Technical
User Guide

Cation-Exchange Adsorbents

SP PuraBead® Edge
Product Code: FG00561

INTRODUCTION

Cation-exchange adsorbents are designed for capture, intermediate, or polishing steps for the purification of target biomolecules by interaction with positively charged species.

SP PuraBead® Edge is a high-performance, strong cation-exchange chromatography adsorbent with a sulphopropyl group (SP).

Strong cation-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 cation-exchange adsorbents

ADSORBENT:	SP PuraBead® Edge
LIGAND:	Sulphopropyl group
TYPE OF ION EXCHANGER:	Strong cation
TOTAL IONIC CAPACITY:	97 to 163 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 95 mg/mL of resin (Lysozyme, when loaded to 10% breakthrough)
RECOMMENDED PACKING CONDITIONS:	Pack at a constant pressure of up to 3.5 bar
RECOMMENDED PACKING SOLUTION:	1 M NaCl solution
RECOMMENDED OPERATIONAL FLOW RATES:	Up to 400 cm/h
OPERATING PH:	pH 4.0 to 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, 0.2 M sodium acetate

COLUMN PACKING

This cation-exchange resin is supplied in a preservative containing 0.2 M sodium acetate, 20% ethanol. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column hardware.

The method below describes the packing of Astrea Bioseparations' SP PuraBead® Edge into axial 1 cm, 1.6 cm and 2.6 cm diameter columns with a 10 cm bed height.

Preparation of adsorbent slurry

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

2. Remove the presence of storage solution by draining and washing at least 3 times with the packing solution. Weigh out a sufficient amount of resin to pack the column. The recommended compression factor (CF) when packing the SP PuraBead® Edge resin is 1.20 -1.26.

   Compression factor is defined as the bed height (BH) achieved after settling by gravity divided by the bed height achieved after packing under flow. The CF is then used to calculate the required resin volume for a target bed height and subsequently the required slurry volume. It is important to accurately determine the required slurry volume in order to achieve a good pack.

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

   Example:

   Column volume (CV) calculation:

$$CV = \pi \times r^2 \times BH$$

$$CV = \pi \times 0.5^2 \times 10 = 7.85\ mL$$

   Where 'r' is the radius of the column hardware in cm and 'BH' is the target bed height in cm.

   Slurry volume (SV) calculation:

$$SV = \frac{CV \times CF}{Slurry\ percentage}$$

$$SV = \frac{7.85 \times 1.26}{0.5} = 19.78\ mL$$

   Where 'CV' is the column volume calculated above, 'CF' is the compression factor, and 'slurry percentage' is the slurry concentration.

   Therefore, for a 10 cm bed height in a 1 cm diameter column, 9.89 g of resin in a 50% slurry with 9.89 mL of packing solution is required.

Column hardware preparation and transfer

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

5. Carefully pour the resin slurry into the column in a single continuous step. Pouring the slurry down the side of the column helps to prevent air becoming trapped within the resin bed. Use a packing reservoir if required.

6. Allow the resin to settle for at least 1 hour in the column, leaving a dead volume of packing solution above the resin bed. Top up the column with packing solution.

Packing the column

7. Attach the (open) top adaptor to the top of the column and adjust the adaptor to approximately 2 mm above the bed; tighten the adaptor and attach to the workstation.

8. Open the column outlet and apply a flow rate until a stable pre-column pressure of 0.25 to 0.35 MPa is achieved. To avoid bed compression on operation, ensure that the packing flow rate is higher than the operational flow rate at the process step with the highest pre-column pressure. Aim for an operational flow rate of up to 75% of the column packing flow rate.

9. Once the resin 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.

10. 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 resin bed).

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

11. Retighten 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 at least 2 CV, ensuring the pre-column pressure does not exceed 0.50 MPa. 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

Test procedure

1. Attach the column packed according to the 'Column Packing' section above to a workstation primed with a mobile phase solution of 300 mM NaCl or buffer of a conductivity of approximately 30 mS/cm.

2. Commence flow of mobile phase at 150 cm/h for at least 1 CV ensuring that the column is equilibrated and a baseline obtained.

3. Inject 2% CV of a 2 M NaCl solution.

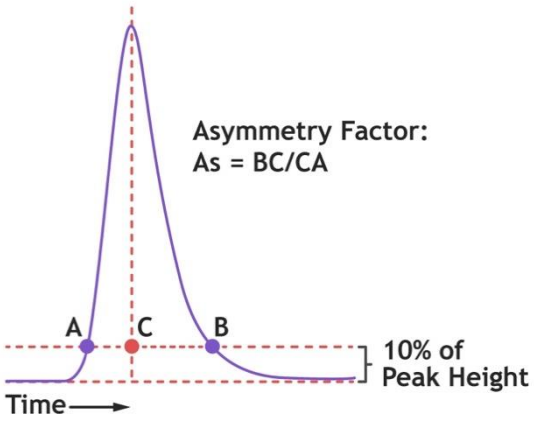
4. Continue flow until a conductivity peak is observed, and the trace has returned to baseline (≥ 1.5 CV).

