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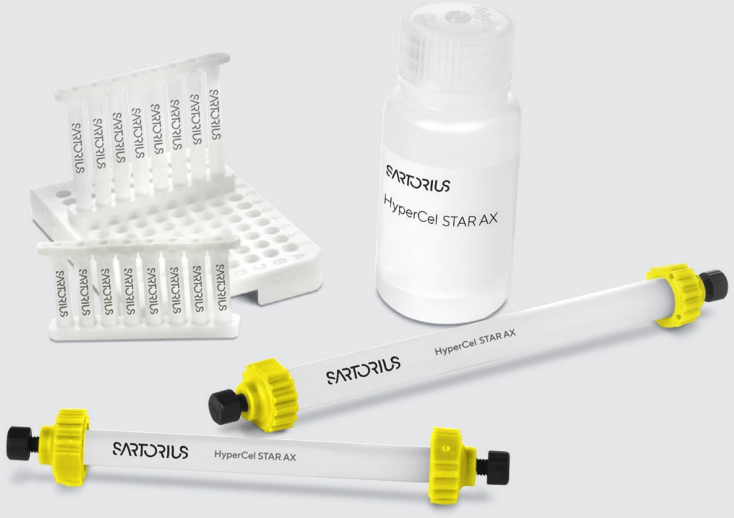
Product Datasheet

HyperCel STAR AX Ion Exchange Resin

Salt Tolerant Advanced Recovery Anion Exchange Chromatography Resin



Product Information

Hypercel STAR AX is an anion exchange chromatography resin designed for high productivity protein capture and impurity removal at moderate or higher conductivity (2 to 15 mS/cm), typical of undiluted biological feedstocks (i.e., mammalian cell culture supernatants, E. coli feedstock, plasma, etc.). HyperCel STAR AX meets the needs of industrial users and regulatory authorities. It is produced at large scale and a Regulatory Support File (RSF) is available.

Features and Benefits

HyperCel STAR AX is a salt tolerant anion exchange resin designed for use in bioprocessing:

- High DBC at short residence times (<2 min)
- Direct capture of protein or impurity removal from undiluted feedstocks at moderate or high conductivity
- Unique selectivity, across a broad conductivity range
- Fast processing and enhanced process economics

Introduction

HyperCel STAR AX resin is composed of a rigid cellulose matrix that has excellent flow properties and generates low backpressure, compatible with the needs of manufacturing-scale protein purification. The resin is available in a variety of packaging configurations:

- As convenient 1 mL and 5 mL PRC prepacked columns designed for fast method optimization, selectivity screening or small preparative work,
- As miniaturized prepacked RoboColumns®* of 200 µL and 600 µL, for fully automated and parallel chromatographic separations.

HyperCel STAR AX resin is supplied as a slurry in 1 M NaCl containing 20% (v/v) ethanol or as a moist cake for process-scale applications. The moist cake resin facilitates the resin transfer, avoiding the agitation and suspension of large material volumes.

HyperCel STAR AX resin has a chemical stability that ensures simple clean-in-place (CIP) and storage. For standard CIP, 0.5 to 1 M NaOH treatment is recommended, while long-term storage in 10 to 100 mM NaOH is possible.

Technical Specifications

Table 1: *Main Properties*

Average particle size	80 µm
Ion exchange ligand	Primary amine
Dynamic binding capacity[1]	>100 mg BSA/mL within pH range 7.5 – 8.0 and conductivity 15 mS/cm
Typical operating range of feedstock conductivity	2 – 15 mS/cm
Recommended cleaning conditions[2]	1 M NaOH

[1] Determined using a 5 mg/mL bovine serum albumin (BSA) in 25 mM Tris-HCl , 0.14 M NaCl at 2 minute residence time.

