

APPLICATION NOTE

Design and Optimization of a Three-Step Monoclonal Antibody Purification Workflow to Improve Yield and Reduce Aggregation

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Abstract

A biopharmaceutical organization was experiencing unacceptable levels of protein aggregation (greater than 30%) in their existing downstream purification process, which relied on legacy Protein A chromatography resins requiring low pH elution. These harsh conditions were denaturing the target protein and generating high molecular weight aggregates, compromising product quality and yield and rendering the process uneconomical.

In response Ecolab developed a complete replacement 3-step chromatographic purification process using Praesto™ JA50 HipH, Praesto Q65, and Praesto SP65 resins. By leveraging the unique high-pH elution capability of HipH resin technology, combined with optimized anion and cation exchange polishing steps operated in flowthrough mode, the new process achieved:

- 41% improvement in overall process yield (from approximately 42% to 59%)
- 97% reduction in aggregate levels (from greater than 30% to less than 1%)
- Greater than 99% final monomer purity
- Effective clearance of residual HCP and DNA impurities

This application note details the systematic development of each purification step and demonstrates the superior performance of Ecolab resins in delivering a robust, high-yielding downstream process.

The Legacy Process

The organization developing the monoclonal antibody drug molecule had tried to implement an existing 3-step purification process to purify the target but had achieved limited success. A number of issues existed with the process that rendered the workflow uneconomical, as outlined below.

The existing Protein A capture step produced greater than 30% high molecular weight aggregates in the eluate. This was the single most critical quality issue in the downstream process, threatening both product efficacy and regulatory compliance. The root cause of this aggregation issue was the low pH elution required of legacy Protein A resins, typically in the range of pH 3.4–3.8. Under these acidic conditions, many antibodies undergo partial unfolding, exposing hydrophobic regions that promote intermolecular association and aggregate formation. The lower the elution pH, the more significant the denaturation and aggregation risk.

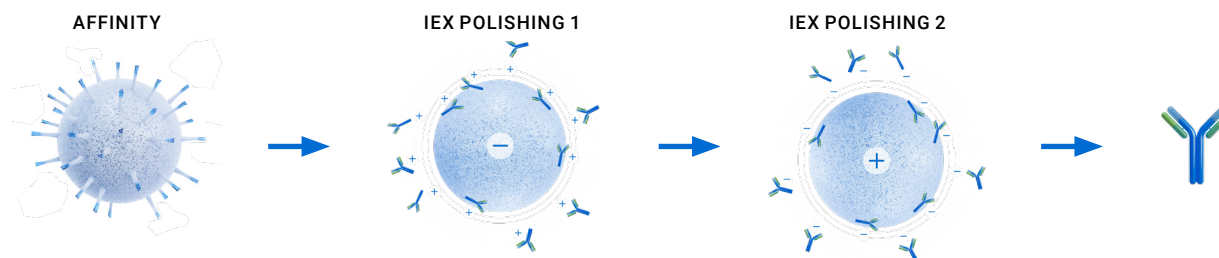
The overall legacy process yield of the 3-column method was approximately 42%, meaning more than half of the valuable target protein was lost during purification. Aggressive downstream polishing was required to remove aggregates, further reducing recoverable product. This made the process and therefore the drug molecule uneconomical to produce.

Previous work had determined that incremental optimization of the legacy resins could not solve the core problem – what was needed was a Protein A resin capable of eluting at significantly milder pH to prevent aggregate formation at the source, paired with efficient polishing steps to achieve final product specifications.

Ecolab, through BPAL (Bioprocessing Applications Laboratory in US), redesigned each step in the legacy 3-step process in an attempt to achieve final product specifications that would be suitable for economically viable development of the molecule. A schematic of the proposed process to be developed and optimized is shown in Figure 1.

Figure 1

Schematic of proposed 3-step purification process.



