

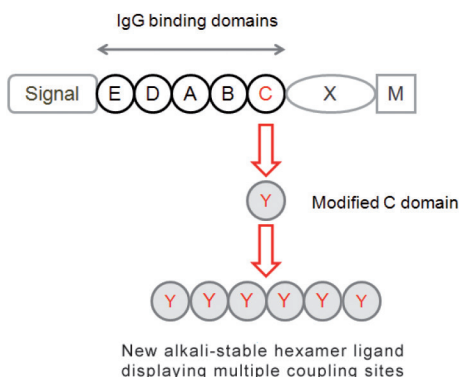


TOYOPEARL® Super A Resin: An Efficient & Durable Protein A Resin

Protein A-based affinity chromatography is the industry standard for capturing monoclonal antibodies (mAbs) in downstream processing, offering high selectivity and yielding a high purity of the target molecule in a single step. The continuous increase in fermentation titers for monoclonal antibody production necessitates resins with high selectivity, capacity, and efficiency.

TOYOPEARL Super A resin delivers a cutting-edge experience in protein A media, combining top-tier performance attributes with exceptional operational flexibility. High binding capacity and alkaline stability are achieved through the optimization of a ligand derived from one of the IgG-binding domains of *Staphylococcus aureus* protein A, which is recombinantly expressed in *E. coli*, ensuring an animal-origin-free product.

➤ **Figure 1.** Ligand structure of TOYOPEARL Super A resin.



Multipoint attachment of the ligand to the TOYOPEARL polymer matrix further enhances the chemical and thermal stability of the resin. This results in minimal protein A ligand leaching and improved resistance to alkaline solutions used during cleaning-in-place (CIP) procedures.

Highlights:

- **State-of-the-art operational flexibility:** Supports high titers with high dynamic binding capacity (DBC), enhanced alkaline stability for rigorous CIP, and excellent pressure-flow behavior to increase process efficiency.
- **Superior critical process performance:** Features mild elution conditions, concentrated elution volumes, and a significant reduction in process impurities, making the process robust, safe, and compliant.

➤ **Table 1.** Technical attributes of TOYOPEARL Super A resin.

Ligand	Modified, code-optimized C domain, hexameric ligand with multipoint attachment
Average particle size	45 µm
Average pore size	100 nm
Dynamic binding capacity	72 mg mAb/mL resin @ 5 min RT 60 mg mAb/mL resin @ 3 min RT
Operating linear flow rate	10 – 600 cm/hr
pH stability (operational)	3 – 12
pH stability (CIP)	2 - 14
Chemical stability	Stable to all commonly used aqueous buffers in Protein A Chromatography
Bacterial count	Max 100 CFU/mL
Endotoxin concentration	Max 10 EU/mL
Delivery conditions	72% slurry in 20% ethanol

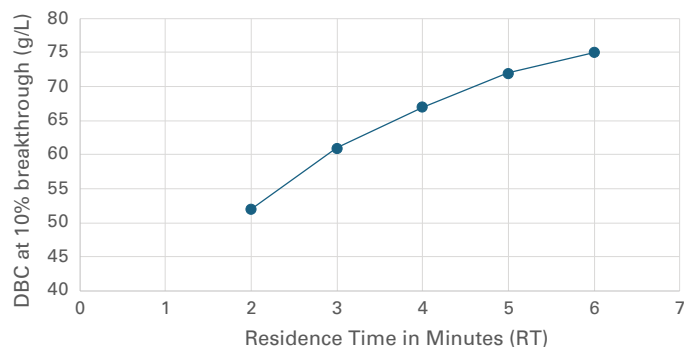
State-of-the-Art Operational Attributes

High Dynamic Binding Capacity (DBC)

The DBC indicates the amount of sample that can be bound to 1 mL of resin at a given residence time (RT). DBC is economically significant because protein A resins require a high capital investment, so it's crucial to use the smallest volume of resin to purify the largest amount of sample. A high binding capacity results in greater product capture, increasing yield and making downstream processing steps more efficient.

Figure 2 shows the dynamic binding capacity of TOYOPEARL Super A resin at different residence times. The DBC at 10% breakthrough is over 70 mg/mL resin at a 5-minute residence time.

