TOSOH

Process Optimization for High-Purity mAb Recovery

Your Challenge

- You are seeing high HCP and low recovery after high-load mAb capture on Protein A resin.
- The standard Protein A purification protocol isn't giving you the results you want.

Our Solution

TOYOPEARL® Super A resin

- Versatility to diverse process conditions

What was done?

- Optimized wash and elution steps to boost recovery and reduce impurities in mAb capture.

What was the result?

- Process adjustments resulted in up to 99% recovery and up to 2000-fold reduction in host cell protein.

TOYOPEARL Super A enables high recovery, low HCP, and minimal Protein A leaching, offering a robust, flexible platform for efficient monoclonal antibody purification.

Your Benefit

Enhanced recovery of pure mAb after process optimization, improving process economics and productivity.

Purification of monoclonal antibodies

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Application Note

TOYOPEARL® Super A Dynamic Binding Capacity and Load, Wash, Elution Condition Optimization

TOYOPEARL Super A is a next-generation recombinant Protein A affinity chromatography resin from Tosoh Bioscience. It has several notable features, such as improved capacity, enhanced alkaline stability—withstanding up to 1 mol/L NaOH clean-in-place (CIP), elution at milder pH, and low elution volume. Here, we also demonstrate that it has high dynamic binding capacity (DBC), high host cell protein (HCP) clearance, and low Protein A ligand leaching. It comes in convenient pre-packed SkillPak™ columns, including sizes specially designed for multi-column chromatography, as well as bulk resin. TOYOPEARL Super A products meet all scales of bioprocessing needs from development and process optimization to plant-scale manufacturing. It is animal-origin-free and certified low-endotoxin (≤ 10 EU/mL).

Since each monoclonal antibody (mAb) is unique, process development is recommended in order to achieve the best performance with TOYOPEARL Super A resin. Variations in mAb characteristics such as sequence, expression level, or stability (for example, pH tolerance or aggregation tendency) may influence purification results. Tailoring the purification method for a particular molecule ensures that maximum recovery and purity are achieved. The adaptability of TOYOPEARL Super A to a variety of process conditions opens up numerous possibilities for method modifications at any phase.

This report details an example workflow of optimizing process purification steps on a TOYOPEARL Super A pre-packed column for a model monoclonal antibody, trastuzumab. DBC as a function of sample load concentration and residence time (RT) was also determined.

Dynamic Binding Capacity

Resin Characteristics

Resin	TOYOPEARL Super A
Mean particle diameter	45 µm (F-grade)
Mean pore diameter	100 nm
Base matrix	Hydroxylated methacrylic polymer
Ligand	Modified, code-optimized C domain, hexameric ligand with multipoint attachment
Caustic stability	Up to 1 mol/L NaOH

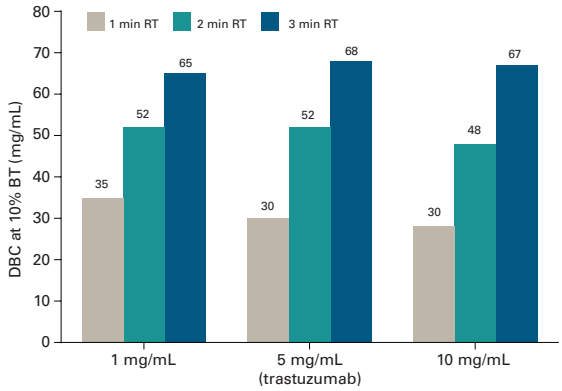
Method

Column:	SkillPak 5 TOYOPEARL Super A
Column dimensions:	0.8 cm ID x 10 cm BH = 5.0 mL
Mobile phase:	20 mmol/L sodium phosphate, pH 7.4, 150 mmol/L NaCl
Sample:	Trastuzumab at 1, 5, 10 mg/mL in mobile phase
Residence times:	1, 2, 5 min

Result

Purified trastuzumab was loaded onto SkillPak 5 TOYOPEARL Super A until 10% breakthrough was observed. Three sample concentrations and three RTs were tested. The result is shown in *Figure 1*.