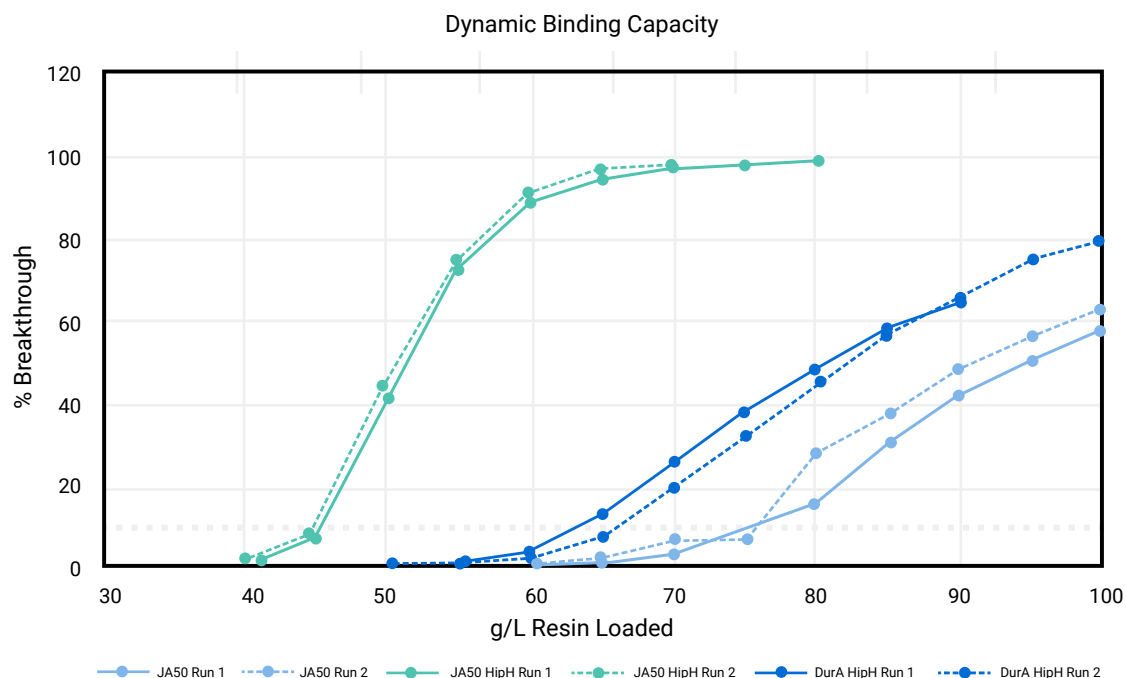
Each step of the process was then developed and assessed against the goal of improved process yield with reduced aggregation.

Step 1 – Affinity Chromatography.

To develop a new affinity capture step, three Ecolab Protein A Purolite resins were screened using high-throughput methods: DurA Cycle A50, Praesto Jetted A50, and Praesto Jetted A50 HipH. Dynamic binding capacity at 10% breakthrough was 43 g/L for HipH, 71 g/L for JA50, and 68 g/L for DurA A50, as shown in Figure 3.

Figure 2

Dynamic binding capacity data for the three affinity resins tested.



Of the three resins tested, Praesto Jetted A50 HipH resin had the lowest DBC as shown in table 1.

TABLE 1 DBC at 10% breakthrough for the three resins.

Resin	10% DBC in mg/ml
JA50 Run 1	71
JA50 Run 2	68
JA50 HipH Run 1	43
JA50 HipH Run 2	42
DurA A50 Run 1	68
DurA A50 Run 2	66

However, despite this lower DBC, Praesto Jetted A50 HipH resin offered a significant advantage as shown in Table 2. Gradient elution from pH 6 to pH 3 showed that the resin elutes at a dramatically higher optimal pH range of 4.0–5.0, compared to pH 3.8–4.2 for DurA Cycle A50 and pH 3.4–3.8 for Praesto Jetted A50. This higher pH elution is the critical differentiator that prevents protein denaturation and aggregate formation. Isocratic elution studies showed that purity and aggregate formation could be tuned by elution pH – at pH 4.0, yield reaches 95.0% with 94.0% purity. At pH 4.5, yield is 77.9% with 98.0% purity. At pH 5.0, yield is 51.3% with 98.9% purity. The optimal pH from this study was pH 4.5, delivering an excellent balance of yield and purity with minimal aggregates. In contrast, Praesto Jetted A50 at pH 3.6 achieved 96.8% yield but only 86.0% purity with 14% aggregates, whereas DurA Cycle A50 gave results in the mid-ground between the two other resins. Praesto Jetted A50 HipH was selected for the process at an elution pH of 4.5 because, as outlined, aggregate reduction was the primary objective of the development process.

TABLE 2 Summarized results for the three resins evaluated for the affinity capture step as a function of elution pH, with selected affinity step highlighted in bold.

Resin	Elution Conditions	% Yield	% Purity
Praesto Jetted A50	pH 3.4	97.40	86.14
	pH 3.6	96.77	86.00
	pH 3.8	91.90	85.97
Praesto Jetted A50 HipH	pH 4.0	95.00	94.00
	pH 4.5	77.94	98.01
	pH 5.0	51.26	98.9
DurA Cycle A50	pH 3.8	98.96	90.89
	pH 4.0	98.41	90.95
	pH 4.2	98.33	91.89

Seven runs on a 15.16 mL JA50 HipH column at slightly adjusted conditions (pH 4.4 elution) confirmed robust performance: average yield of 86.2%, average purity of 95.0% by SEC, and average rDNA of 149 copies/ μ L. The material generated was used for downstream IEX development.

