Technical
User Guide

AlbuPure®

Product Code: 3151

Search: Astrea Bioseparations

Astrea Bioseparations

PURITY by DESIGN

INTRODUCTION

AlbuPure® is a selective affinity chromatography adsorbent developed by Astrea Bioseparations Ltd and its affiliates (Astrea Bioseparations) in collaboration with Albumedix (formerly Novozymes Biopharma UK Ltd) for the purification of albumin-fusion proteins.

AlbuPure® is manufactured exclusively by Astrea Bioseparations in a controlled environment to ISO 9001 quality standard and is produced and designed for use in cGMP manufacturing of biological molecules.

The AlbuPure® adsorbent comprises a novel synthetic triazine ligand derived from Astrea Bioseparations' Mimetic Ligand™ technology and is coupled to Astrea Bioseparations' proprietary PuraBead® 6HF base matrix (a highly cross-linked 6% near monodisperse beaded agarose). This proven technology provides a stable ligand and attachment chemistry with low ligand leakage that enables the use of up to 1.0 M NaOH for cleaning and sanitization ensuring extended adsorbent lifetimes.

Properties of AlbuPure®:

LIGAND:	Synthetic triazine
FUNCTION:	For the purification of Albumin Fusion Proteins
ADSORBENT APPEARANCE:	White
MEAN PARTICLE SIZE:	90 ± 10 µm
BASE MATRIX:	Highly cross-linked 6% near monodisperse agarose (PuraBead® 6HF)
BINDING CAPACITY:	> 35 g/L*
RECOMMENDED PACKING CONDITIONS:	At a constant pressure of 1.5 bar (~ 22 psi)
RECOMMENDED PACKING SOLUTION:	0.1 M NaCl solution (saline)
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 600 cm/h
CHEMICAL STABILITY:	Stable in all commonly used buffers and solutions
CLEANING / SANITIZATION:	0.5 – 1.0 M NaOH
RECOMMENDED STORAGE CONDITION:	2 – 30 °C, 20% ethanol : 80% 0.1 M NaCl (v/v)

* Performed using scFv albumin-fusion fermentation supernatant loading to 10% breakthrough at 240 cm/h, pH 4.5.

COLUMN PACKING

AlbuPure® is supplied in 20% ethanol : 80% 0.1 M NaCl (v/v) solution. There is no requirement to remove the storage solution prior to packing. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of AlbuPure® 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 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 leaving a dead volume of packing solution above the adsorbent bed.

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 of 1.5 bar (~ 22 psi).

7. Once the adsorbent has packed (after ~ 2 CV), measure and mark the bed height under packing flow, close the column outlet and stop the liquid flow through the bed.

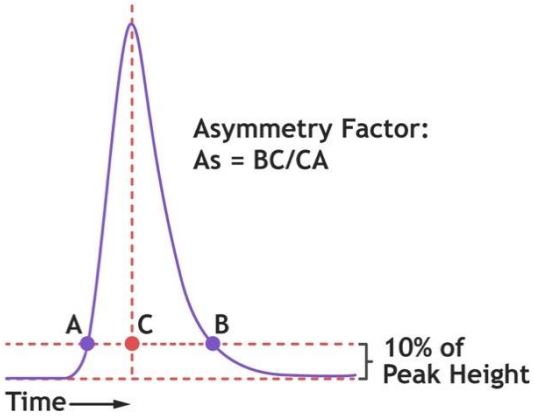
8. Lower the top adaptor by loosening the top adaptor seal (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to the position of the marked bed height (do not push the top adaptor further into the adsorbent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

   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. AlbuPure® 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. AlbuPure® 1- and 5-mL Column Kits (PC6626 & PC6627) are available for scouting experiments.

The following instructions are recommended (as a starting point) for the purification of albumin-fusion proteins. 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 column volumes (CV) of equilibration buffer.

   It is recommended that the pH of the load and equilibration buffer is between pH 4.5 and pH 8.0. Between pH 4.5 and pH 5.5; a 50 mM sodium citrate buffer is recommended and between pH 6.0 and pH 8.0, a 50 mM sodium phosphate buffer is recommended.

   Note: Higher binding capacities will be achieved using a lower pH equilibration buffer and load, therefore starting equilibration buffer of 50 mM sodium citrate buffer, pH 5.5 is recommended.

2. Apply the clarified (and pH adjusted, if required) protein feedstock onto the equilibrated column. A minimum residence time of ≥ 3 minutes is recommended.

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.

   Note: The use of a wash buffer may be employed to increase the selectivity of AlbuPure® by removing potential non-specifically bound material from the column. Wash steps can be included after the post load wash with incremental pH increases.

   For example, if the pH of the load and equilibration is pH 5.5, the column could be washed with 50 mM sodium acetate or sodium phosphate buffer at range of pH 4.0 to pH 6.0 followed by a second wash using 50 mM sodium phosphate or Tris buffer at a range of pH 7.5 to pH 9.0.

5. Elute the bound protein using 5 CV of elution buffer.

   It is recommended to use a neutral to mildly alkali buffer with the addition of sodium octanoate (caprylate) e.g. 50 mM ammonium acetate, 10 mM sodium octanoate, pH 7.0.

   Note: Up to 100 mM sodium octanoate (caprylate) can be used for tightly bound target materials.

6. If a clean-in-place is required, use up to 5 CV 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 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 the column with 5 CV of equilibration solution/buffer (to remove the CIP solution) and check pH and conductivity of column eluate is equal to that of the buffer entering the column before storage or re-use.

8. For long term storage, it is recommended to store AlbuPure® in 20% ethanol : 80% 0.1 M NaCl (v/v) at 2 – 30 °C.

   Note: If the material is stored for a length of time (> 3 months) at room temperature, it is recommended that the adsorbent is washed with 2 – 3 CV of either 0.5 M sodium hydroxide (recommended solution) or 20 mM sodium hydroxide prior to use.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3151-00025	AlbuPure®	25 mL
3151-00100	AlbuPure®	100 mL
3151-00500	AlbuPure®	500 mL
3151-01000	AlbuPure®	1000 mL

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

Pre-packed Column Format

Code	Description	Pack Size
6626	AlbuPure® HT 1 mL Column Kit	4 x 1 mL columns
6627	AlbuPure® HT 5 mL Column Kit	4 x 5 mL columns
6628	HT-1 Albumin purification selection kit	2 x 1 mL AlbuPure®, 1 x 1 mL Mimetic Blue® SA P6HF, 1 x 1 mL Mimetic Blue® SA HL P6HF
6629	HT-5 Albumin purification selection kit	2 x 5 mL AlbuPure®, 1 x 5 mL Mimetic Blue® SA P6HF, 1 x 5 mL Mimetic Blue® SA HL P6HF

Astrea Bioseparations can also offer column 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: 30 June 2023
CCR Number: CCR-2488
Author Name: R Hawkins
QA Reviewer Name: R Hawkins

Astrea Bioseparations