Figure 1. DBC at 10% breakthrough (BT) reported in mg/mL resin for three RTs and three sample concentrations of trastuzumab monoclonal antibody.



RT had the largest impact on binding capacity, with longer RT resulting in greater binding. TOYOPEARL Super A had an average DBC of 67 mg/mL across all three sample concentrations at 5 min RT. Titer of mAb in the sample load did not significantly impact capacity.

Process Optimization

As is standard with conventional affinity chromatography workflows, the familiar bind–wash–elute sequence was employed; this served as the foundation for method optimization. To ensure pH control and protein stability, eluate fractions were immediately neutralized using 1 mol/L Tris base, preloaded into the collection block at 1/20th of the fraction volume.

Materials and Methods

Column: SkillPak 5 TOYOPEARL Super A
Column dimensions: 0.8 cm ID x 10 cm BH = 5.0 mL
Flow rate: 1.25 mL/min (4 min RT) except equilibration phases—2.5 mL/min (2 min RT)
Sample: CHO cell culture supernatant containing trastuzumab monoclonal antibody, 6.9 mg/mL
Injection vol.: 39.53 mL
Load level: 55 mg/mL resin (≈90% capacity)

Process Conditions

Phase	Buffer	Length
Equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV
Load	Trastuzumab CHO feedstock	
Wash 1	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV
Wash 2	25 mmol/L sodium citrate, pH 5.2 + experimental addition(s)	5 CV
Elution	25 mmol/L sodium citrate, pH 3.2 + experimental addition(s)	5 CV
CIP	0.5 mol/L NaOH	4 CV
Re-equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV

Analytics – mAb titer

mAb titer was quantified using a TSKgel® Protein A-5PW analytical column. This column rapidly (2-minute runtime) and effectively quantifies non-purified mAb in complex matrices (e.g. CHO feedstock) during recovery analysis. UV spectroscopy was used to validate the titer in eluate samples, which were highly consistent with titers obtained from the analytical method.

Analytical Method

Column: TSKgel Protein A-5PW (4.6 mm ID × 3.5 cm L, 20 µm particle)
Mobile Phase: A: 20 mmol/L NaH_2PO_4/Na_2HPO_4, pH 7.4
B: 12 mmol/L HCl
Gradient: 0 min – 0.5 min 0% B
0.51 min – 2.0 min, 100% B (step elution)
Flow rate: 2.0 mL/min
Injection vol.: 2.5 - 5.0 µL

Analytics – ELISA for HCP and Protein A

HCP concentration was determined with the CHO HCP ELISA Kit, 3G, from Cygnus Technologies. Protein A content in eluate was evaluated using the Tosoh R50, R40 and R28 Protein A Mix-N-Go™ ELISA kit from Cygnus Technologies.

Process Optimization – Wash Step

To simulate biomanufacturing conditions, trastuzumab was loaded to approximately 90% resin capacity (55 mg/mL) during wash step optimization. This high-load scenario reflects typical production environments where maximizing resin utilization is critical for throughput. Under such conditions, HCP levels are proportionally elevated, presenting a greater purification challenge. The objective was to evaluate the performance of TOYOPEARL Super A resin under realistic loading conditions and identify wash buffer modifications that effectively reduce HCP content.

