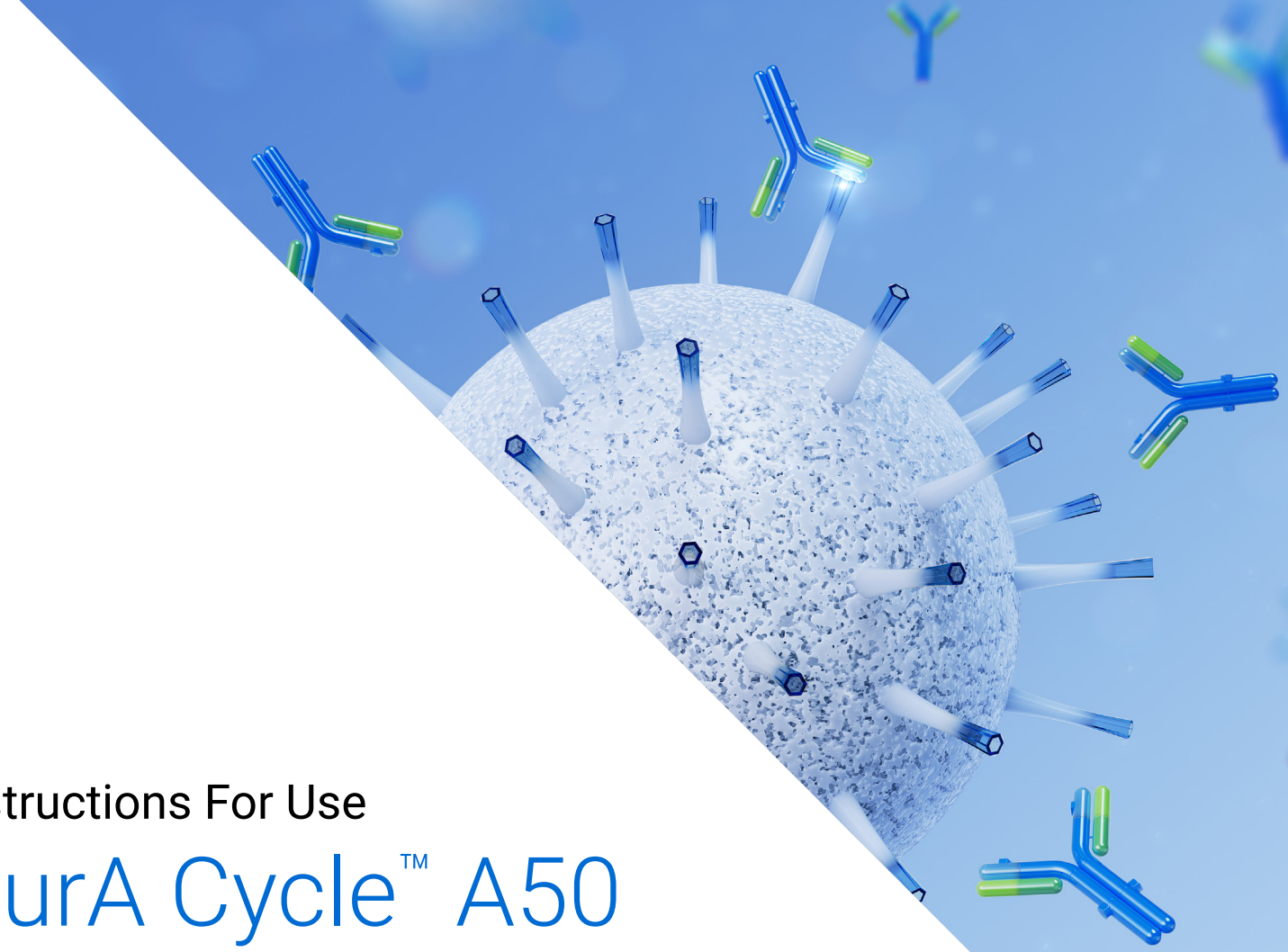


Instructions For Use

DurA Cycle™ A50

ECOLAB®

DurA Cycle™ A50

Contents

Introduction

The first protein A resins were based on protein A antibodies expressed by a pathogenic Staphylococcus aureus strain. Since then, the manufacturing of monoclonal antibodies has grown tremendously and is now the most important group of molecules in the pharmaceutical industry.

Alongside this development, protein A resins have been significantly improved 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.