Praesto Jetted A50 HipH

The first mild-elution pH affinity capture resin on the market, Praesto Jetted A50 HipH has several properties that render it particularly appropriate for aggregation prone molecules.

Dramatically higher elution pH (4.0–5.0)

Praesto Jetted A50 HipH resin technology enables effective protein elution at pH 4.0–5.0, a full unit higher than conventional Protein A resins (pH 3.4–3.8). This is achieved through proprietary modifications to the Protein A ligand that weaken antibody binding while maintaining strong capture at neutral pH.

Prevention of acid-induced aggregation

The higher elution pH means the antibody remains in its native conformation during elution, avoiding the acid-induced unfolding that leads to aggregate formation.

Reduced burden on downstream polishing

Because the Protein A eluate already contains minimal aggregates, the downstream AEX and CEX steps do not need to perform aggressive aggregate removal. This enables the use of modes which maximize yield. The entire downstream process becomes simpler, more efficient, and higher-yielding.

Tunable yield-purity trade-off

HipH provides flexibility to tune elution pH within the 4.0–5.0 range. Process developers can select the optimal balance for their specific product requirements.

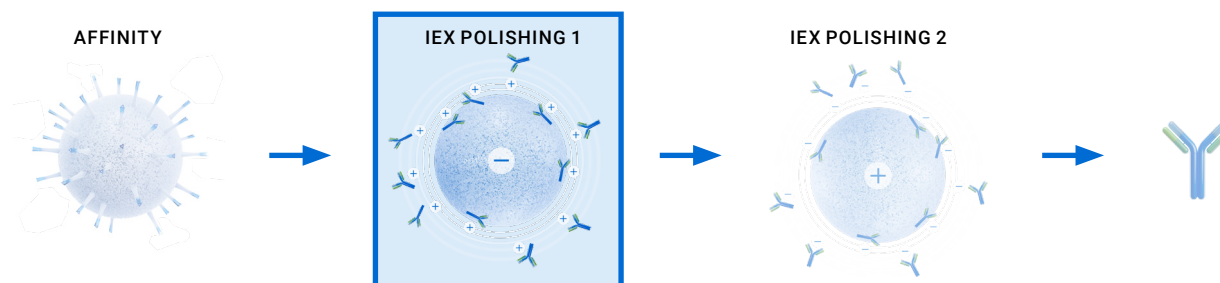
Improved product quality and stability

By eliminating acid-induced conformational damage at the capture step, Praesto Jetted A50 HipH resin produces a higher quality intermediate that retains its native structure throughout downstream processing. This translates to better final product stability, reduced particulate formation, and a stronger regulatory filing position.

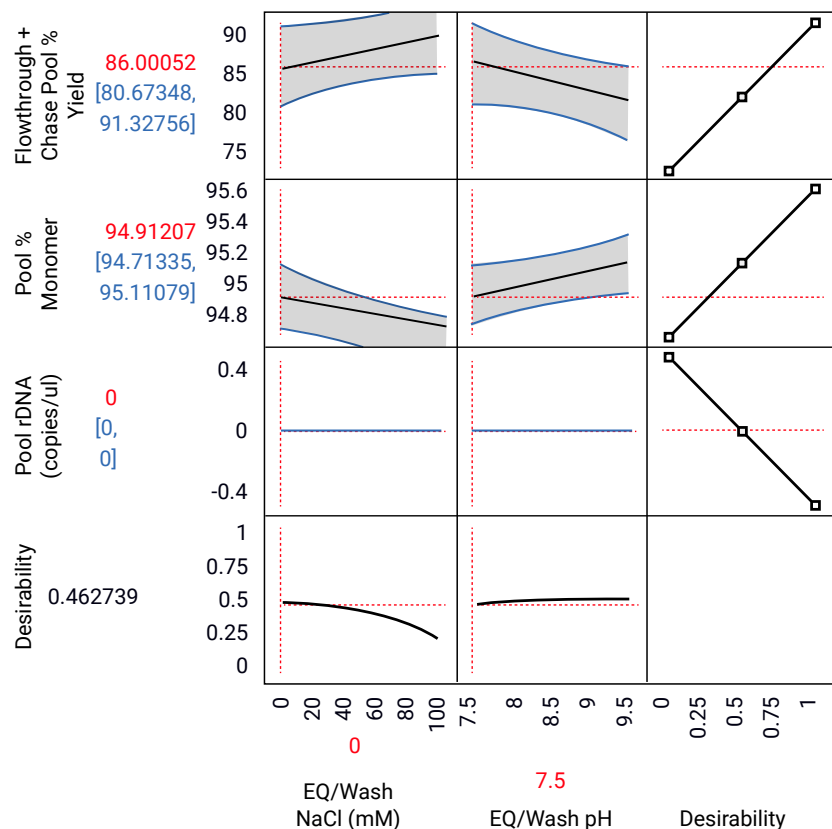
Step 2 – Anion Exchange Polishing

Figure 3

Schematic of monoclonal antibody purification highlighting the first polishing step post-affinity with anion ion-exchange resin.



