TOSOH

Performance Data

TOYOPEARL® Super A Resin: Unmatched Performance in Protein A Chromatography

Protein A-based affinity chromatography is the industry standard for capturing monoclonal antibodies (mAbs) in downstream processing as it offers high selectivity, yielding a high purity of the target molecule in one step. The continuous increase in fermentation titers in monoclonal antibody production necessitates resins with higher downstream capacity and efficiency. TOYOPEARL Super A resin provides advanced performance, blending critical performance attributes with exceptional operational flexibility, making it an ideal protein A resin for capturing mAbs in downstream processing.

The performance of TOYOPEARL Super A resin is compared with an agarose-based, alkaline-resistant protein A resin (Resin X).

Part of the data shown in this document has been kindly provided by Rentschler Biopharma SE.

Key Features of TOYOPEARL Super A Resin:

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

Superior Process Performance

Critical Quality Attributes of Protein A Purification Step

The critical performance aspects of protein A resin are crucial for producing a safer, purer product that meets the ever-increasing regulatory standards and has a significant impact on the overall process economics and efficiency.

The capture step of a humanized mAb (IgG 1) harvest using TOYOPEARL Super A resin and Resin X is shown in **Figure 1**. The same experimental conditions (column volume, method, buffers, sample) are used for both resins.
TOYOPEARL Super A resin demonstrated efficient and consistent performance across various titers, showcasing its performance and robustness (**Table 1**).

Figure 1. Chromatogram of the capture step of humanized mAb (IgG 1) harvest.

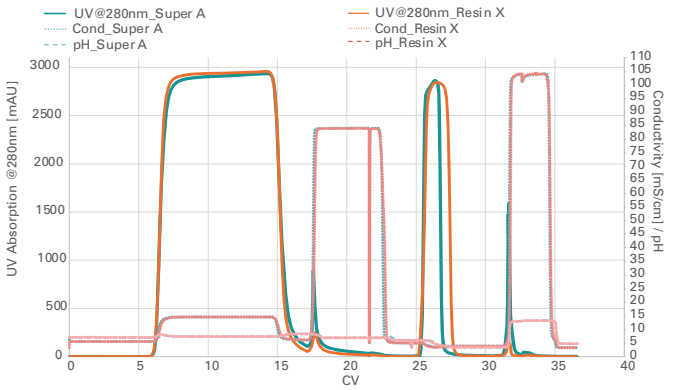


Table 1. Critical performance aspects of both resins.

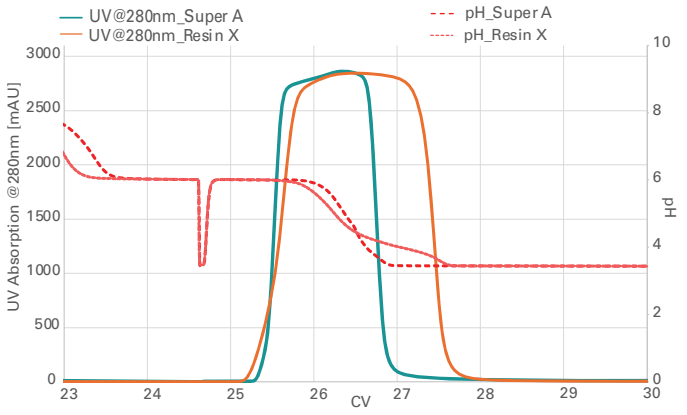
	TOYOPEARL Super A			Resin X		
mAb Titer (g/L)	2.09	4.24	8.31	2.09	4.24	8.31
HCP Load (ppm)	111181	58136	31976	111181	58136	31976
			Elution			
Eluate Volume (CV)	1.7	1.7	1.7	2.5	2.5	2.5
Elution Concentration (g/L)	20.5	21	19.8	13.8	14.4	12.7
Elution pH	5.0	5.0	4.9	4.7	4.7	4.7
Yield (%)	98.9	95.6	100.6	99.8	98.0	97.6
HCP (ppm)	750	777	859	1472	1520	1546
Monomer (%)	96.3	96.4	96.4	95.8	95.9	95.9
Aggregate (%)	2.3	2.3	2.2	2.5	2.5	2.4
Protein A (ppm)	8	2	1	4	2	2

Low Elution Volume

The elution capabilities and profiles of chromatographic media directly impact the efficiency of a purification process. Reducing the buffer volume required to fully elute a target species produces a more concentrated eluate, which ultimately decreases liquid handling and increases the efficiency of later downstream steps. Additionally, the reduced buffer volume reduces the processing time. These factors collectively lead to a more efficient purification process and increase the process economics.

As depicted in **Figure 2**, the elution volume of the TOYOPEARL Super A resin was ~30% lower (1.7 CV vs. 2.5 CV) compared to Resin X. Using TOYOPEARL Super A resin reduces overall process time and liquid handling, thereby improving process efficiency and economics.

Figure 2. Elution profiles of a humanized monoclonal antibody (IgG 1) harvest with both resins.