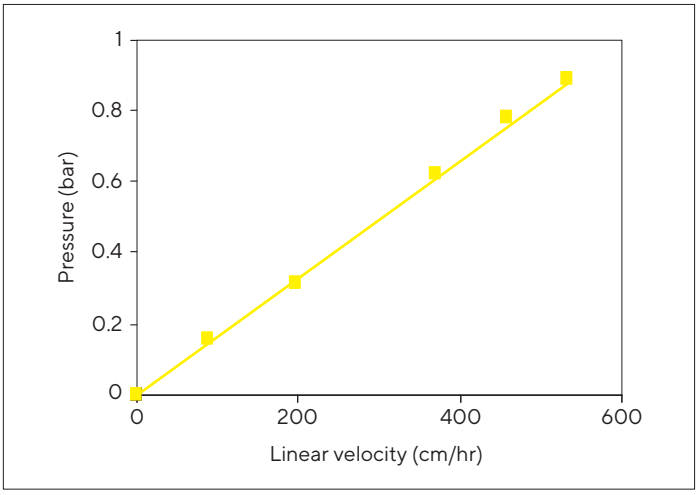
[2] Injection of 5 column volumes (CV) of 0.5 – 1 M NaOH, 1 hour contact time.

HyperCel STAR AX resin is very easy to pack and unpack in laboratory, pilot and production-scale columns, and shows excellent flow properties, compatible with the requirements of advanced production processes.

The resin can be packed in standard inexpensive buffers. For example, a 200 mm I.D. × 150 mm height column packed in a 10 mM NaCl buffer can be operated at less than 1.5 bar (22 psi) backpressure (Figure 1).

Packing performance is consistent from laboratory to large scale (400 mm I.D.) columns, Typical performance values achieved are N/m >2000 plates and asymmetry factor 1.0<AF<1.4.

Figure 1: *Pressure vs. Flow Rate of HyperCel STAR AX Resin*

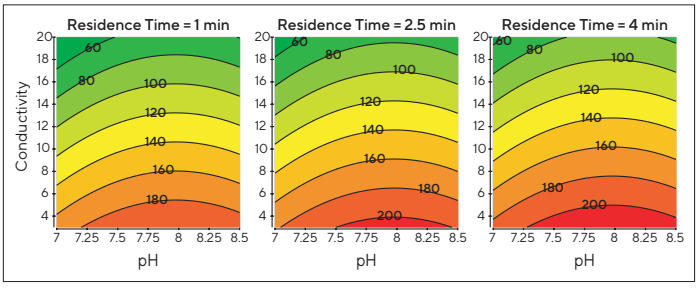


Column: 200 mm I.D. × 150 mm bed height. Packing Buffer: 10 mM NaCl

Features and Benefits

High Dynamic Binding Capacity Across Broad Conductivity Range: Avoids Feedstock Dilution And Streamlines Downstream Processing

Figure 2: *Dynamic Binding Capacity vs. Residence Time as a Function of pH and Conductivity of HyperCel STAR AX Resin*

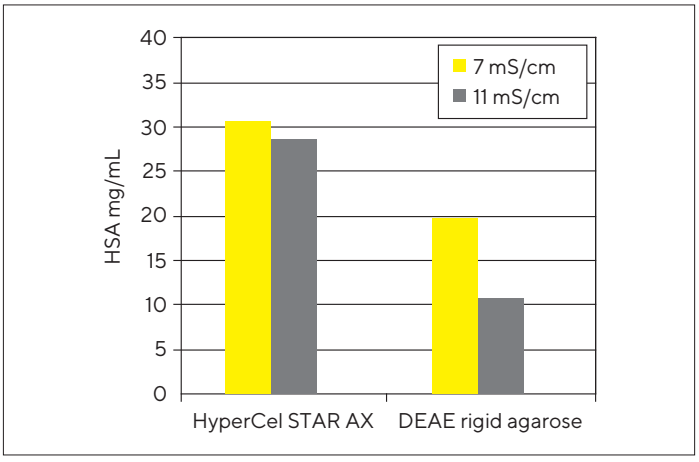


Column: 0.5 cm I.D. × 5 cm bed height (~1 mL).
Sample: 5 mg/mL BSA in equilibration buffer.
Equilibration buffer: 25 mM Tris-HCl, pH 7.0 – 8.5.
Conductivity 3 – 20 mS/cm. Residence time: 1 – 4 min (0.25 – 1 mL/min).
Numbers indicate binding capacity for BSA in mg/mL of resin.

A Design of Experiments study (DoE) was done to explore the influence of various pHs (7.0 to 8.5), conductivities (3 to 20 mS/cm) and residence times (1 to 4 minutes) on the dynamic binding capacity for BSA used as a model.

The data in figure 2 shows the impact of pH and conductivity on DBC for BSA on HyperCel STAR AX resin. The contour plots show that the resin achieves a high DBC (> 100 mg/mL) over a wide range of pHs and conductivities at short residence time, allowing optimal process flexibility and productivity.