Wash buffer additives

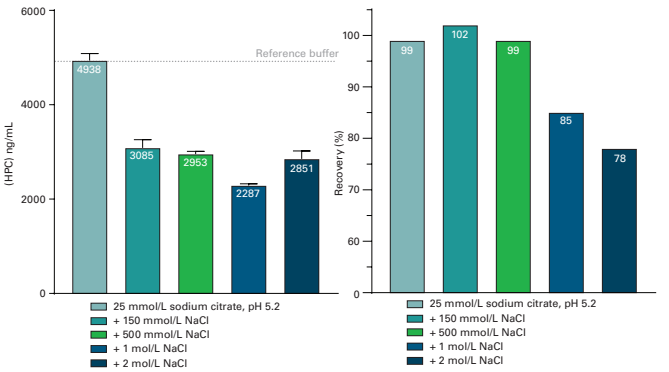
Two wash buffer additives, NaCl and arginine-HCl, were investigated for wash step enhancement. NaCl should prevent non-specific, charge-based interactions; arginine is a gentle chaotrope that reduces aggregation and may disrupt binding of HCPs to the column.

Different concentrations of the additives for the Wash 2 step were tested, measuring recovery and HCP reduction in the eluate relative to the reference wash buffer.

Wash buffer additives – Results

The method used was as previously described, except different concentrations of NaCl were included in the Wash 2 buffer (25 mmol/L sodium citrate, pH 5.2). We tested 150, 500, 1000, and 2000 mmol/L NaCl. The result is shown in *Figure 2*.

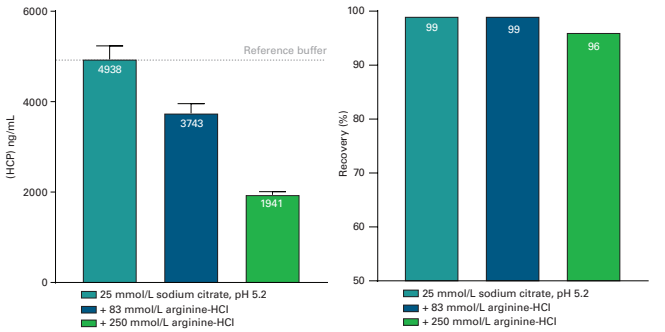
Figure 2. Left panel: HCP concentration in eluates with varying NaCl concentrations in the second wash step, shown as a mean of 4 ELISA replicates (except 150 mmol/L condition, in which n=2), with standard deviation shown in error bars. Right panel: Recovery of mAb in eluates after including varying NaCl concentrations in the second wash step. Recovery was determined by quantifying mAb concentration in feedstock and eluate using TSKgel Protein A column, and dividing mAb mass in the eluate by the mAb mass loaded.



Including NaCl in the wash buffer at 150 mmol/L provided the best balance between recovery and HCP reduction. 500 mmol/L was also acceptable and had comparable performance, but we wanted to include the minimum concentration of additive necessary to achieve the purity and recovery goals.

Arginine-HCl was also a highly effective additive at reducing HCP at 83 mmol/L and 250 mmol/L compared to sodium citrate alone *(Figure 3)*. We focused on NaCl to simplify further development.

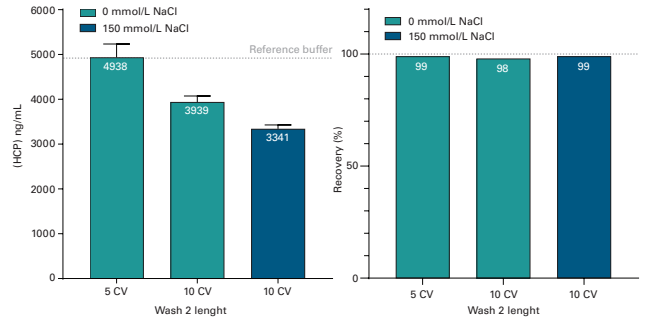
Figure 3. Left panel: HCP concentration in eluates with two arginine-HCl concentrations in the second wash step, shown as a mean of 4 ELISA replicates, with standard deviation shown in error bars. Right panel: Recovery of mAb in eluates after including two arginine-HCl concentrations in the second wash step.