DurA Cycle A50 is the latest generation protein A resin, designed for the purification of a wide range of mAbs and related constructs. DurA Cycle A50 has been designed with high capacity, improved stability, low protein A leakage, and innovative pH elution in mind.

DurA Cycle A50 is part of the Purolite™ bioprocessing resin family based on highly cross-linked agarose, the bead rigidity allows for process and column design flexibility that can be optimized for capacity utilization and economic performance.

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 mAb, Fab or fragments, are unique, and will differ both in chemical and physical characteristics. Optimization of washing and elution steps are important in the development stage.

All Purolite 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 DurA Cycle A50. 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. DurA Cycle A50 binds to both the Fc and VH domains as with other protein A resins.

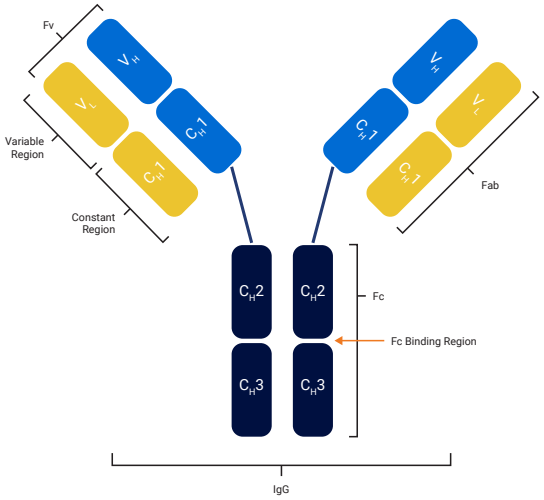


TABLE 1 Product Specifications

Product Characteristics	DurA Cycle A50
Matrix	Highly cross-linked agarose
Dynamic Binding Capacity	Up to 80 mg hIgG/mL resin
Jetted 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 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 a diffusion-driven process. 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 6–8 minutes for DurA Cycle A50 when using a 20 cm bed height, due to the 50-micron bead size.

Higher residence times can be used with shorter bed heights, as demonstrated in Figure 3.

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 DurA Cycle A50*

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 (DBC)	N/A
Intermediate Wash 1	20 mM Sodium phosphate, 0.15 M NaCl, pH 7.0–7.4	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 and Regeneration	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, 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.

Figure 3 shows operational flows at a range of bed heights with DurA Cycle A50*. 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 DurA Cycle A50 over a range of residence times.

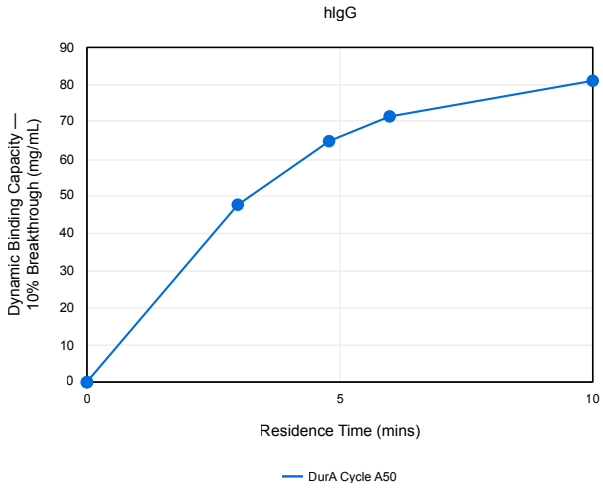


FIGURE 3

Expected operational flow window for DurA Cycle A50*, generated at 30 cm inner diameter.