Figure 3: *Dynamic Binding Capacity of HyperCel STAR AX Resin for Human Serum Albumin (HSA) from Undiluted and Diluted Plasma*



Comparison of the DBC for HSA of HyperCel STAR AX resin and a conventional rigid agarose DEAE anion exchange resin at conductivities corresponding to undiluted (11 mS/cm) and diluted (7 mS/cm) plasma.

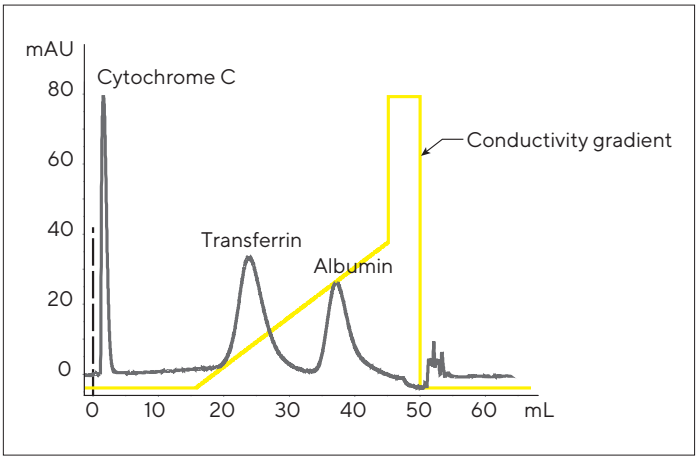
Data shown in Figure 3 confirms that DBC is less affected by conductivity in the 7 – 11 mS/cm range, compared to conventional resin. This allows direct load of undiluted plasma on HyperCel STAR AX resin.

Combined with excellent flow characteristics at low backpressures (Figure 1), large volumes of feedstock can be processed directly and quickly, increasing the overall process throughput, and limiting the risk of protein degradation.

High binding capacity facilitates operation using columns of moderate volume and footprint, allowing further reduction in buffer-volume requirements, and leading to equipment savings and reduced investment costs for resins.

Excellent Selectivity and Separation Efficiency Over a Broad Range of Conductivities

Figure 4: *Separation of a Protein Mix on HyperCel STAR AX Resin at 10 mS/cm*



HyperCel STAR AX PRC prepacked column of 1 mL; 100 µL mix (2 mg/mL cytochrome C, 10 mg/mL human transferrin, 10 mg/mL BSA. Load: 25 mM Tris-HCl pH 8.0, 10 mS/cm. Elute: gradient 0 – 50% 25 mM Tris-HCl, pH 8.0 + 1 M NaCl.

Resin selectivity is a key parameter to discriminate between the target protein and contaminants in the feedstock. Screening of resin selectivity is critical and should be done at early stages of process development.

Rapid screening and condition optimization can be achieved using a 1 mL PRC prepacked column. Once the appropriate chemistry is selected, the conditions of use can be optimized in a 5 mL PRC column by doubling the height. Two 5 mL columns can be connected in series to increase the column bed height to 20 cm, and more closely model real conditions in pilot scale or for scale-down applications. Columns of 1 mL can also be connected in series.

Due to the difference in the bead structure, ligand chemistry and the specific ionic charge density, as shown in Figure 4, the selectivity and separation efficiency of HyperCel STAR AX resin is maintained in a broad range of conductivities.

Applications and Examples

Applications include direct capture of recombinant proteins, monoclonal and polyclonal antibodies, plasma derivatives or other biopharmaceuticals.

Due to its ability to capture proteins directly from undiluted feedstreams, HyperCel STAR AX resin can also be used for early contaminant removal (e.g., CHO host cell proteins), before target purification, e.g., before monoclonal antibody (MAb) capture by a Protein A affinity step.

Application 1. Direct Capture of Albumin from Undiluted Plasma

The objective is to evaluate HyperCel STAR AX resin as the first step in a two-step purification sequence to capture HSA from undiluted plasma (conductivity 11 mS/cm). HyperCel STAR AX resin was used as a first capture step, followed by an orthogonal cation exchange step without pH or conductivity adjustment of the plasma. Neat undiluted plasma (pH 7.6, 11 mS/cm), was loaded with DBC of 30 mg/mL (refer to Figure 3).