Wash Step Extension

We selected the 150 mmol/L NaCl condition for further development because it preserved recovery and was effective at reducing HCP. We extended the Wash 2 step length from 5 CV to 10 CV to investigate if HCP content could be further reduced by simply increasing the duration. We also compared this condition (10 CV, 150 mmol/L NaCl) to a standard length (5 CV) wash and an extended wash (10 CV) with the reference wash buffer (0 mmol/L NaCl), with the results shown in *Figure 4*.

Figure 4. Left panel: HCP concentration in eluates with either 0 mmol/L NaCl (pink bars) or 150 mmol/L NaCl (blue bar) included in the second wash step, shown as a mean of 4 ELISA replicates, with standard deviation shown in error bars. Wash step duration is indicated on the x-axis. Right panel: Recovery of mAb in eluates for each run.



Increasing the wash step from 5 CV to 10 CV provided further HCP reduction without loss in recovery. This wash buffer composition and length was used in the following elution and load optimization experiments.

Process Optimization – Elution Buffer Additives

The TOYOPEARL Super A runs had high recoveries, around 99%, when loaded to approximately 90% of DBC during the wash optimization studies. We sought an elution condition that might enhance recovery, even at low load levels at which recoveries tend to be lower. For this, we loaded mAb to approximately 10% of binding capacity, where recovery was 78% without additives, and tested arginine-HCl, glycerol, and ethanol as elution buffer additives.

We used arginine because it disrupts protein-protein interactions. We also observed arginine causing mAb leakage in the wash step at 250 mmol/L and above; we hypothesized that the arginine might act similarly if used in the elution step, which would be advantageous for increasing recovery. We tested the addition of arginine-HCl in the elution buffer at 100, 500, and 1000 mmol/L concentrations.

We selected glycerol and ethanol because they alter solvent polarity, which may disrupt hydrophobic interactions between the mAb and the Protein A ligand. These are also relatively safe process reagents, compared to more toxic alternatives such as propylene glycol or isopropanol, which are not favored in biopharmaceutical processes and necessitate extensive removal procedures. For the glycerol and ethanol additives, we preceded the NaOH sanitization step with 5 CV ultrafiltered H_2O to remove the additive. This was to prevent contact between NaOH and the additive, as this may result in unwanted reactive nucleophilic species such as ethoxide ions. For both glycerol and ethanol, concentrations of 5, 10, and 20% (v/v) were tested as elution buffer additives.

Elution Buffer Additives – Results

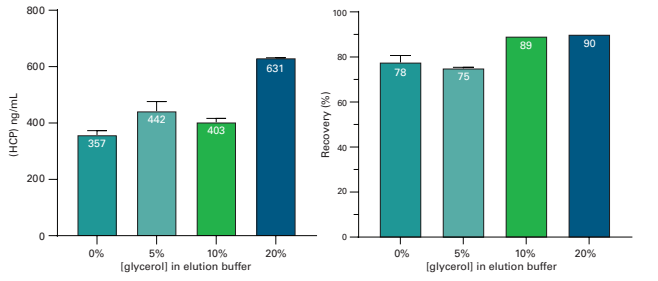
Arginine and Ethanol

Arginine-HCl was a poor elution enhancer that resulted in higher HCP content in the eluate with no benefit to recovery. Ethanol marginally improved recovery (up to 13% with 10% ethanol), but at a severe cost to HCP reduction (nearly 3-fold increase).

Glycerol

Glycerol demonstrated great performance as an elution additive. We observed a 14% increase in recovery when using 10% glycerol, without significantly increasing HCP content *(Figure 5)*.

Figure 5. Left panel: HCP concentration in eluates with various concentrations of glycerol included in the elution buffer, shown as a mean of 2 ELISA replicates (except for 0% glycerol, in which case n=4) with standard deviation shown in error bars. Recovery of mAb in eluates for each run; n=1 except for 0 mmol/L condition, in which n=3 replicate runs, and 5% in which n=2 replicate runs, with standard deviations shown in error bars.



