

PuraBead® HF Ion Exchange Adsorbents

Near mono-disperse high performance **anion** & **cation** exchange adsorbents with excellent flow properties designed for capture, intermediate purification and polishing steps - engineered for use in demanding process applications



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Ion Exchange Principles

lon exchange (IEX) chromatography is used to separate proteins based on their net charge at a particular pH. A protein is positively charged when the pH is below the pl of the protein and is negatively charged when the pH is above the pl of the protein.

IEX chromatography separates proteins by a reversible interaction between the charged protein and an oppositely charged adsorbent. Typically, binding is performed in a low salt buffer and elution is achieved by the addition of salt and/or adjustment of pH using either a step-wise or gradient elution.

Anion exchangers (positively charged adsorbents) are used when the protein(s) to be bound are negatively charged, whereas cation exchangers (negatively charged adsorbents) are used when the protein(s) to be bound are positively charged.

lon exchangers are categorised as either strong or weak, defined by the pH range over which the adsorbent remains charged, with strong ion exchangers being charged over a wider pH range than weak ion exchangers.

Astrea Bioseparations Ltd has developed four IEX adsorbents which have broad applicability: DEAE PuraBead® HF (weak anion exchanger); Q PuraBead® HF (strong anion exchanger); CM PuraBead® HF (weak cation exchanger) and SP PuraBead® HF (strong cation exchanger).

PuraBead[®] 6 Agarose Beads

PuraBead® 6 is a 6% agarose bead material manufactured using a novel method which does not require the use of organic solvents in the beading process and provides uniform near mono-disperse beads with excellent flow properties.

After beading, the matrix is cross-linked to provide a material capable of supporting very high flow rates and which can be further derivatized with synthetic ligands. The plot below demonstrates the different particle size distribution between Astrea's PuraBead® 6% near mono-disperse agarose beads.



PuraBead® 6% Near Mono-disperse Agarose Beads





x 1000 magnification

ficación

Conventional Poly-disperse Agarose Beads



PuraBead® HF IEX Adsorbents

Astrea's ion exchange products are agarose-based chromatography adsorbents with excellent flow properties and high binding capacities. SP PuraBead® HF is intended for use in downstream process capture steps but can also be used for polishing applications. Q, DEAE and CM PuraBead® HF have been designed for use in capture, intermediate purification and polishing applications. They benefit from Astrea's proprietary PuraBead® 6HF base matrix technology with a near mono-disperse particle size distribution which provides high resolution, reduces the risk of column fouling and renders the adsorbents easy to pack.

Key Benefits

- Uniform particle size (PuraBead® 6HF) and easy to pack
- Excellent flow properties
- Good resolution & high binding capacities
- Cost effective & reliable
- Easy to clean (robust, long life adsorbents)
- PuraBead® 6HF base matrix manufactured using a solvent free process

Superior Performance

Easy to pack

The chromatogram below shows typical packing results for Astrea's PuraBead® HF IEX adsorbents (10 cm diameter column, ~ 14 cm bed height), providing asymmetry results in the range of 0.8 to 1.2.



Plates/meter (N / m): 3854 Asymmetry: 0.96

Excellent Flow Properties

PuraBead® HF IEX adsorbents provide excellent flow rates and low back pressures. In comparison to competitor agarose IEX resins the Astrea near mono-disperse beads provide improved flow rates (10 cm diameter column, 20 cm bed height).



Buffer conditions: 10 mM sodium acetate, 100 mM NaCl, pH 5.2

Anion & Cation Exchange

Astrea PuraBead® HF IEX adsorbents enable high resolution separations. The chromatograms below show the resolution of three proteins from a protein mixture using DEAE PuraBead® HF (Anion) and four proteins from a protein mixture using CM PuraBead® HF (Cation).

