



Life Sciences

USD 2831(2)

HyperCel™ STAR AX Ion Exchange Sorbent



Salt Tolerant Advanced Recovery Anion Exchange Chromatography Sorbent

An industry-scalable anion exchange chromatography sorbent designed for high productivity protein capture and impurity removal at moderate or higher salt conductivity (2 to 15 mS/cm), typical of undiluted biological feedstocks (i.e., mammalian cell culture supernatants, E. coli feedstock, plasma, others...).

Filtration. Separation. Solution.SM

HyperCel STAR AX sorbent is manufactured at large scale and meets the needs of industrial users and regulatory authorities. A Regulatory Support File (RSF) is available to assist users in the development of validation procedures. This sorbent offers:

- ▶ High dynamic binding capacity (DBC) at short residence time (2 minutes or lower)
- ▶ Direct capture of protein or impurity removal from undiluted feedstock at moderate or high conductivity
- ▶ Excellent flow rate properties for fast feedstock processing
- ▶ Distinctive selectivity, consistent in a broad conductivity range (2 – 15 mS/cm)
- ▶ Enhanced process economics

Description

HyperCel STAR AX sorbent is composed of a rigid cellulose matrix that has excellent flow properties and generates low backpressure, compatible with the needs of manufacturing-scale protein production. The sorbent is available in a variety of packaging configurations, as AcroPrep™ ScreenExpert 96-well Plates designed for early screening of resins suitable for protein purification, and also as convenient 1 mL and 5 mL PRC prepacked columns designed for fast method optimization, selectivity screening or small preparative work. HyperCel STAR AX sorbent is supplied as a slurry/suspension in 1 M NaCl containing 20% (v/v) ethanol or as a moist cake for process-scale applications (the moist cake sorbent facilitates the sorbent transfer, avoiding the agitation and suspension of large material volumes).

HyperCel STAR AX sorbent 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.

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 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.

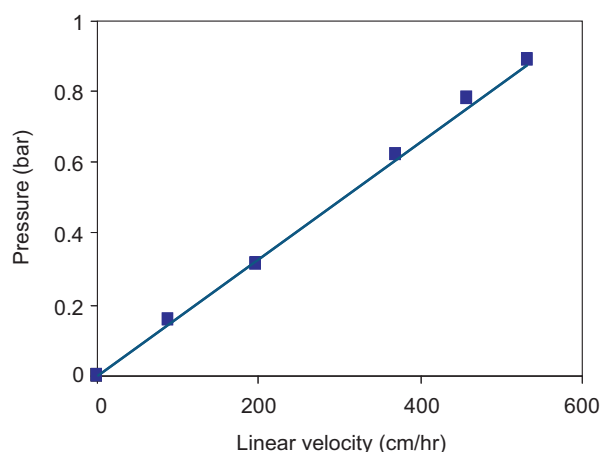
HyperCel STAR AX sorbent is very easy to pack and unpack in laboratory, pilot and production-scale columns, and shows excellent flow properties, compatible with the requirements of advanced production processes.

The sorbent can be packed in standard inexpensive buffers. For example, a 200 mm I.D. x 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 values of N/m > 2000 plates and asymmetry factors (AF) are: 1.0 < AF < 1.4.

Figure 1

Pressure vs. Flow Rate of HyperCel STAR AX Sorbent



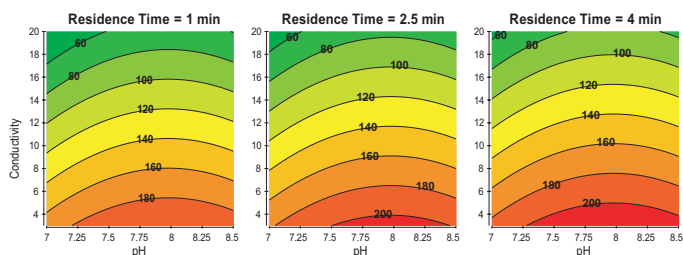
Column: 200 mm I.D. x 150 mm bed height. Packing Buffer: 10 mM NaCl

Features and Benefits

High Dynamic Binding Capacity in Broad Conductivity Operating Conditions: 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 Sorbent



Column: 0.5 cm I.D. x 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 sorbent.

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 bovine serum albumin (BSA) used as a model.

