Technical
User Guide

Anion Exchange Adsorbents

Q PURABEAD® Edge
Product Code: FG00476

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PURITY by DESIGN

INTRODUCTION

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

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

Strong anion exchange ligands are coupled to highly cross-linked near monodisperse 6% beaded agarose (PuraBead® P60HF) which has excellent peak separation and flow properties. PuraBead® Edge 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® Edge
LIGAND:	Quaternary ammonium
TYPE OF ION EXCHANGER:	Strong Anion
TOTAL IONIC CAPACITY:	80-117 µmol/g settled gel
MEAN PARTICLE SIZE (µm):	65 ± 10 µm
MATRIX:	PuraBead® P60HF (Highly cross-linked 6% near monodisperse agarose)
BINDING CAPACITY:	Up to 72 mg/ml 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 300 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). 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) then close the column outlet.

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

3. Remove the excess of storage solution by draining. Weigh out a sufficient amount of resin to pack the column. The expected compression factor for the column is 1.11 -1.15.

4. Resuspend resin to create a 50% slurry in packing solution.

5. 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.

6. Allow the adsorbent to settle in the column leaving a dead volume of packing solution above the adsorbent bed. Top column up with packing solution.

7. Attach the (open) top adaptor to the top of the column and adjust the adaptor to just above the bed, tighten the adaptor and attach to the workstation. Open the column outlet and apply a flow rate of 700 cm/h.

8. Once the adsorbent has packed (after ~ 5 CV), measure and mark the bed height under packing flow, close the column outlet and stop the liquid flow through the bed.

9. Lower the top adaptor by loosening the top adaptor seal (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 1 mm below the marked bed height (do not push the top adaptor further into the adsorbent bed).

10. **Note:** Once the flow is paused the bed may relax and rise.

11. Re-tighten the top adaptor (if loosened) and attach back to the workstation (or switch valve back in-line). Open the bottom outlet and apply the packing flow to the column again for 5 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 75 cm/h.

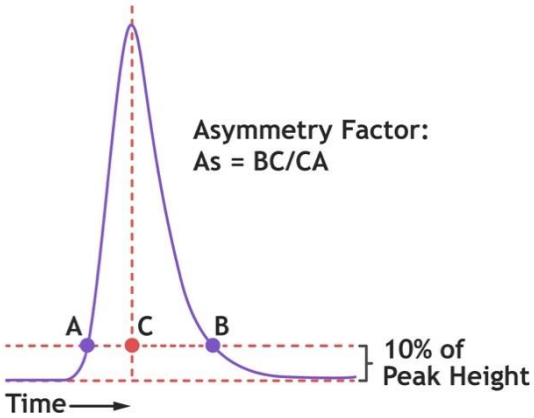
2. Attach the column to an primed workstation.

3. Commence flow for at least 1 CV ensuring that the column is equilibrated and A baseline obtained.

4. Inject 2% to 5% CV of a 2% acetone or 0.8 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 5minutes (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 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
FG00476-00025	Q PuraBead® Edge	25 mL
FG00476-00100	Q PuraBead® Edge	100 mL
FG00476-00500	Q PuraBead® Edge	500 mL
FG00476-01000	Q PuraBead® Edge	1000 mL

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

Column Kits

Code	Description	Pack Size
6752	Q PuraBead® Edge	1 x 1 mL column
6753	Q PuraBead® Edge	1 x 5 mL column
6652	Q PuraBead® Edge	4 x 1 mL columns
6653	Q PuraBead® Edge	4 x 5 mL columns

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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