



# Long-term performance and stability of TOYOPEARL® Super A over 200 cleaning cycles

Protein A purification is the gold standard for affinity chromatography capture of monoclonal antibodies (mAb). The newest addition to the portfolio of mAb purification solutions from Tosoh Bioscience is the TOYOPEARL Super A resin. With enhanced alkaline stability, it withstands harsh CIP conditions up to 0.5 mol/L NaOH.

The long-term performance of Protein A resins under repeated use is critical to process economics and product consistency. This study focuses on measuring the stability and performance of the TOYOPEARL Super A Resin over 200 runs with cleaning cycles.

## Materials and Methods

### Column Characteristics

Column	SkillPak™ 5 TOYOPEARL Super A, PN: 0045400
Column dimensions	0.8 cm ID × 10 cm H
Column volume	5.0 mL
Mean particle size	45 µm
Mean pore size	100 nm
Maximum flow rate	2.5 mL/min

### Method

Flow rate	1.25 mL/min (4 min residence time), except equilibration phases (2.5 mL/min)
Detection	Absorbance @ 280 nm (mAU), conductivity (mS/cm), pH
Temperature	Ambient
Sample	CHO cell culture supernatant containing trastuzumab monoclonal antibody, 6.4 mg/mL; conductivity-adjusted and filtered
Load Level	1.28 mg/mL
Instrument	ÅKTA pure™ 25 (Unicorn 7.3)

Phase	Buffer	Length
Equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV
Load	Trastuzumab CHO clarified feedstock, loaded to 1.28 mg/mL	1 mL
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	5 CV
Elution	25 mmol/L sodium citrate, pH 3.2 Collection Start: 11.6 CV Collection Stop: 12.0 CV	5 CV
CIP	0.5 mol/L NaOH (16 min total exposure)	4 CV
	Water (upflow)	2 CV
Re-equilibration	20 mmol/L sodium phosphate pH 7.4, 150 mmol/L NaCl	5 CV

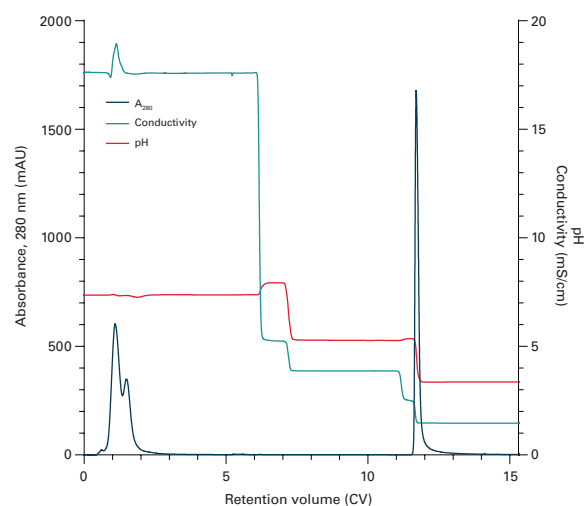
Eluate fractions of 1000 µL were collected in a 96-well deep well plate block containing 50 µL of 1 mol/L Tris to neutralize; the peak fractions were pooled for a volume of 2.1 mL.

The column was cleaned in downflow with 0.5 mol/L NaOH for 4 column volumes (CV) at 4 min residence time, for a total exposure time of 16 minutes. The method was repeated 200 times. Eluates from the first two runs were collected; afterwards, only the eluates of every tenth run were collected and analyzed. Cycles 50 and 70 were not collected due to equipment error.

## Results

A representative chromatogram is displayed in *Figure 1*.

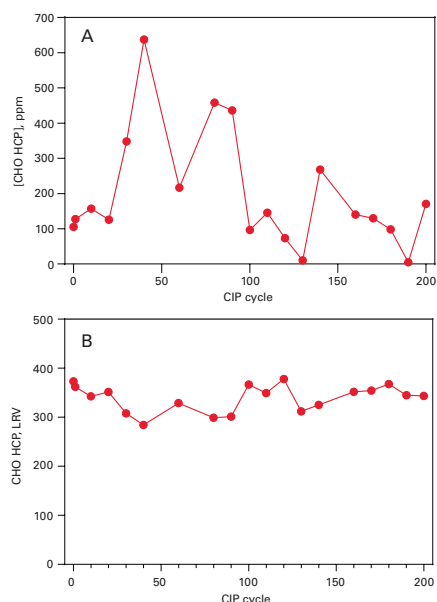
➤ **Figure 1.** A typical purification of trastuzumab from CHO feedstock on SkillPak 5 TOYOPEARL Super A.