Design of Experiments (DoE) in 96-well filter plates was performed to determine optimal conditions to achieve the best yield | purity ratio (one example of the optimization of wash | elution conditions is shown in Figure 5).

These conditions were then transferred to column chromatography on PRC prepacked columns (Figure 6).

Figure 5 shows that HSA yield is impacted mainly by elution conditions (optimal zone: pH 3.5 – 4.2 and conductivity 2 – 27 mS/cm) while purity is impacted mainly by wash conditions (high conductivity wash at >15 mS/cm improves purity).

Figure 5: *Impact of Washing (W) and Elution (E) Conditions on Purity of HSA and Elution Yield (in percentage) on HyperCel STAR AX Resin (Design of Experiments in 96-Well Filter Plates)*

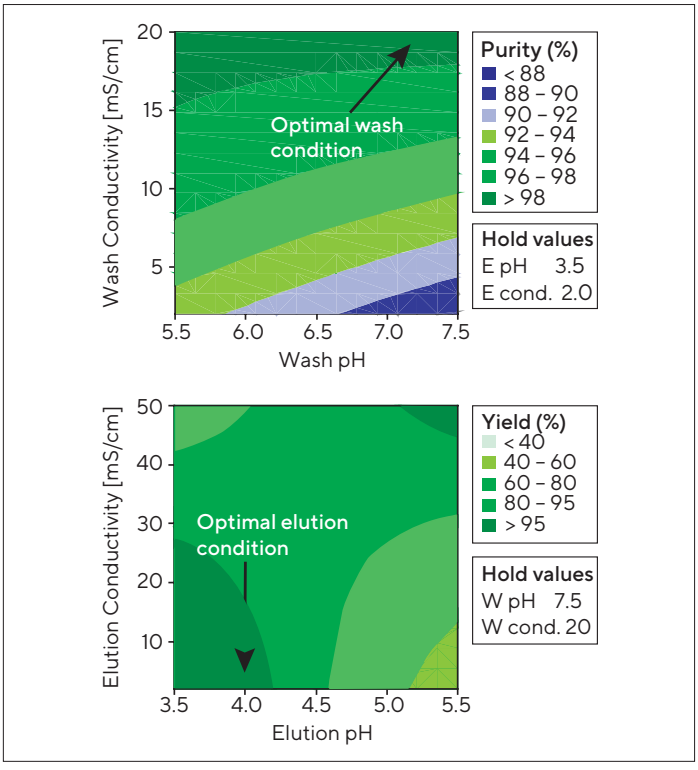
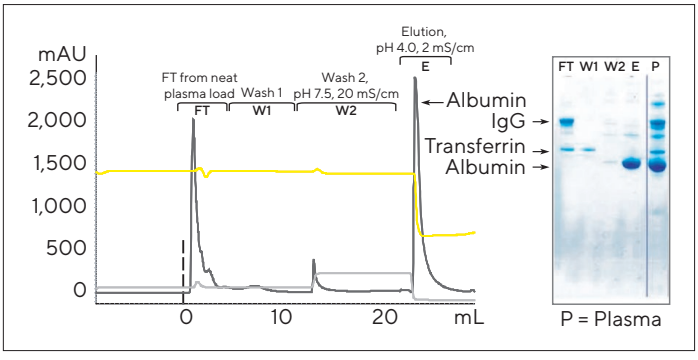


Figure 6: *Transfer of Conditions Determined in 96-Well Plates to 1 mL HyperCel STAR AX PRC Prepacked Column*



Conditions determined in 96-well plates were applied to chromatography of undiluted plasma on HyperCel STAR AX resin in a 1 mL PRC prepacked column. Chromatogram and SDS-PAGE analysis of fractions in Figure 6 confirmed that most contaminants were eliminated by high conductivity wash, leading to a 99% pure HSA fraction in a single step, with 90% yield.

In addition, as shown before in Figure 3, HyperCel STAR AX resin used at capture step with undiluted plasma had a DBC >30 mg/mL, more than 2-fold higher than that of a conventional DEAE agarose resin tested in these conditions.

HSA was eluted by a simple decrease of pH (4.0), without addition of salt, allowing a direct load on an orthogonal cation exchange column.

This last polishing step on cation exchange resin had a capacity around 65 mg/mL, and led to a purified fraction of HSA eluted at pH 7 with a purity >99% (Table 2).