After the affinity capture step, anion ion exchange was implemented as the first of two polishing steps for further impurity removal. Ecolab Praesto Q65 anion exchange resin was evaluated in flowthrough mode using a 16-run high-throughput screen on a Tecan Fluent robotic platform. The screen explored equilibration/wash buffer pH (7.5–9.5) and NaCl concentration (0–100 mM) to identify conditions that maximize impurity clearance while maintaining high product recovery. An example of the output of the multivariate analysis performed is shown in Figure 5. In the figure, desirability analysis integrates the individual responses to identify operating conditions that balance yield preservation with robust impurity clearance, supporting selection of mild, low risk flowthrough conditions.

**Figure 4**

Multivariate response analysis of flowthrough anion exchange (AEX) screening on Q65 resin. Response surfaces illustrate the effect of equilibration/wash buffer sodium chloride concentration and pH on pooled flowthrough yield, monomer purity (by SEC), residual rDNA content, and overall desirability. Black lines indicate predicted trends, grey shading represents confidence intervals, and red dashed lines denote target performance thresholds.

The results of the analysis show excellent DNA clearance across all conditions. Residual DNA was reduced to 0 copies/ μ L across every condition tested, demonstrating the robust DNA binding capacity of Praesto Q65 resin even in flowthrough mode. This provides high confidence in DNA clearance regardless of the specific operating conditions selected.

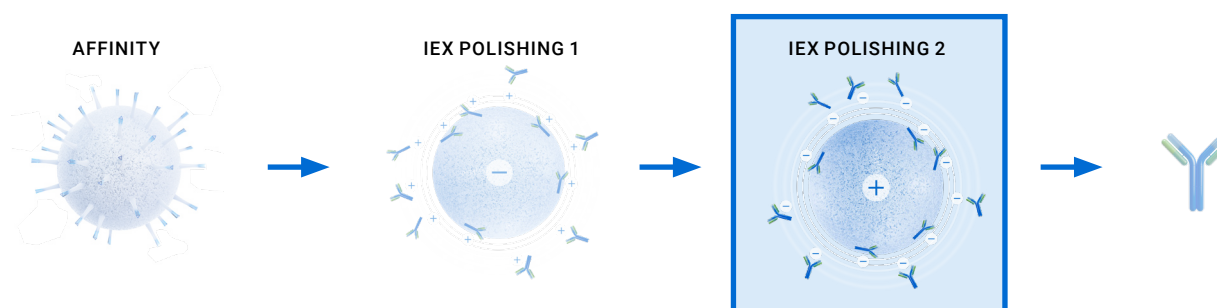
Based on the screening data, the optimal AEX conditions were determined to be 50 mM Tris pH 7.5. Salt concentration had minimal impact on performance, and aggregate clearance was similar across most conditions (approximately 5% aggregates in the flowthrough). At pH 9.5, some additional aggregate clearance was observed but at the cost of significant yield loss, making this approach unfavorable.

At lab scale on a 3.42 mL Q65 column loaded at 100 g/L resin density, the AEX step delivered 98.6% yield with 96.48% monomer purity, while reducing rHCP from 394 to 283 ppm and completely eliminating residual DNA. The flowthrough mode eliminates the need for gradient elution development, simplifying the process and maximizing throughput.

Step 3 – Cation Exchange Polishing

Figure 5

Schematic of monoclonal antibody purification highlighting the second polishing step post-affinity with cation ion-exchange resin.



A similar approach was used to develop the final step in the process, the second cationic polishing step. Ecolab Praesto SP65 cation exchange resin was initially evaluated in traditional bind-and-elute mode. A 32-condition binding screen identified optimal binding at pH 5.0 with 0 mM NaCl, and a 16-run elution screen determined the best elution at 50 mM Tris, 100 mM NaCl, pH 6.0. In bind-and-elute mode, CEX achieved 83% yield with 94.79% monomer purity (4.92% aggregates).

