Technical User Guide

p-Aminobenzamidine Agarose 6XL

Product Code: 0320

Astrea Bioseparations

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Introduction

p-Aminobenzamidine Agarose 6XL is an affinity adsorbent for the purification of serine proteases and esterases (i.e. trypsin, thrombin, kallikrein, urokinase, acetyl cholinesterase and alkaline phosphatase). p-Aminobenzamidine Agarose 6XL can be used in the separation of closely related proteases, for example, trypsin from chymotrypsin, or the removal of trace protease contamination from a final product.

p-Aminobenzamidine Agarose 6XL is immobilised onto a 6% cross-linked agarose via a stable spacer arm to provide a robust adsorbent. This product is manufactured in a controlled environment in accordance with an ISO 9001 quality system and is designed for use in cGMP manufacturing.

Properties of p-Aminobenzamidine Agarose 6XL:

Appearance:	White to off-white
Ligand:	para-Aminobenzamidine
Particle size:	45-165 µm (≥ 95% of beads within this range)
Base Matrix:	Cross-linked 6% agarose (Agarose 6XL)
Dynamic Binding capacity:	25 - 40 mg of Trypsin/mL of adsorbent
Recommended packing conditions:	0.1 M NaCl solution (at a constant pressure of up to 3 bar)
Recommended operational flow rates:	Up to 200 cm/h (up to 1 bar)
Chemical Stability:	All commonly used aqueous buffers and solutions within the operating pH
Operating pH:	pH 2 to 12
pH stability:	The guanidine moiety of the Aminobenzamidine ligand can degrade if exposed to pH values higher than pH 12.0, therefore **DO NOT** use NaOH (alkali conditions) for cleaning
Cleaning:	8 M Urea Low (pH 2.0) / High (pH 12.0) wash cycles Low (0 M) / High (1 M) NaCl wash cycles
Recommended storage condition*:	2 – 8 °C, 24% ethanol / 76% 0.1 NaCl (v/v) solution

* For short term storage and shipment (up to 1 month), pABA A6XL can be kept at ambient conditions (up to 25 °C) without any negative impact to adsorbent performance or ligand leachates

Column Packing

p-Aminobenzamidine Agarose 6XL is supplied in 24% ethanol / 0.1 M NaCl (v/v) solution. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of p-Aminobenzamidine Agarose 6XL 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. 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.

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 until a dead volume of packing solution above the adsorbent has formed (~ 2 cm).

6. 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 (to obtain a uniform pack) is at a constant pressure up to but not exceeding 3 bar (~ 45 psi).

   Note: The flow rate will be dependent on column dimensions, however, will range from 200 to 400 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.

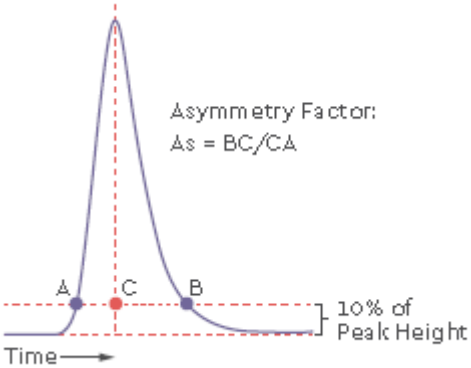
8. Lower the top adaptor by loosening the top adaptor seal (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to the position of the marked bed height.

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

9. 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. p-Aminobenzamidine Agarose 6XL 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 should be conducted to reveal the level of flexibility that can be tolerated with the chromatography adsorbent, buffer and protein combination selected.

p-Aminobenzamidine Agarose 6XL 1 mL and 5 mL Column Kits (6618 and 6619) are available for high throughput process optimization and scouting experiments.

The preferred option is to use p-Aminobenzamidine Agarose 6XL with a liquid chromatography system or automated workstation. Note: the adsorbents can also be operated manually using a peristaltic pump or even a syringe.

An initial flow rate of 50 cm/h for the column chromatography steps is recommended. Subsequent changes to the flow rate can be investigated to improve binding capacity / resolution or decrease processing times.

1. Equilibrate the column with 5 column volumes (CV) of equilibration buffer (e.g. 100 mM Sodium acetate, 10 mM calcium chloride, pH 5.5). Allow the column buffers and sample to reach the operational temperature.

2. Apply the clarified feedstock / sample onto the column. A minimum residence time of ≥ 3 minutes is recommended.

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

4. To remove any non-specifically bound material, p-Aminobenzamidine Agarose 6XL can be washed with up to 5 CV of 100 mM Sodium acetate, 10 mM calcium chloride, 900 mM sodium chloride, pH 5.5

5. Elute the bound enzyme/material with up to 5CV using 50 mM glycine-HCl, pH 2.2.

6. Depending on the nature of the feed and the extent of column fouling, conditions for CIP will require optimization depending on which of the recommended solutions is employed. If a Clean-in-Place (CIP) or sanitization is required, the following solutions are recommended for CIP of the column:

    i. 8.0 M Urea.

    ii. Low/high pH (i.e. pH 2.0/pH 12.0) wash cycles.

    iii. Low/high salt (i.e. 0 to 1.0 M NaCl) wash cycles.

    The adsorbent can also be cleaned using 20% ethanol/1.0 M acetic acid

    Note: Do **NOT** use sodium hydroxide (NaOH) as the ligand is not stable in alkali conditions (> pH 12.0).

7. Post CIP, wash the column with at least 5 CV of equilibration buffer to remove the CIP solution and check pH and conductivity of the column eluate is equal to that of the buffer entering the column before re-use or storage.

8. If required at a later date, store the column into the storage solution at 2 – 8 ºC. The recommended preservative is 24% ethanol / 76% 0.1 M NaCl.

Order information

Gel Slurry

Code	Description	Pack Size
0320-00025	p-Aminobenzamidine Agarose 6XL	25 mL
0320-00100	p-Aminobenzamidine Agarose 6XL	100 mL
0320-00500	p-Aminobenzamidine Agarose 6XL	500 mL
0320-01000	p-Aminobenzamidine Agarose 6XL	1000 mL

Please visit our webshop at https://www.astreabioseparations.com/product-category/serine-proteases-purification-removal/. We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes.

Column Kits

Code	Description	Pack Size
6618	p-Aminobenzamidine Agarose 6XL 1 mL HT Column Kit	4 x 1 mL columns
6619	p-Aminobenzamidine Agarose 6XL 5 mL HT Column Kit	4 x 5 mL columns

Astrea can also supply larger sized pre-packed columns for process development or small-scale manufacture. For more information on this or any other supply related matters please do not hesitate to contact us on **sales@astrea-bio.com**

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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