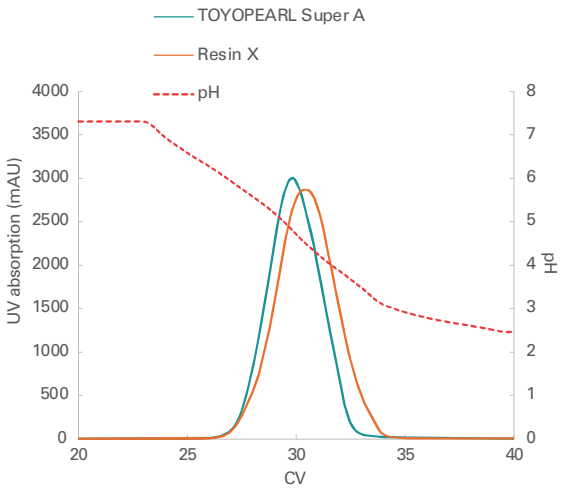
Mild Elution pH

A typical platform protein A capture step elutes mAbs under acidic conditions, typically 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, the low elution pH may cause unwanted protein aggregation. As aggregates are considered contaminants with increased immunogenicity and thus compromise safety and efficacy, aggregates must be reduced to an acceptable level in the final drug product. Traditional protein A resins requiring low pH conditions for elution can challenge the development and manufacturing of proteins sensitive to low pH conditions.
A pH gradient was run to assess the required pH for elution of a humanized monoclonal antibody from both resins (**Figure 3**).

The data shows that elution was achieved at a higher pH value of 5.0 compared to 4.6 for Resin X. TOYOPEARL Super A resin improves the overall efficiency of the protein A chromatography process as the eluate contains a highly pure and stable product and also offers a greater flexibility to pH-sensitive molecules.

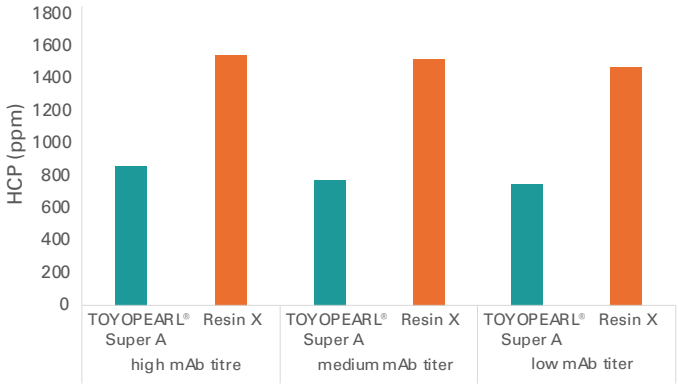
Advanced Impurity Removal and Monomer Recovery

Figure 3. Comparison of elution pH.



Lower levels of host cell protein (HCP), a common impurity in the mAb manufacturing process, results in improved purity of mAb therapeutics. As depicted in **Figure 4**, TOYOPEARL Super A resin demonstrates approximately 50% greater effectiveness in HCP removal compared to the Resin X across all mAb titers.

Figure 4. Comparison of HCP reduction with TOYOPEARL Super A resin and Resin X.



As shown in **Figure 5**, the eluate pool with TOYOPEARL Super A resin contained lower levels of aggregates for all mAb titers compared to Resin X.

Figure 5. Comparison of aggregate content in the eluate pool for both resins.

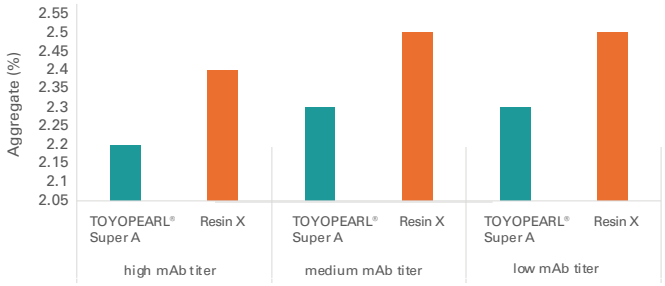
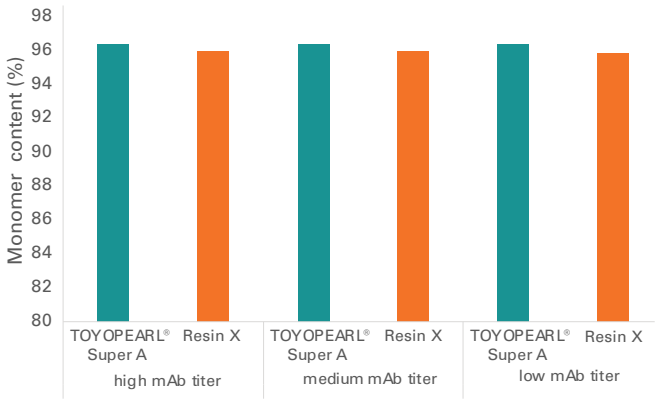


Figure 6 demonstrates that TOYOPEARL Super A resin and Resin X yield similar monomer concentrations.

Figure 6. Monomer content with TOYOPEARL Super A resin and Resin X.



This data demonstrates the ability of TOYOPEARL Super A resin to remove impurities during the capture step more effectively than the Resin X, thus improving process efficiency.

