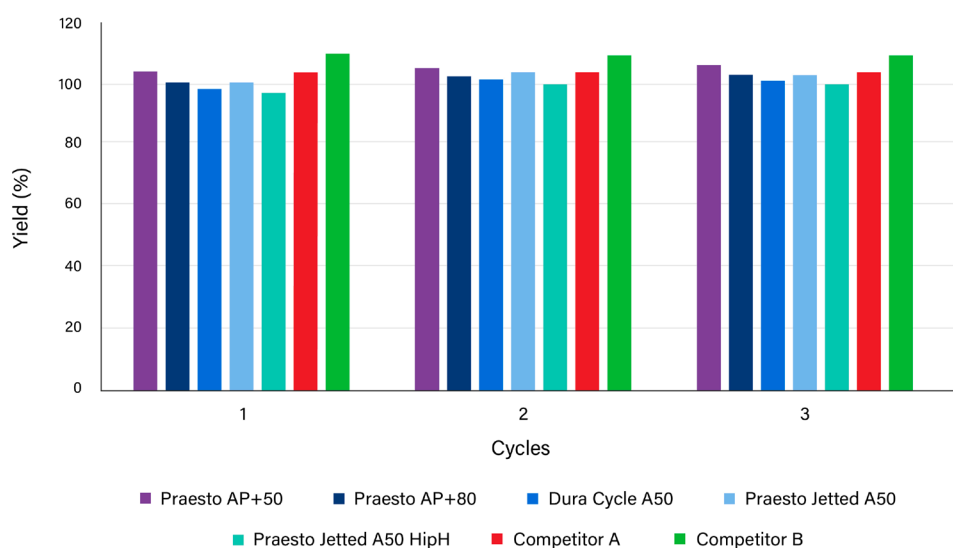


FIGURE 14

Log reduction HCP (%) from protein A capture of an IgG1 mAb.

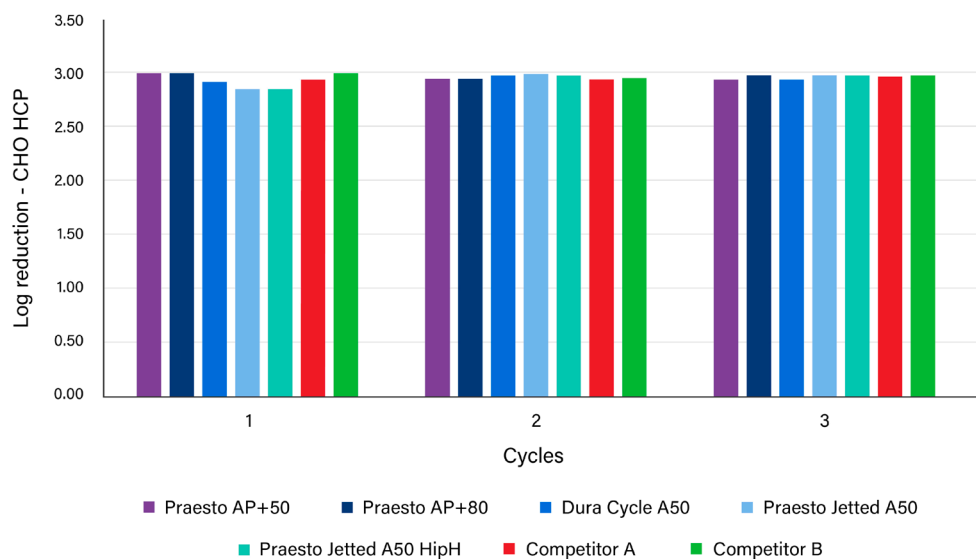
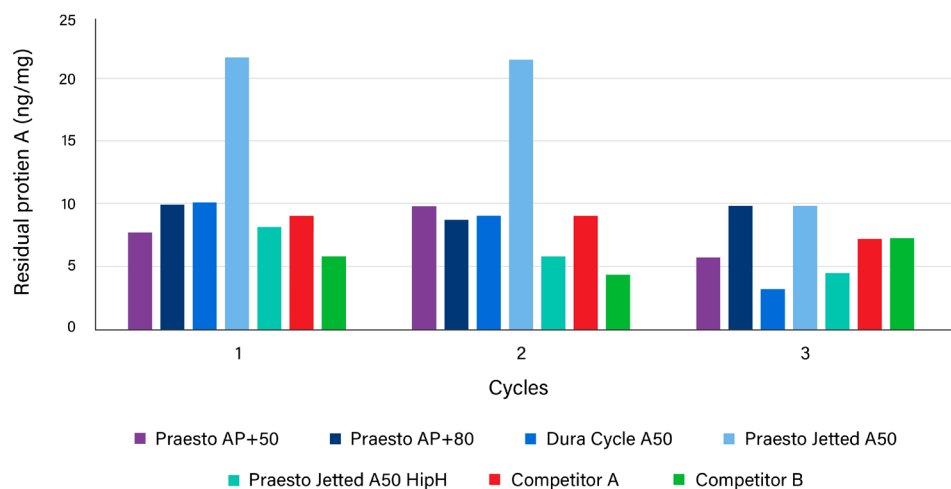


FIGURE 15

Leached protein A from protein A capture of an IgG1 mAb.





Innovative Solutions for Bioprocessing

In partnership with Repligen, Ecolab 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.



Ecolab Affinity Resin Toolbox

Ecolab's diverse toolbox offers Protein A resins, [Praesto Jetted A50](#), [Praesto AP+50](#) 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.



Ecolab 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.

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Notes

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Ecolab is a global developer, manufacturer, and supplier of Purolite™ Resins including ion exchange, catalyst adsorbent, and advanced polymers that make the world cleaner and healthier.



www.puroliteresins.com



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