Note regarding operating pressure and glycerol. Glycerol increases elution buffer viscosity, resulting in slightly higher pre-column pressure; nonetheless, it stayed below the maximum operating pressure. Occasionally, high pressure occurred after CIP before re-equilibration after pump and column washes. Including a system wash (bypassing the column) at high flow before re-equilibration restored baseline pressure. Residual glycerol may have remained in the system hardware that did not disperse at low flow rates. No delta-column pressure increase was observed with 10% glycerol.

Process Optimization – Final Conditions

Optimized Process: Changes from reference method highlighted in red

Phase	Buffer	Length
Equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV
Load	CHO feedstock, 6.9 mg/mL trastuzumab	
Injection volume	4.94 mL / 39.5mL	
Load level	6.8 mg/mL resin (≈10%) / 55 mg/mL resin (≈90%)	
Wash 1	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV
Wash 2	25 mmol/L sodium citrate, pH 5.2, 150 mmol/L NaCl	10 CV
Elution	25 mmol/L sodium citrate, pH 3.2, 10% glycerol	5 CV
CIP	Water (downflow)	5 CV
	0.5 mol/L NaOH	4 CV
	Water (upflow)	2 CV
Re-equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV

We performed this optimized process at two different load levels corresponding to approximately 10% and 90% DBC to ascertain performance at optimized conditions at a larger scale. *(Table 1)* Because the elution optimization was carried out with a low load level only, we sought to quantify the performance of finalized conditions with the more production-relevant high load level.

Table 1. Comparative performance of optimized TOYOPEARL Super A method at low and high mAb mass loads.

Load, % of maximum capacity (approx.)	10%	90%
Recovery	89%	97%
[Protein A], ng/mL	19.56	29.11
[Protein A], ppm	2.62	1.04
[HCP], ng/mL	402	7709
[HCP], ppm	54	276
HCP log reduction	3.34	2.06

Processes at both load levels had exceptionally low Protein A ligand leaching, showcasing the stability of the TOYOPEARL Super A ligand. HCP reduction was also extensive, with over 2000-fold decrease observed in the 10% load condition. The balance of purity and recovery in preparative chromatography should be considered, with lower mass loads resulting in higher purity at the cost of lower recovery. The excellent capture performance and contaminant clearance of TOYOPEARL Super A reduces the burden of extensive post-affinity polishing steps, optimizing process economics overall.

Conclusions

TOYOPEARL Super A resin is adaptable to various process conditions, expanding the potential experimental space and allowing for powerful downstream process development.

We identified arginine-HCl and NaCl as effective wash buffer additives, and optimized the wash step to maximize recovery, up to 99%, and effectively reduce HCP up to 2000-fold.

We identified glycerol as an ideal elution-enhancing additive for situations in which high recovery is critical, even at low loads. Using 10% glycerol, we increased recovery by 14%.

We showcased examples of performance from final optimized processes at both high and low load levels; they demonstrated exceptionally low Protein A leaching and excellent clearance of HCP contaminants.

TOYOPEARL Super A is a flexible and robust solution for preparative purification of monoclonal antibodies. Its excellent stability and overall reliability under diverse process conditions make it an excellent choice for a variety of mAb purifications.

Featured Products

Part #	Product name	Resin vol.
0023580	TOYOPEARL Super A	10 mL
0023581	TOYOPEARL Super A	25 mL
0023582	TOYOPEARL Super A	100 mL
0023583	TOYOPEARL Super A	1 L
0023584	TOYOPEARL Super A	5 L

Part #	Product name	Resin vol.	Column dim.
0045398	SkillPak 1 TOYOPEARL Super A	1 mL (ea.)	7 mm ID × 2.5 cm
0045399	SkillPak 1 TOYOPEARL Super A (qty 5)	1 mL (ea.)	7 mm ID × 2.5 cm
0045400	SkillPak 5 TOYOPEARL Super A	5 mL (ea.)	8 mm ID × 10 cm
0023483	TSKgel Protein A-5PW		4.6 mm ID × 3.5 cm