Technical
User Guide

Anion Exchange Adsorbents

Q PURABEAD® HF
Product Code: 3452

DEAE PURABEAD® HF
Product Code: 3451

INTRODUCTION

Anion exchange adsorbents have been designed for capture, intermediate or polishing steps for the purification of negatively charged bio-molecules.

Q PuraBead® HF is a high performance strong anion exchange chromatography adsorbent with a quaternary ammonium group (Q).

DEAE PuraBead® HF is a high performance weak anion exchange chromatography adsorbent with a diethylaminoethyl group.

Both ligands are coupled to highly cross-linked near monodisperse 6% beaded agarose (PuraBead® 6HF) which has excellent flow properties. Both adsorbents are stable in up to 1.0 M sodium hydroxide which allows for stringent cleaning and sanitization protocols.

Properties of anion exchange adsorbents:

ADSORBENT:	**Q PuraBead® HF**	**DEAE PuraBead® HF**
LIGAND:	Quaternary ammonium	Diethylaminoethyl
TYPE OF ION EXCHANGER:	Strong Anion	Weak Anion
TOTAL IONIC CAPACITY:	44 - 68 µmol/g settled gel	110 to 160 µmol/g settled gel
MEAN PARTICLE SIZE (µm):	90 ± 10 µm	
MATRIX:	PuraBead® 6HF (Highly cross-linked 6% near monodisperse agarose	
BINDING CAPACITY:	75 - 110 mg/g of adsorbent (BSA)	76 - 120 mg/g of adsorbent (BSA)
RECOMMENDED PACKING CONDITIONS:	Pack at a constant pressure of 1.5 bar (~ 22 psi)	
RECOMMENDED PACKING SOLUTION:	0.1 M NaCl solution or equilibration buffer	
RECOMMENDED OPERATIONAL FLOW RATES:	Up to 600 cm/h	
OPERATING PH:	pH 2.0 – pH 14.0	
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents	
CLEANING/SANITIZATION:	0.5 to 1.0 M NaOH	
STERILIZATION:	Autoclavable in 0.1 M NaCl solution at 121 °C for 30 minutes	
STORAGE:	2 – 30 °C, 20% ethanol	

COLUMN PACKING

The anion exchange adsorbents are supplied in a preservative containing 20% ethanol. Due to the presence of ethanol, there may initially be an increased back pressure during the pack; however, this should reduce after ~ 1 column volume (CV). There is no requirement to remove the preservative prior to packing. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of Astrea Bioseparations' anion exchange adsorbents into axial columns:

1. Assemble the column and remove air from the dead spaces by flushing the end piece and adaptor with packing solution (0.1 M NaCl solution or equilibration buffer) then close the column outlet.

2. Allow all materials to equilibrate to the temperature at which the chromatography process is to be performed.

3. If required to obtain a fixed bed height (i.e. for larger column packs), it is recommended to determine the slurry percentage. For example, weigh a sample of the complete slurry, drain away the preservative and re-weigh the adsorbent. The final weight over the total weight will determine the slurry percentage. The compression factor of this adsorbent is 1.15 to 1.20.

4. Carefully pour the adsorbent slurry into the column in a single continuous step. Pouring the adsorbent down the side of the column helps to prevent air becoming trapped within the adsorbent bed.

5. Allow the adsorbent to settle in the column leaving a dead volume of packing solution above the adsorbent bed.

6. Attach the (open) top adaptor to the top of the column and adjust the adaptor to ~1 cm above the bed, tighten the adaptor and attach to the workstation. Open the column outlet and apply the desired flow to the bed (for ~ 2 CV). The recommended packing condition (to obtain a uniform pack) is at a constant pressure of 1.5 bar (~ 22 psi). The flow rate is dependent on column dimensions, however, will range from 700 to 1000 cm/h.

7. Once the adsorbent has packed (after ~ 2 CV), measure and mark the bed height under packing flow and close the column outlet and stop the liquid flow through the bed.

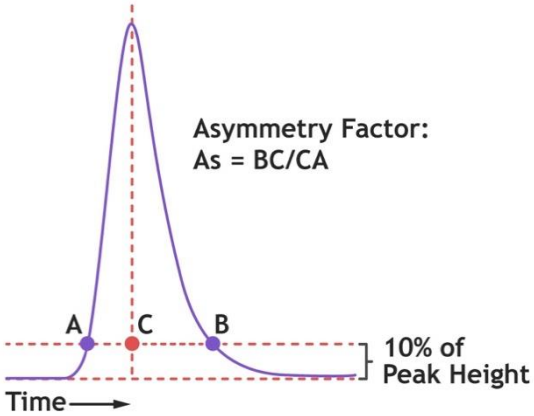
8. Lower the top adaptor (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to up to ~1 cm below the position of the marked bed height.

   Note: once the flow is paused the bed may relax and rise. The seal of the top adaptor may need to be loosened to allow the adaptor to be lowered.