Data shows the impact of pH and conductivity on DBC for BSA on HyperCel STAR AX sorbent. The contour plots in Figure 2 show that the sorbent 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 Sorbent for Human Serum Albumin (HSA) from Undiluted and Diluted Plasma

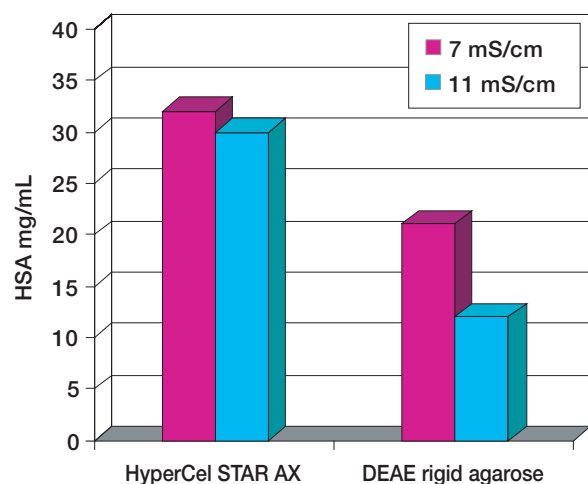


Figure 3 shows the DBC for HSA of HyperCel STAR AX sorbent compared to a conventional rigid agarose DEAE anion exchange sorbent at conductivities corresponding to undiluted (11 mS/cm) and diluted (7 mS/cm) plasma.

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

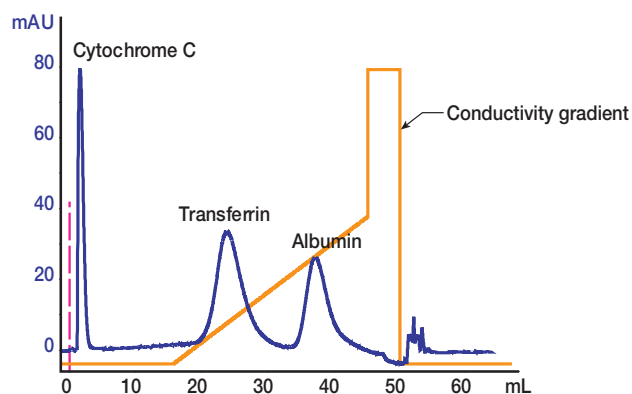
Combined with excellent flow characteristics at low backpressures (Figure 1), large volumes of feedstock can be processed directly and fast, increasing the overall process throughput, and limiting the risk of protein degradation.

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 sorbents.

Excellent Selectivity and Separation Efficiency over a Broad Range of Conductivities

Figure 4

Separation of a Protein Mix on HyperCel STAR AX Sorbent 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 bovine serum albumin [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.

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

Rapid screening and condition optimization can be achieved using a Pall 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 sorbent 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 directly proteins from undiluted feedstreams, HyperCel STAR AX sorbent can also be used for early contaminant removal (i.e., CHO Host Cell Proteins), before target purification, i.e., before 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 sorbent as the first step in a two-step purification sequence to capture human serum albumin (HSA) from undiluted plasma (conductivity 11 mS/cm). HyperCel STAR AX sorbent was used as a first capture step, followed by an orthogonal cation exchange step performed on S HyperCel sorbent, 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 (AcroPrep™ Advance filter plates, Pall) 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 Pall PRC prepacked columns (Figure 6).