Asymmetry determination

Determine the asymmetry (As) factor as follows:

$$As = \frac{BC}{CA}$$

Where 'BC' is the peak tail width at 10% peak height and 'CA' is the peak front width at 10% peak height.



A typical acceptable range for asymmetry factor for packed cation-exchange adsorbents is between 0.8 to 1.2.

Asymmetry trouble shooting

12. As > 1.2 (Tailing) indicates that the column is **under packed**. For asymmetry values significantly above 1.2, aim for a higher compression factor, target a higher packing pressure (> 0.25 MPa), or extend the number of CV's that the packing flow is applied for.

13. As < 0.8 (Fronting) indicates that the column is **over packed**. For asymmetry values significantly below 0.8, ensure a high-salt packing solution is being used, target a lower compression factor, or ensure that the packing pressure does not exceed 0.50 MPa.

Efficiency (HETP) determination

Determine the HETP and N values as follows:

Theoretical plates (N) value is a measure of the peak broadening and can be used to determine the column efficiency. The higher the plates value, the less dispersion and the more efficient peaks and separation. The plates (N) value is calculated by:

$$N = 5.545 \times \left(\frac{V_R}{W_h}\right)^2$$

Where 'V_R' is the retention volume and 'W_h' is the peak width at half of the peak height.

HETP (H) is used to determine the column efficiency and corresponds to the distance between each plate. HETP is calculated by:

$$H = \frac{L}{N}$$

Where 'L' is the length of the column (bed height) and 'N' is the number of theoretical plates (as calculated above).

Plate count range required for the column should be verified for each process/application. The typical theoretical plates per meter count for an acceptable pack is ≥ 2000 N/m.

HETP trouble shooting

If the calculated plates/meter value is significantly below 2000 N/m, repack the column and repeat column efficiency testing.

The lower the HETP value (the higher the plate number), the more efficient the column is. When measured over time, the HETP can be used to monitor the column performance. If the HETP value increases, this indicates a reduction in the column performance and the column should be repacked.

OPERATING INSTRUCTIONS

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

The following method is recommended (as a starting point) using a 10 cm bed height, 1 cm diameter column, 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. Aim for an operational flow rate of up to 75% of the column packing flow rate.

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

1. Equilibration

Equilibrate the column with up to 5 CV of equilibration buffer. The equilibration buffer pH and conductivity should be chosen to allow selectivity with respect to the target biomolecule. CIEX resins are designed for adsorption of positively charged biomolecules (pH < pI). It is recommended to use an equilibration buffer with a pH of at least 0.5 units (but ideally 1 unit) below the isoelectric point (pI) of the target species. The ionic strength of the equilibration buffer should also be low, with preferably no or minimal salt present.

2. Load and post-load wash

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

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.

3. Elution

Elute the bound protein by increasing the conductivity or the pH 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) or a pH of at least 2 units above the equilibration solution would be suitable.

For initial investigations, it is recommended to carry out an 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 biomolecule and identify a purification strategy (i.e. separation of non-target proteins).

4. Regeneration

After each use the resin should be regenerated with at least 2 CV of a high-salt buffer (1.0 M NaCl). This is generally included in the elution phase of the run, but if a pH gradient is used, a separate regeneration phase should be implemented.

5. Cleaning and sanitization

A clean-in-place (CIP) step is recommended to avoid buildup of process impurities over multiple cycles. This maintains column efficiency, capacity, and separation performance.

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.

When a more intensive cleaning cycle is required, the following are recommended:

- If lipid fouling is a major issue, use 30%–40% isopropanol in combination with NaOH.
- If iron and calcium fouling is an issue, use 50% citric acid.
- If aggregated/precipitated proteins are an issue or a crude lysate feedstock has been loaded onto the column, wash the resin with either 8 M urea or 6 M Guanidine-HCl.

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

6. Storage

If the column is to be stored for future use, place the column into the storage solution (20% ethanol, 0.2 M sodium acetate recommended; 0.01 M NaOH acceptable for short-term storage up to 1 week) and store at 2–30 °C.

ORDER INFORMATION

Gel slurry

Code	Description	Pack Size
FG00561-00025	SP PuraBead® Edge	25 mL
FG00561-00100	SP PuraBead® Edge	100 mL
FG00561-00500	SP PuraBead® Edge	500 mL
FG00561-01000	SP 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
6754	SP PuraBead® Edge	1 x 1 mL column
6755	SP PuraBead® Edge	1 x 5 mL column
6654	SP PuraBead® Edge	4 x 1 mL columns
6655	SP PuraBead® Edge	4 x 5 mL columns
4300-00005-FG00561	SP PuraBead® Edge	Evolve® R 5 mL

Astrea Bioseparations also offers column packing services. For more information on this or any other inquiries, please feel free to contact us at sales@astrea-bio.com

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