Figure 2. Dynamic binding capacity of TOYOPEARL Super A resin (0.6 cm ID x 4.0 cm L column).



Enhanced Alkaline Stability Extends Resin Lifetime and Boosts Cost-Effectiveness

Sodium hydroxide (NaOH) solutions are widely used for cleaning-in-place (CIP) in the bioprocessing industry due to their strong alkaline nature, which denatures a wide range of biological contaminants. Chromatography columns and systems are often flushed with 0.1 – 1 M NaOH solutions to remove residual proteins, lipids, nucleic acids, and inactivate microorganisms or endotoxins that might remain in the system.

Figure 3. Relative DBC in dependence of the number of CIP cycles performed with 0.5 M and 1 M NaOH. The contact time for each cycle was 15 min.

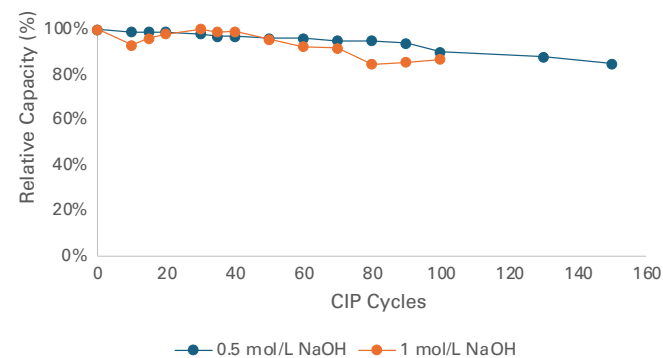


Table 2. Critical performance aspects of TOYOPEARL Super A resin.

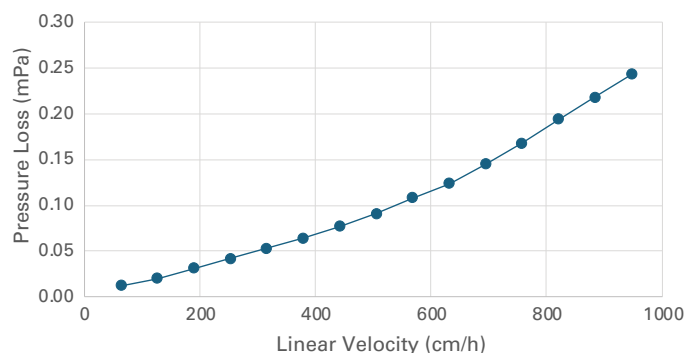
mAb Concentration (g/L)	Load Capacity [CV]	Eluate Volume [mL]	Eluate Volume [CV]	Concentration [g/L]	Elution pH	Yield [%]	HCP (ppm)	Monomer (%)	Aggregate (%)	Protein A (ppm)
8.31	32.6	8.3	1.7	19.8	4.9	100.6	859	96.4	2.2	1

Prolonged exposure of conventional protein A ligands to NaOH degrades the ligands, leading to reduced binding capacity and shortened resin lifetime. However, the structure of the recombinant ligand in TOYOPEARL Super A resin, along with its multipoint attachment to the base matrix, enhances the resin's stability in 0.5 - 1 M NaOH. TOYOPEARL Super A resin shows high alkaline stability, retaining 90% binding capacity after over 100 CIP cycles at 0.5 M NaOH and 70 CIP cycles at 1 M NaOH (Figure 3).

Pressure-Flow Behavior

TOYOPEARL Super A resin is optimized to withstand high flow velocities in process-scale operations, allowing for high-throughput purification of mAbs and other antibody molecules. The resin is also optimized for continuous manufacturing and large feed volumes. Figure 4 displays pressure-flow curves for TOYOPEARL Super A resin.

Figure 4. Pressure-flow curve for TOYOPEARL Super A resin packed in 2.2 cm ID x 20 cm, generated in water.