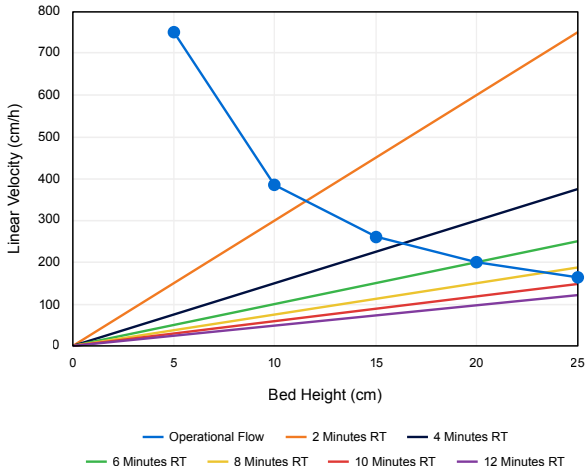


FIGURE 4

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

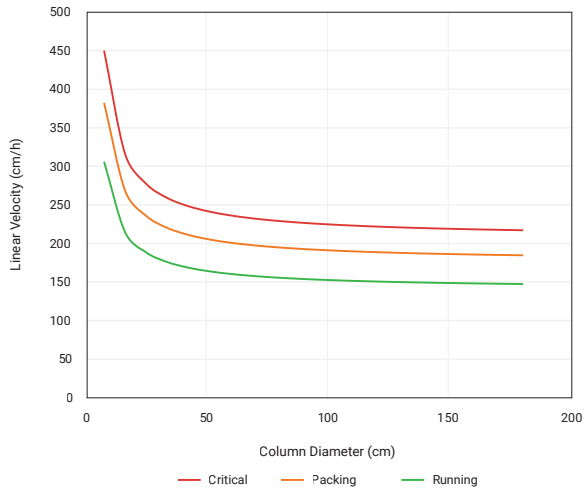


FIGURE 5

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

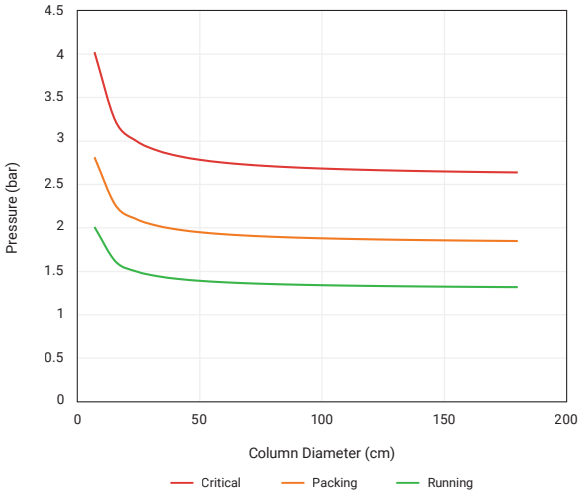


TABLE 3 Recommended packing factor and compression factors for use at 20 cm bed height with DurA Cycle A50*.

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 versus flow for DurA Cycle A50* 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.

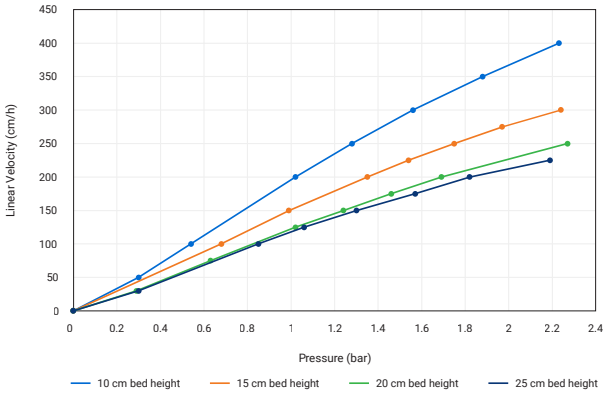


FIGURE 7

Pressure versus flow for DurA Cycle A50* at 60 cm inner diameter with bed heights of 12, 15 & 20 cm. Packed by axial compression in an AxiChrom 600 column.

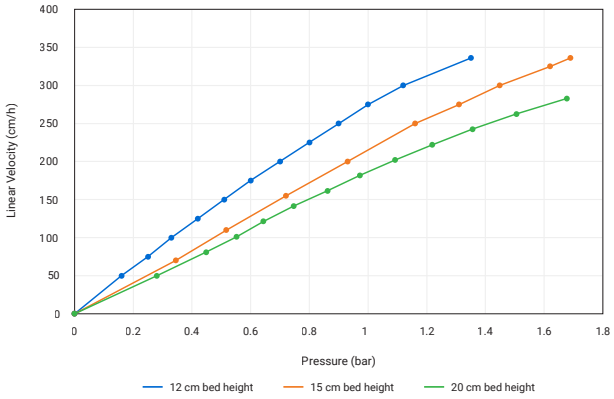


TABLE 4

Pressure versus flow data for DurA Cycle A50* 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.

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

TABLE 5 Pressure versus flow data for DurA Cycle A50 at 60 cm inner diameter with bed heights of 12, 15 & 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 during test*

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

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.

DurA Cycle A50 ligand engineering allows for milder pH elution for non-VH3 containing molecules.

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.