9. Re-tighten the top adaptor (if loosened) and attach back to the workstation (or switch valve back in-line). Apply the packing flow to the column again for ~ 1 CV. If a space is formed between the top of the bed and the adaptor repeat the steps above. If no space forms the column is packed and ready to use.

COLUMN EFFICIENCY TEST

1. Test the column at a flow rate of 100 cm/h.
2. Attach the column to an equilibrated workstation.
3. Commence flow for 1 CV (i.e. to equilibrate and obtain baseline).
4. Inject 2% to 5% CV of a 2% acetone or 2 M NaCl solution.
5. Continue flow until a UV (or conductivity) peak is observed and the trace has returned to baseline (1 to 1.5 CV).
6. End run and determine the asymmetry factor:



7. The recommended asymmetry factor for packed anion exchange adsorbents is between 0.8 to 1.2. The recommended plate count for an acceptable pack is ≥ 2000 N/m.

OPERATING INSTRUCTIONS

Note: The following recommendations are not prescriptive and thorough investigation of these parameters at small-scale should be conducted to reveal the level of flexibility that can be tolerated with the chromatography adsorbent, buffer and protein combination selected. AIEX column kits are also available for screening experiments.

The following method is recommended (as a starting point), using an initial flow rate of 100 cm/h for the column chromatography steps. Subsequent increases/decreases in the flow rate can be investigated to improve binding capacity/resolution or decrease processing times.

Filter all buffers and feedstock through an appropriate filter, prior to running the column.

1. Equilibrate the column with up to 5 CV of equilibration buffer 20 mM Tris buffer, pH 7.5. Other buffers suitable for use with Astrea Bioseparations' anion exchange adsorbents to obtain optimal binding include sodium phosphate, sodium citrate and HEPES.

   Note: The equilibration buffer pH and conductivity should match that of the protein feedstock. AIEX adsorbents are designed for adsorption of negatively charged proteins (pH > pI). It is recommended to use an equilibration buffer with a pH of at least 0.5 units above the isoelectric point (pI) of the target protein. The ionic strength of the equilibration buffer should also be low, with preferably no or minimal salt present.

2. Apply the clarified / filtered protein feedstock onto the equilibrated column. Recommended residence time of 3 minutes (or greater).

3. Remove any non-bound material in the column with up to 5 CV of equilibration solution/ buffer, or until the UV trace returns to baseline.

4. Elute the bound protein by increasing the conductivity of the solution with up to 5 CV of elution buffer. Any of the recommended equilibration buffers with the addition of up to 1.0 M sodium chloride (NaCl) would be suitable.

   For initial investigations, it is recommended to carry out a salt elution gradient (e.g. 20 CV from 0 to 1.0 M NaCl in equilibration buffer) to determine the appropriate elution condition for your target bio-molecule and will also identify a purification strategy (i.e. separation of non-target proteins).

5. If a CIP is required, use up to 5 CV of 0.5 to 1.0 M NaOH. A contact time of 1 hour will normally suffice to ensure destruction of viable organisms, although up to 5 hours contact time may be required. No less than 5 column volumes are recommended.

6. Re-equilibrate column with up to 5 CV of equilibration buffer (to remove sodium hydroxide) and check pH and conductivity of the column eluate is equal to that of the buffer entering the column before storage or re-use.

7. If the column is to be stored for future use, place the column into the storage solution (20% ethanol recommended, 0.1 M NaOH acceptable for short term storage) and store at 2 – 30 °C.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3452-00025	Q PuraBead® HF	25 mL
3452-00100	Q PuraBead® HF	100 mL
3452-00500	Q PuraBead® HF	500 mL
3452-01000	Q PuraBead® HF	1000 mL

Code	Description	Pack Size
3451-00025	DEAE PuraBead® HF	25 mL
3451-00100	DEAE PuraBead® HF	100 mL
3451-00500	DEAE PuraBead® HF	500 mL
3451-01000	DEAE PuraBead® HF	1000 mL

Astrea Bioseparations also supplies larger volumes of bulk resins for cGMP development and manufacturing scale processes.

Column Kits

Code	Description	Pack Size
6600	Q PuraBead® HF	4 x 1 mL columns
6601	Q PuraBead® HF	4 x 5 mL columns
6602	DEAE PuraBead® HF	4 x 1 mL columns
6603	DEAE PuraBead® HF	4 x 5 mL columns
6608	IEX selection kit (1 mL columns)	1 x 1 mL Q PuraBead® HF 1 x 1 mL SP PuraBead® HF 1 x 1 mL CM PuraBead® HF 1 x 1 mL DEAE PuraBead® HF
6609	IEX selection kit (5 mL columns)	1 x 5 mL Q PuraBead® HF 1 x 5 mL SP PuraBead® HF 1 x 5 mL CM PuraBead® HF 1 x 5 mL DEAE PuraBead® HF

Astrea Bioseparations can also provide column packing services. For more information on this, or any other matters please do not hesitate to contact us at sales@astrea-bio.com

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