Superior Purification Efficiency with Critical Performance Attributes

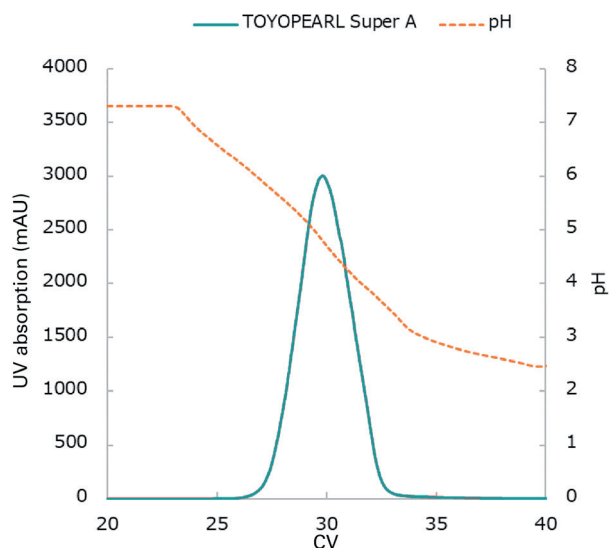
TOYOPEARL Super A resin offers superior performance in protein purification by eluting at slightly acidic pH levels with low elution volumes. High reduction in host cell protein (HCP) concentrations guarantee a highly pure product. Additionally, the resin maintains the structural integrity of the target protein, with monomer percentages consistently exceeding 95% and minimal aggregation. The consistently low levels of protein A leaching (< 10 ppm) further ensure product safety and efficacy. These features make TOYOPEARL Super A resin the perfect choice for large-scale bioprocessing, delivering reliable and high-quality results every time.

Mild Elution pH

Typically, protein A capture steps elute mAb target species under acidic conditions, around pH 3.5. For most mAbs, these elution conditions effectively achieve high yields without affecting product quality. However, some mAbs are more sensitive to low pH; consequently, low elution pH may cause unwanted protein aggregation. Aggregates, which are considered contaminants with increased immunogenicity, must be reduced to acceptable levels in the final drug product.

A pH gradient was run to assess the required pH for elution of a humanized monoclonal antibody from TOYOPEARL Super A resin (Figure 5). Elution was achieved at a mild acidic pH value of 5.0. TOYOPEARL Super A resin improves the overall efficiency of the protein A chromatography process, providing a highly pure and stable product while offering greater flexibility for pH-sensitive molecules.

Figure 5. pH gradient elution with TOYOPEARL Super A resin (0.6 cm ID x 4.0 cm L column).

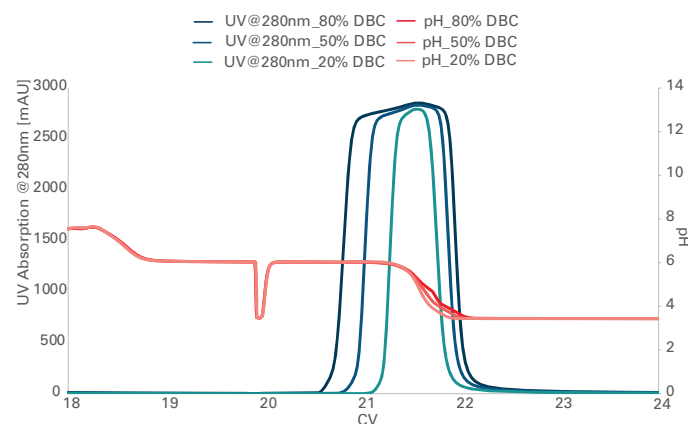


Low Elution Volume

Evaluating the elution volume of chromatographic media can further optimize process effectiveness. Low elution volume results in a more concentrated sample, ultimately improving handling and efficiency in subsequent chromatographic or filtration steps. Additionally, the processing time is shortened and buffer consumption reduced. These factors collectively lead to a more cost-effective and efficient purification process.

When eluting from TOYOPEARL Super A resin at load levels of 20%, 50%, and 80%, the elution volume was consistently low (0.8 CV, 1.3 CV, and 1.6 CV, respectively) with high recovery, making the purification process more economical (Figure 6).

Figure 6. Elution volume of TOYOPEARL Super A resin at load levels of 20%, 50% and 80% of DBC_{10%} (0.8 cm ID. x 10 cm L column).



Summary

The efficiency, durability, and flexibility of TOYOPEARL Super A resin make it an ideal choice for large-scale bioprocessing. Its advanced features improve process economics and ensure the manufacturing of high-quality monoclonal antibody therapeutics.

Ordering Information

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

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