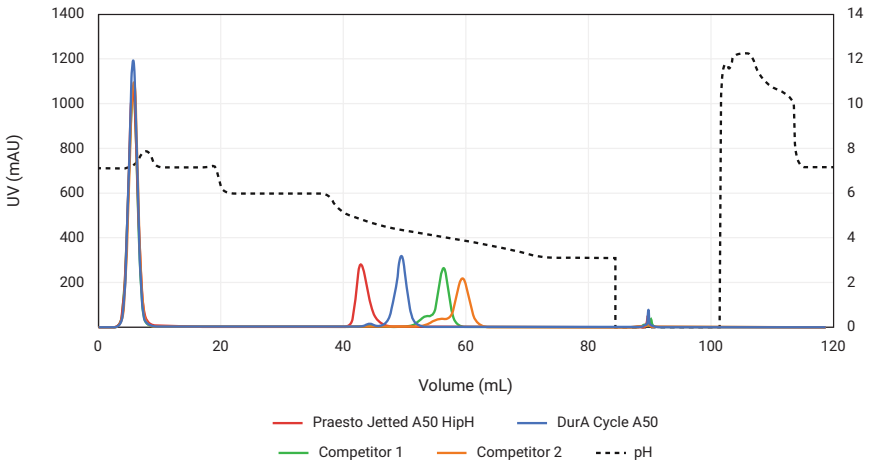
Buffers containing A: 50 mM Citrate, pH 7.0, and B: 50 mM Citrate, pH 3.0, can be used for gradient evaluation. If running a gradient is not possible, a pH of 3.6–4.2 is a good starting point for screening.

TABLE 6 Suggested Protocol for Determining Maximum pH Elution

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

FIGURE 8

Elution pH determination for an IgG1 mAb on DurA Cycle A50, Praesto™ Jetted A50, and Praesto Jetted A50 HipH using a gradient elution from pH 6–3.



Storage

It is recommended that DurA Cycle A50 should be stored between 2–8 °C in 20% Ethanol or 2% Benzyl alcohol. 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

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 DurA Cycle A50 can be analyzed using the commercially available ELISA kit — NGL-Impact® A DurA Affinity Ligand ELISA Kit UG 9-EL-0060.

Residual protein levels can be effectively removed through the use of Praesto anion exchange and Praesto cation exchange resins.

FIGURE 9

Representative protein A levels — Determined using an IgG4 clarified harvest batch column chromatography format.

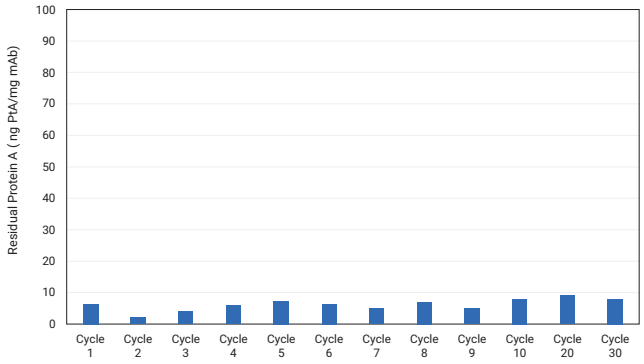
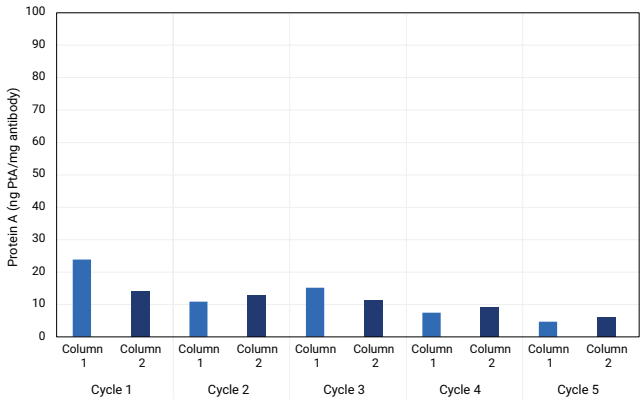


FIGURE 10

Representative protein A levels — Determined using an Fc-fusion protein in a two-column continuous chromatography format.



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.

0.1 M sodium hydroxide (NaOH) 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. Higher concentrations of sodium hydroxide may be used between campaigns and when more substantial cleaning is required. DurA Cycle A50 is alkaline stable for over 60 hours of exposure to 0.5 M NaOH, and over 300 hours of exposure to 0.1 M NaOH.