DEAE PuraBead® HF



Elution buffer: 20 mM Tris, 0 to 0.5 M NaCl gradient, pH 7.4 Column dimensions: 1 cm diameter, 10.6 cm bed height (8.3 mL CV) Flow rate: 150 cm/h

CM PuraBead® HF



Elution buffer: 20 mM sodium phosphate, 0 to 0.5 M NaCl gradient, pH 6.8

Column dimensions: 1 cm diameter, 10.6 cm bed height (8.3 mL CV) Flow rate: 150 cm/h

Properties

	DEAE PuraBead® HF	Q PuraBead® HF	CM PuraBead® HF	SP PuraBead® HF
Type of ion exchanger:	Weak anion	Strong anion	Weak cation	Strong cation
Functional group:	Diethylaminoethyl (DEAE)	Quarternary ammonium (Q)	Carboxymethyl (CM)	Sulphonic acid with a propyl linker (SP)
Base matrix:	PuraBead® 6HF (6% cross-linked near monodisperse agarose)			
Total ionic capacity:	110 - 160 μmol/g settled gel	40 - 60 μmol/g settled gel	90 to 130 µmol/g settled gel	≥160 µmol/g settled gel
Operational flow rate:	Up to 600 cm/h (up to 1 bar)			
Dynamic binding capacity (10% breakthrough):	≥ 50 mg/mL of adsorbent (100 cm/h, BSA)	> 64 mg/mL of adsorbent (100 cm/h, HSA)	> 95 mg/mL of adsorbent (60 cm/h, lysozyme)	≥ 95 mg/mL of adsorbent (300 cm/h, lysozyme)
Working pH range:	pH 2.0 to pH 9.0	pH 2.0 to pH 12.0	pH 5.0 to pH 10.0	pH 4.0 to pH 12.0
Operational pH range (short term):	pH 2.0 to pH 14.0	pH 2.0 to pH 14.0	pH 4.0 to pH 14.0	pH 4.0 to pH 14.0
Chemical stability:	Stable in all commonly used buffers and solutions			
Clean in place/ sterilization:	0.5 to 1.0 M NaOH (autoclavable in 0.1 M saline at 121 $^\circ\text{C}$ for 30 minutes)			
Storage:	2 - 30 °C, 20% ethanol	2 - 30 °C, 20% ethanol	2 - 30 °C, 20% ethanol	2 - 30 °C, 20% ethanol/ 0.2 M sodium acetate

Purification Solutions

Recombinant insulin

The SDS-PAGE images below show the capture and purification of recombinant single chain insulin using a two-step process -

(1) capture using Astrea's Insulin Adsorbent and

(2) polishing using Astrea's SP PuraBead® HF.

Step 1



Non-reduced SDS-PAGE of the process fractions for the purification of recombinant single chain insulin using Astrea's Insulin Adsorbent. Lane 1 - mw marker; Lane 2 - load; Lane 3 - flow through; Lane 4 - post load wash; Lane 5 - elution (dilution 1 in 3); Lane 6 - strip; Lane 7 - CIP



Non-reduced SDS-PAGE of the process fractions for the purification of recombinant single chain insulin using Astrea's SP PuraBead® HF. Lane 1 - mw marker; Lane 2 - load (Insulin Adsorbent elution fraction); Lane 3 - post load wash; Lane 4 elution; Lane 5 - CIP; Lane 6 - blank; Lane 7 - insulin standard

Easy to Clean

Robust & Long-Life Adsorbents

The chromatograms below show the performance of SP PuraBead $^{\odot}$ HF over 200 cycles when used for purification of a plasma protein. The column was cleaned with 0.5 M NaOH every cycle.



215 cycle reuse study with a PuraBead® ion exchanger



1ml and 5ml pre-packed disposable columns

Ordering Information

For small orders up to 500 mL, please visit our webshop.

We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes.

Adsorbent Gel Slurry

Pack Size	DEAE PuraBead® HF	Q PuraBead® HF
25 mL	3451-00025	3452-00025
100 mL	3451-00100	3452-00100
500 mL	3451-00500	3452-00500
1 L	3451-01000	3452-01000

Pack Size	CM PuraBead® HF	SP PuraBead® HF
25 mL	3450-00025	3453-00025
100 mL	3450-00100	3453-00100
500 mL	3450-00500	3453-00500
1 L	3450-01000	3453-01000

Pre-Packed Columns

Astrea offer a range of pre-packed columns from a 1 mL and 5 mL column kits and 5 mL and 50 mL EvolveR columns for research and development as well as a range of up to 6.28 L GMP ready columns for pilot to process scale manufacturing.

For more details please visit our website or for more information on this or any other supply related matters please do not hesitate to contact us.

Contact Us:

With sales and support offices in North America and Europe, R&D facilities in Cambridge, UK and manufacturing facilities located on the Isle of Man, British Isles and in Joliette, QC, Canada we are able to meet your needs and support your application wherever you are.

Sales and Technical Support

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IEX Adsorbent Brochure - V6

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