Table 2: *Two-step Purification of Human Serum Albumin from Undiluted Plasma*

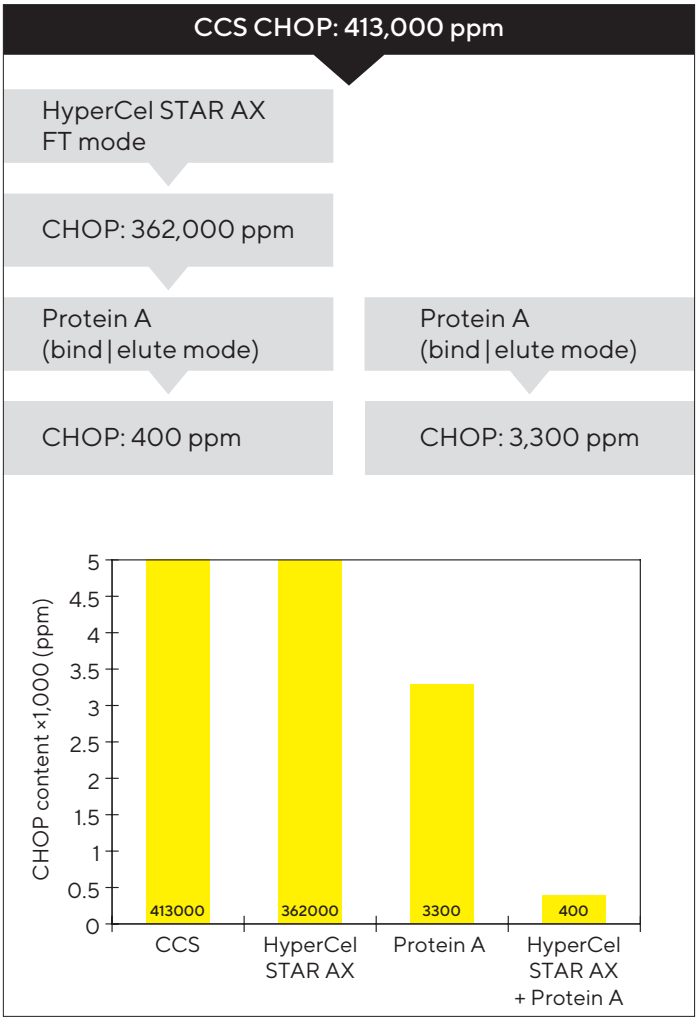
	Load	Capacity (mg/mL)	Yield	Purity
Capture on HyperCel STAR AX resin	Undiluted plasma	> 30 mg/mL	90%	99%
Polishing on cation exchange resin	pH 4.0 eluate from HyperCel STAR AX resin	65 mg/mL	95%	> 99%

Application 2. Early Removal of CHOPs before Protein A Capture of a MAb

The objective of this study was to evaluate the impact of a pre-purification step using HyperCel STAR AX resin before conventional Protein A resin capture of a MAb from mammalian cell culture feedstock (Figure 7).

The content of contaminating CHOPs (Chinese Hamster Ovary Proteins) was compared using a commercial ELISA assay for the two purification schemes shown in Figure 7.

Figure 7: *Conventional and Alternative MAb Purification Scheme Including Pre-purification Step on HyperCel STAR AX Resin*



Data shows that using HyperCel STAR AX resin prior to Protein A results in a better CHOP reduction (>8-fold). Due to this synergistic effect, starting from an initial Host Cell Protein (HCP) content of 413,000 ppm, the final CHOP level is reduced to ~400 ppm (3-Log reduction), with a MAb recovery of 90%.

A pre-purification step can impact positively process economics by extending the lifetime of an expensive Protein A column used as standard step for MAb capture.

Ordering Information

Description	Part Number
HyperCel STAR AX Resin	
25 mL	20197-026
100 mL	20197-032
1 L	20197-046
5 L	20197-058
10 L	20197-064
Prepacked Columns	
PRC Column 5 × 50 HyperCel STAR AX, 1 mL	PRCSTARAX1ML
PRC Column 8 × 100 HyperCel STAR AX, 5 mL	PRCSTARAX5ML
RoboColumn®* HyperCel STAR AX 200 µL, row of 8	SR2STARAX
RoboColumn®* HyperCel STAR AX 600 µL, row of 8	SR6STARAX

*RoboColumn is a registered trademark of Repligen GmbH

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DIR: 2578783-000-01
Status: 06 | 2024