To extend lifetime of DurA Cycle A50, a combination of 0.1 M NaOH and 0.5 M NaOH cleaning can be applied depending on the nature and challenge of the material applied.

FIGURE 11

Dynamic binding capacity determination after 20 hour incubations in 0.1 M NaOH — tested using polyclonal hIgG in a 0.66 x 10 cm column.

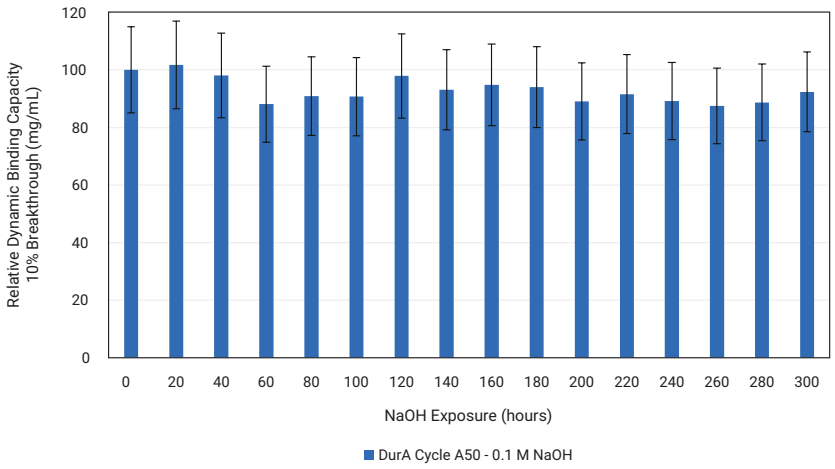
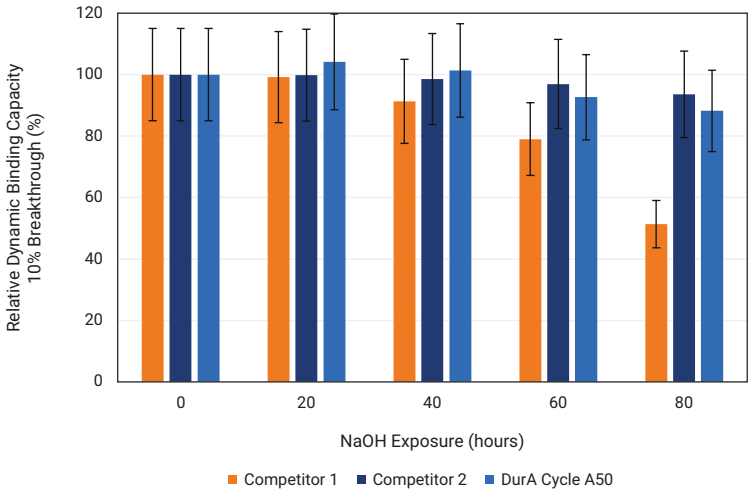


FIGURE 12

Dynamic binding capacity determination after 20 hour incubations in 0.5 M NaOH — tested using polyclonal hIgG in a 0.66 x 10 cm column.



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 (Wash 2)

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.

Conclusion

This instruction provides a starting point for protein A purification using DurA Cycle A50. 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
DurA Cycle A50 — 25mL	PR00555-166
DurA Cycle A50 — 100mL	PR00555-164
DurA Cycle A50 — 500mL	PR00555-165
DurA Cycle A50 — 1L	PR00555-310
DurA Cycle A50 — 5L	PR00555-311
DurA Cycle A50 — 10L	PR00555-312
DurA Cycle A50 (1mL) MiniChrom Columns	PR00555-175
DurA Cycle A50 (5mL) MiniChrom Columns	PR00555-176
DurA Cycle A50 (200uL) RoboColumn Column	PR00555-174
DurA Cycle A50 (600uL) RoboColumn Column	PR00555-279
DurA Cycle A50 (1mL) HT Column	PR00555-275
DurA Cycle A50 (5mL) HT Column	PR00555-276

**Performance data at scale was executed with an un-coupled, 50µm 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.*

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.

PuroliteResins.com

We're ready to solve your process challenges.

For further information on products and services, visit PuroliteResins.com or complete a Contact Us form via PuroliteResins.com/contact-us or use the QR code.

Contact Us Form:

P-000142-100PP-60324-ENG-R1-BP