Technical User Guide

Aminophenylboronate A6XL

Product Code: 0355

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Astrea Bioseparations

Introduction

Aminophenylboronate affinity adsorbents are used for the purification of a diverse range of macromolecules which possess 1,2-diols, 1,3-diols, 1,2-hydroxy acids and 1,2-hydroxylamine groups. These functionalities are present in glycoproteins, carbohydrates, nucleic acids (nucleosides, nucleotides and RNA's) and polyphenols (catechols, flavonoids). Aminophenylboronate Agarose affinity adsorbents are particularly suited to the purification of glycoproteins, or the removal of glycoprotein and carbohydrate impurities form non-glycosylated molecules.

These adsorbents can also be used for the purification and removal of certain enzymes such as proteases and hydrolases where the boronic acid group has affinity for the active site.

The chemically stable affinity ligand is bonded to 6% cross-linked agarose beads to produce a highly robust adsorbent which is resistant to concentrated sodium hydroxide and suitable for use in downstream purification process applications and incorporation into diagnostic assays.

Properties of Aminophenylboronate A6XL:

Ligand:	m-Aminophenylboronic acid
Adsorbent appearance:	White
Mean particle size (µm)	90 µm (45-165 µm ≥95% of beads within this range)
Matrix:	Highly cross-linked 6% agarose
Binding capacity:	Glycoproteins – in the range of 10-20 g/L Carbohydrates – up to 30 g/L
Recommended packing conditions:	Packing pressure – up to 3 bar
Recommended packing solution:	0.1 M NaCl solution or any process buffer (not NaOH)
Recommended operational flow rates:	Up to 500 cm/hr with column diameter less than 3 cm Up to 200 cm/hr with column diameter greater than 5 cm
Operating pH:	pH 2 to 14 (intermittent)
pH stability:	Long term (3 months) pH 3 to 12
Chemical stability:	All commonly used aqueous buffers and co-solvents
Cleaning/sanitization:	0.5 to 1.0 M NaOH
Storage:	2 – 30 °C, 20% ethanol

Column Packing

Aminophenylboronate A6XL is supplied in 20% ethanol solution. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of Aminophenylboronate A6XL into axial columns:

1. Decant off the shipping preservative and prepare a 50% slurry of the adsorbent with either 0.1 M NaCl solution or equilibration buffer (packing solution).

2. 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.

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

4. If required to obtain a fixed bed height (i.e. for larger column packs), it is recommended to accurately 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.

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.

7. 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 up to but not exceeding 3 bar (~45 psi).

8. 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.

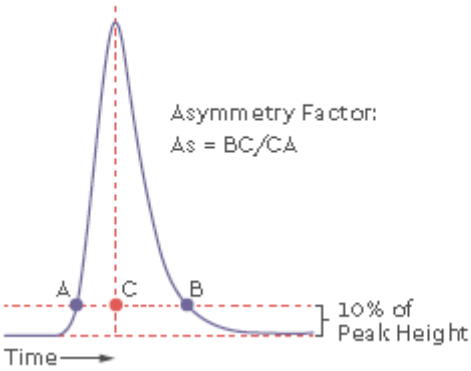
9. Lower the top adaptor (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to up to ~1 cm below the position of the marked bed height.

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 Aminophenylboronate A6XL 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. Aminophenylboronate A6XL 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 is recommended. 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.

- **Sample treatment:**

  Clarify the feedstock/protein solution using an appropriate filter and adjust the pH and or conductivity of the solution if required.

- **Equilibration buffer:**

  50mM Glycine NaOH buffer at pH 9.0 or 50mM sodium phosphate buffer at pH 8.0.

- **Wash buffer**:

  If a wash step is required to remove any loosely bound non-target material, initially a moderate amount of salt (0.2 – 0.6 M NaCl) can be added to the equilibration buffer to remove non-specifically bound proteins. Alternatively, the addition of up to 1.0 M Tris to the equilibration buffer can also have the same desired effect.

- **Elution buffer(s):**

  For selective desorption of the target protein use up to 200mM sorbitol in the equilibration buffer. Other recommendations are using buffers at low pH, see below for details:

  50 mM sodium acetate buffer at pH 4.0 or 50mM Glycine HCl buffer at pH 3.0

- **Cleaning-in-Place (CIP) and Sanitization:**

  Removal of any residual adsorbed material including micro-organisms, viruses and endotoxins can be achieved by washing the column with 0.5 M 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.

  Once cleaning with NaOH is complete, wash with at least 5 bed volumes of equilibration buffer until the pH and conductivity of the column eluate is equal to that of the buffer entering the column. Complete this process prior to further use or storage in the storage buffer.

- **Storage:**

  Aminophenylboronate A6XL should be stored in 20% ethanol at 2-30°C.

Recommended Column Chromatography Conditions for Using Aminophenylboronate A6XL

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

1. Equilibrate the column with 3 to 5 CV of equilibration buffer or until the pH/conductivity is at baseline (Note: ensure the equilibration buffer of the column is comparable to the protein feedstock).

2. Apply the protein feedstock onto the equilibrated column. A residence time of 3 minutes (or greater) is recommended.

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

4. Elute the bound protein using up to 5 CV of an appropriate elution buffer.

5. If a clean-in-place is required, use 5 CV of 0.5 M NaOH.

6. Re-equilibrate column with 5 CV of equilibration buffer (until the pH of the eluent is comparable to the equilibration buffer entering the column).

7. It is recommended to store the column in 20% ethanol at 2-30°C.

Order information

Gel Slurry

Code	Description	Pack Size
0355-00025	Aminophenylboronate A6XL	25 mL
0355-00100	Aminophenylboronate A6XL	100 mL
0355-00500	Aminophenylboronate A6XL	500 mL
0355-01000	Aminophenylboronate A6XL	1000 mL

We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes. For more information on this or any other supply related matters please do not hesitate to contact us on **sales@astrea-bio.com**

Column Kits

Code	Description	Pack Size
4444-0001	Aminophenylboronate A6XL Column kit	4 x 1 mL columns

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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

Please contact us for further sales or technical support information regarding any of our innovative biochromatography products and services.

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