Technical
User Guide

Aminophenylboronate P6XL

Product Code: 3355

Search: Astrea Bioseparations

Astrea Bioseparations

PURITY by DESIGN

INTRODUCTION

Aminophenylboronate P6XL is an affinity adsorbent 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 P6XL is particularly suited to the purification of glycoproteins, or the removal of glycoprotein and carbohydrate impurities form non-glycosylated molecules.

The adsorbent 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 covalently attached to a near monodisperse 6% cross-linked agarose bead (PuraBead® 6XL) to produce a 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 P6XL:

LIGAND:	m-Aminophenylboronic acid
ADSORBENT APPEARANCE:	White
PARTICLE SIZE:	100µm ± 10µm
BASE MATRIX:	PuraBead® 6XL (6% cross-linked near monodisperse agarose)
LIGAND DENSITY (IMMOBILIZED BORON):	900 ± 150 µmol/g dry gel
BINDING CAPACITY:	Glycoproteins – in the range of 10 - 20 g/L Sorbitol Binding Capacity: ≥ 20 µmol/mL
RECOMMENDED PACKING CONDITIONS:	At a constant pressure – up to 3 bar
RECOMMENDED PACKING SOLUTION:	0.1 M NaCl solution or any process buffer (not NaOH)
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 500 cm/hr with column diameter less than 3 cm Up to 200 cm/hr with column diameter greater than 5 cm
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents
OPERATING PH:	pH 2 to 14 (intermittent)
PH STABILITY:	Long term (3 months) pH 3 to 12
CLEANING / SANITIZATION:	0.5 - 1.0 M NaOH, 25 °C
STORAGE:	2 – 30 °C, 20% ethanol

COLUMN PACKING

Aminophenylboronate P6XL 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 P6XL 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 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 just above the bed, tighten the adaptor and attach to the workstation. Open the column outlet and apply the desired flow to the bed. The recommended packing conditions is to flow pack (to obtain a uniform pack) at a 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.

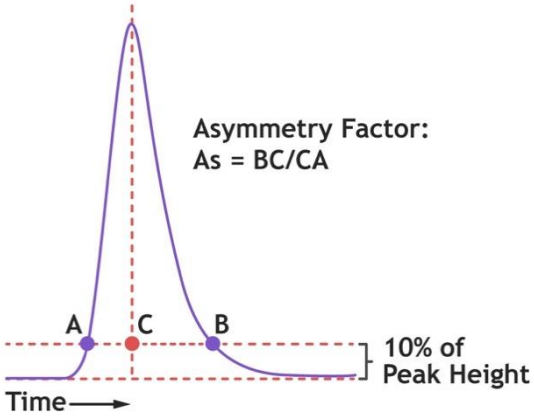
9. Lower the top adaptor by loosening the top adaptor seal (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to the position of the marked bed height (do not push the top adaptor further into the adsorbent bed).

   Note: once the flow is paused the bed may relax and rise. Depending on the pressure attained you may need to apply up to 1 cm axial compression.

10. 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 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. Aminophenylboronate P6XL is an affinity adsorbent, therefore an asymmetry factor for an acceptable pack is between 0.8 to 1.6. 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 recommended to reveal the level of flexibility that can be tolerated with the chromatography adsorbent, buffer and protein combination selected.

The following instructions are recommended (as a starting point). Filter all buffers and feedstock through an appropriate filter, prior to running the column.

An initial flow rate of 100 cm/h for all 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 up 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).

   Recommended equilibration buffers are 50 mM Glycine.NaOH, pH 9.0 or 50 mM sodium phosphate, pH 8.0.

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

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

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

4. If required, use an appropriate wash strategy to remove non-specifically bound material prior to elution.

   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.

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

   For selective desorption of the target protein use up to 200 mM sorbitol in the equilibration buffer.

   Other recommendations are using buffers at low pH, such as 50 mM sodium acetate, pH 4.0 or 50 mM Glycine.HCl, pH 3.0.

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

   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.

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

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

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3355-00025	Aminophenylboronate P6XL	25 mL
3355-00100	Aminophenylboronate P6XL	100 mL
3355-00500	Aminophenylboronate P6XL	500 mL
3355-01000	Aminophenylboronate P6XL	1000 mL

In addition, Astrea Bioseparations supply a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes as well as 1 mL and 5 mL column kits for scouting experiments.

Pre-packed Column Format

Code	Description
6616	Aminophenylboronate P6XL 4 x 1 mL Column kit
6617	Aminophenylboronate P6XL 4 x 5 mL Column kit

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

+44 (0) 1223 433 800 | astreabioseparations.com

sales@astrea-bio.com | techsupport@astrea-bio.com | quality@astrea-bio.com

Global bases in North America, Canada and Cambridge UK HQ:
Horizon Park, Barton Road, Comberton, Cambridge, CB23 7AJ, UK

Issue Date: 29 Mar 2022
CCR Number: CCR-1231
Author Name: R Dodd
QA Reviewer Name: R Hawkins

Astrea Bioseparations