Figure 5

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

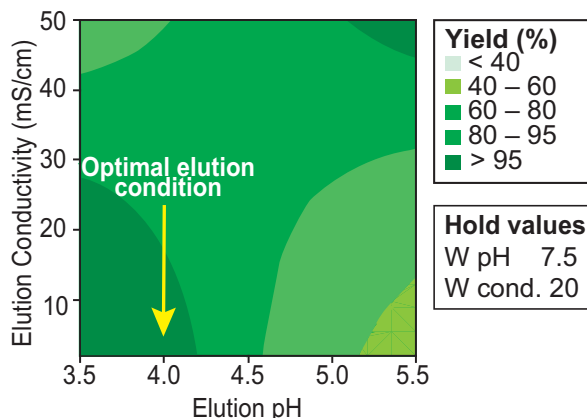
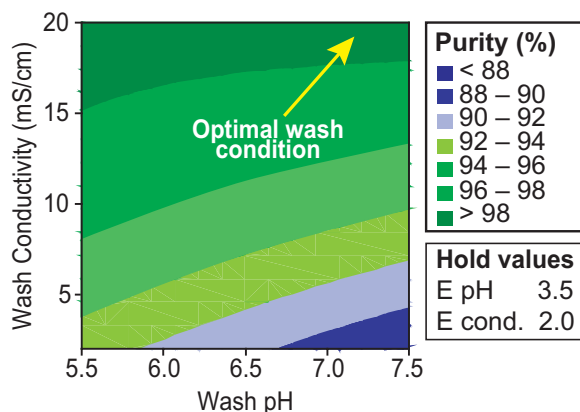
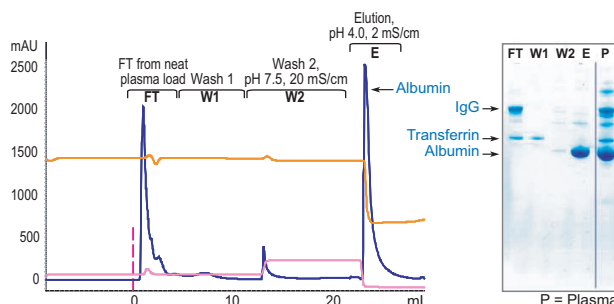


Figure 5 shows that HSA yield is impacted mainly by elution conditions (optimal zone: pH 3.5 – 4.2 and conductivity 2 – 27 mS/cm) while purity is impacted mainly by wash conditions (high conductivity wash at >15 mS/cm improves purity).

Figure 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 sorbent in a 1 mL PRC prepacked column. Chromatogram (Figure 6) and SDS-PAGE analysis of fractions 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 sorbent 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 sorbent 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 (S HyperCel sorbent).

This last polishing step on S HyperCel sorbent led to a purified fraction of HSA eluted at pH 7.0 with purity >99%, and a capacity around 65 mg/mL (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 sorbent	Undiluted plasma	> 30 mg/mL	90%	99%
Polishing on S HyperCel sorbent	pH 4.0 eluate from HyperCel STAR AX sorbent	65 mg/mL	95%	> 99%

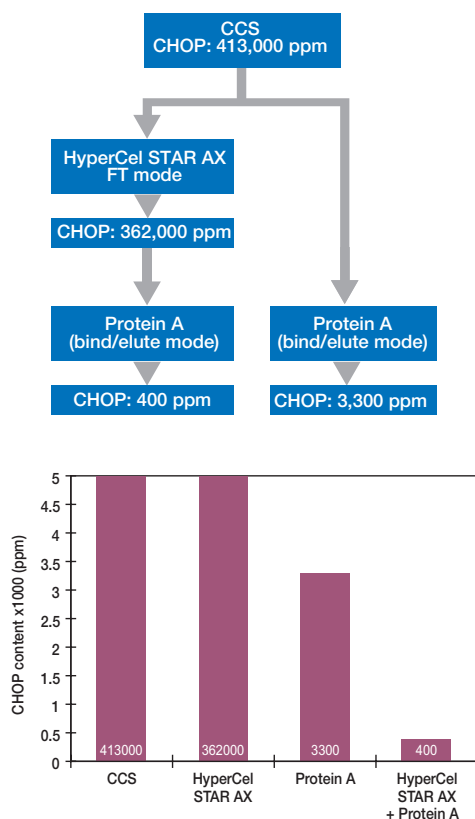
Application 2. Early Removal of CHOPs before Protein A Capture of a Monoclonal Antibody (MAb)

The objective of this study was to evaluate the impact of a pre-purification step using HyperCel STAR AX sorbent before conventional Protein A sorbent capture of a monoclonal antibody (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 Sorbent



Data shows that using HyperCel STAR AX sorbent 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

HyperCel STAR AX Sorbent

Description	Part Number
5 mL	20197-018
25 mL	20197-026
100 mL	20197-032
1 L	20197-046
5 L	20197-058
10 L	20197-064

HyperCel STAR AX PRC Prepacked Columns

Description	Part Number
PRC Column 5x50 HyperCel STAR AX, 1 mL	PRCSTARAX1ML
PRC Column 8x100 HyperCel STAR AX, 5 mL	PRCSTARAX5ML

HyperCel STAR AX Sorbent Plates

Description	Part Number
AcroPrep ScreenExpert Plates : HyperCel STAR AX Anion Exchange Sorbent	96WPSTARAX50



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