During the binding screen, analysis of flowthrough fractions at pH 7.4 (unadjusted load) revealed an unexpected finding: the flowthrough contained protein at 68% yield with exceptional 99.19% monomer purity and only 0.81% aggregates. The aggregates and impurities were preferentially binding to the resin while the monomer passed through. Table 3 summarizes the data from both bind and elute and flowthrough cation polishing.

TABLE 3 Summary of results from cation polishing using bind and elute or flowthrough mode.

Run Type	Load pH	% Yield	% Aggregates	% Monomer
Bind and Elute	5	83	4.92	94.79
Flowthrough	7.4	68	0.81	99.19

Although flowthrough yield (68%) was lower than bind-and-elute (83%), the dramatic improvement in purity (99.19% vs. 94.79%) made it the clear choice. The primary concern was aggregate clearance, and flowthrough CEX on SP65 delivered a final product with less than 1% aggregates, well below the specification threshold. The flowthrough CEX step also reduced rHCP from 283 ppm to 83 ppm, providing an additional 3.4-fold HCP reduction on top of the clearance already achieved by Protein A and AEX. Combined with complete DNA elimination at the AEX step, the final product meets stringent impurity specifications.

The Complete End-to-End Process

The full 3-column process was run end-to-end: Protein A capture on Praesto Jetted A50 HipH (15.16 mL column, 34 g/L load, pH 4.5 elution), followed by AEX flowthrough on Praesto Q65 (3.42 mL column, 100 g/L load, 50 mM Tris pH 7.5), then CEX flowthrough on Praesto SP65 (3.42 mL column, 13 g/L load, 50 mM Acetate pH 5.0 equilibration, load at pH 7.2). The results are summarized in Table 4.

TABLE 4 Summary of the results for the complete end-to-end purification workflow.

Resin	Load Material	Load pH	% Yield	% Aggregates	% Monomer	rHCP (ppm)	rDNA (copies/ul)
Feed	n/a	n/a	n/a	n/a	n/a	1147929.9	>1000000
Affinity capture – Praesto Jetted A50 HipH	HCCF	7.57	76.4	3.72	96.28	394.3	149
First polishing – Praesto Q65 flowthrough	Neutralized VI	7.49	98.6	3.53	96.48	283.3	0
Second polishing – Praesto SP65 flowthrough	AEX FT diluted 1:1 with EQ buffer	7.21	78.7	0.78	99.22	83.2	0

Step-by-step yield was as follows: Protein A (JA50 HipH): 76.4% yield, 96.28% purity. AEX (Q65 flowthrough): 98.6% yield, 96.48% purity. CEX (SP65 flowthrough): 78.7% yield, 99.22% purity. The AEX flowthrough step is notably efficient, losing only 1.4% of product while eliminating all residual DNA. The cumulative yield of the 3-column Ecolab process is 59.3%, representing a 41% improvement over the legacy process yield of approximately 42%. This improvement is driven by the ability to use mild flowthrough polishing steps rather than aggressive bind-and-elute chromatography for aggregate removal.

Final product aggregates were reduced from greater than 30% to 0.78%, representing a 97% reduction in aggregate levels. The final product was 99.22% monomer by SEC, with rHCP reduced to 83 ppm and rDNA at 0 copies/ μ L. These results demonstrate a complete solution to the aggregation challenge.

For applications where yield is prioritized over purity, a bind-and-elute CEX option was also validated, delivering 75.6% overall process yield with 96.08% final purity. This demonstrates the flexibility of the Ecolab platform to be optimized for different product requirements.

Conclusion

This application note demonstrates how Ecolab's chromatography resin portfolio – anchored by Praesto Jetted A50 HipH Protein A resin – can solve critical aggregation challenges in downstream bioprocessing. By replacing a legacy 3-column purification process with an optimized Ecolab process, transformative improvements were achieved in both product quality and process economics.

- Praesto Jetted A50 HipH enables high-pH elution (pH 4.0–5.0), eliminating acid-induced aggregation at the source
- Clean Protein A eluate enables efficient flowthrough AEX (Praesto Q65) and CEX (Praesto SP65) polishing
- Overall yield improved by 41% (from ~42% to 59.3%) compared to the legacy process
- Aggregates reduced by 97% (from >30% to 0.78%), achieving >99% monomer purity
- rHCP and rDNA effectively cleared to meet stringent quality specifications
- Flowthrough polishing simplifies process development and scale-up

The Ecolab chromatography resin platform provides a comprehensive toolkit for biopharmaceutical manufacturers seeking to overcome aggregation challenges and improve downstream process efficiency. Contact your Ecolab representative to discuss how Praesto Jetted A50 HipH and our complete resin portfolio can optimize your purification process.

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