## Host Cell Protein Reduction

CHO host cell protein (HCP) content was assayed using CHO HCP ELISA Kit, 3G, from Cygnus Technologies. HCP in the eluates was quantified via ELISA and compared to that of the feedstock sample to determine the degree of reduction. The results are shown in *Figure 2*. Although the HCP content was variable between cycles, there was no trend of increasing HCP, indicating that consistent column performance can be expected for at least 200 cycles. Across the series, the CHO HCP log<sub>10</sub> reduction values were consistent, with an approximate range of between 3 – 4 (*Figure 2*). This corresponds to a fold reduction from 700-fold (Cycle 40) up to 6000-fold (Cycle 120).

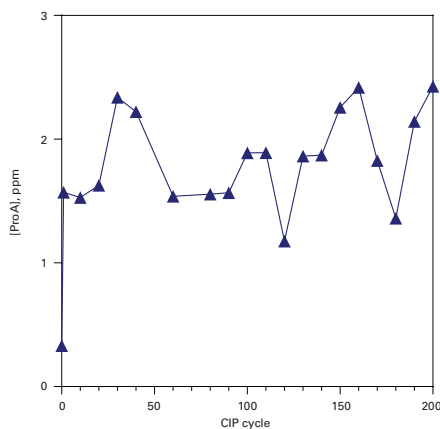
➤ **Figure 2.** (A) CHO host cell protein (HCP) content across Super A eluates over 200 CIP cycles, as quantified by ELISA. (B) Degree of CHO HCP reduction, reported as  $\log_{10}$  reduction value (LRV).



### Protein A ligand leaching

Protein A content in the eluates was quantified via the Tosoh R50, R40 and R28 Protein A Mix-N-Go™ ELISA kit from Cygnus Technologies. The results are shown in [Figure 3](#).

➤ **Figure 3.** Presence of Protein A ligand in Super A eluates over 200 cycles, reported in ppm.



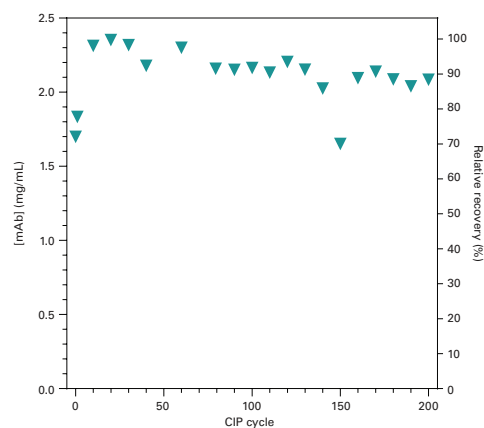
The first run, with no exposure to NaOH, had 0.33 ppm Protein A leaching; after one CIP cycle, it rose to 1.5 ppm. For CIP cycles 1-200, the average was  $1.85 \pm 0.37$  ppm Protein A present in the eluate. The low levels of Protein A content may minimize the number of further polishing steps to remove the residual ligand.

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### Recovery

mAb concentration in the eluates was quantified using UV absorption spectroscopy at 280 nm. Relative recovery and concentration results are reported in [Figure 4](#).

➤ **Figure 4.** Concentration of mAb in the eluate, and recovery relative to the maximum observed value (Cycle 20).



mAb concentration in the eluates was quantified using UV absorption spectroscopy at 280 nm. Runs 1 and 2 had lower relative recovery, presumably because of a preconditioning effect in which the irreversible binding sites had not yet been saturated. There was one outlier at Cycle 150.

TOYOPEARL Super A retained nearly 90% of its original binding capacity after 200 CIP cycles, highlighting its exceptional durability over extended use.

### Conclusions

TOYOPEARL Super A retains process consistency without deterioration in performance over the column's lifetime. There was minimal Protein A ligand leaching observed despite a harsh CIP condition. Stable HCP reduction was observed even after 200 CIP cycles. Recovery remained approximately 90% of maximum capacity. The stability and reusability of Super A makes it a sustainable and cost-effective choice for mAb purification.

### Ordering Information

Part #	Product name	Resin vol.	Pore size	Particle size
0023580	TOYOPEARL Super A	10 mL	100 nm	45 $\mu$ m
0023581	TOYOPEARL Super A	25 mL	100 nm	45 $\mu$ m
0023582	TOYOPEARL Super A	100 mL	100 nm	45 $\mu$ m
0023583	TOYOPEARL Super A	1 L	100 nm	45 $\mu$ m
0023584	TOYOPEARL Super A	5 L	100 nm	45 $\mu$ m

#### Process Development Columns

Part #	Product name	Resin vol.	Column dim.
0045398	SkillPak 1 TOYOPEARL Super A	1 mL (ea.)	7 mm ID $\times$ 2.5 cm
0045399	SkillPak 1 TOYOPEARL Super A (qty 5)	1 mL (ea.)	7 mm ID $\times$ 2.5 cm
0045400	SkillPak 5 TOYOPEARL Super A	5 mL (ea.)	8 mm ID $\times$ 10 cm