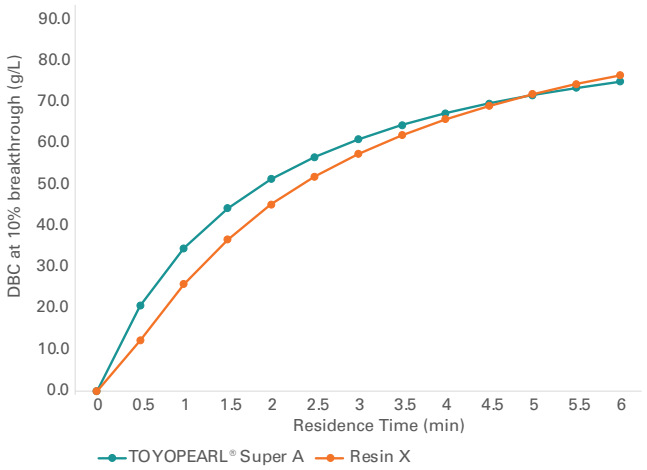
State-of-the-Art Operational Flexibility

Market Leading 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). The DBC value is economically significant because protein A resins require a high capital investment, so the minimum amount of resin must be used to purify the largest amount of product. A high binding capacity improves product capture, thereby increasing the yield and making subsequent downstream processing steps more efficient.

Figure 7 depicts the dynamic binding capacity of TOYOPEARL Super A resin at different residence times. The DBC at 10% breakthrough is 72 mg/mL resin at 5 minutes residence time.

Figure 7. Dynamic binding capacity comparison of both resins using humanized mAb (IgG 1) at a titer of 1.0 g/L.



Enhanced Alkaline Stability Extends Resin Lifetime and Boosts Cost-Effectiveness

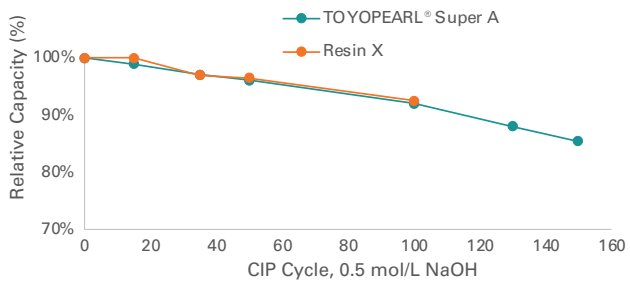
Sodium hydroxide (NaOH) solutions are widely used for cleaning purposes in the bioprocessing industry due to their strong alkaline nature, which denatures a wide range of biological contaminants. Chromatography columns and systems are often flushed with 0.1 – 1.0 M NaOH solutions to remove residual proteins, lipids, or nucleic acids and to inactivate microorganisms or endotoxins that might remain in the system.

Prolonged exposure of conventional protein A ligands to NaOH degrades the ligands, which leads to reduced binding capacity. In contrast, the recombinant ligand structure and multipoint attachment to the base matrix of TOYOPEARL Super A resin enhances its 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.

For long term use, protein A resins are often used until the threshold of remaining capacity is reached. Typically, this threshold is set at 80% remaining capacity. Following the general alkaline stability, it becomes apparent that TOYOPEARL Super A resin can withstand a minimum of 150 cycles before the resin batch needs to be discarded.

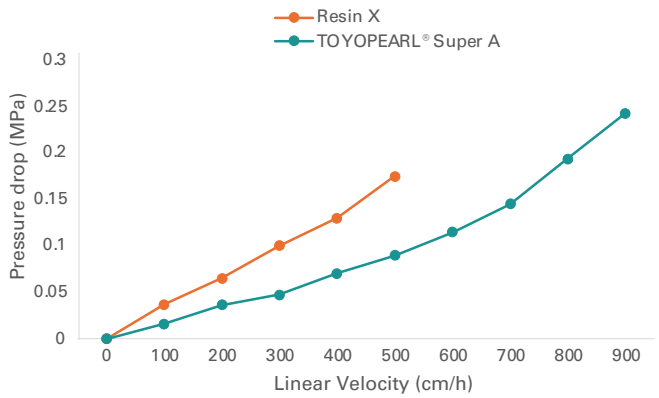
Figure 8. Relative DBC in dependence of the number of CIP cycles performed with 0.5 M NaOH and 15-minute contact time per cycle.



Pressure-Flow Behavior

TOYOPEARL Super A resin is optimized to withstand high flow velocities. This allows for high-throughput purification of mAbs and other antibody molecules. This translates to optimal performance for large feed volumes or in continuous manufacturing. **Figure 9** displays pressure-flow curves for different protein A resins.

Figure 9. Pressure-flow curve for both resins. TOYOPEARL Super A resin was packed in 2.2 cm ID x 20 cm L column. Competitor data is taken from marketing material.



Summary

TOYOPEARL Super A resin offers industry-leading critical performance attributes along with flexible operational parameters making it an ideal choice for small and large-scale bioprocessing. Its advanced features improve process economics and ensure high-quality monoclonal antibody purification.

*Data has been generated by Rentschler Biopharma SE.

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