Technical User Guide

Insulin Adsorbent

Product Code: 3160

Astrea Bioseparations

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Introduction

Insulin Adsorbent is a high-performance synthetic ligand affinity chromatography adsorbent for the capture and purification of insulin and insulin analogues from a variety of sources.

The Insulin Adsorbent ligand is coupled to highly cross-linked near monodisperse 6% beaded agarose (PuraBead® 6HF) which has excellent flow properties. The adsorbent is stable in up to 0.5 M sodium hydroxide which allows for stringent cleaning and sanitization protocols.

Properties of Insulin Adsorbent:

Ligand:	Synthetic derivative structure
Function:	For the purification of Insulin and Insulin analogues
Adsorbent appearance:	Brown
Mean particle size (µm)	90 ± 10 µm
Matrix:	Highly cross-linked 6% near monodisperse agarose (PuraBead® 6HF)
Binding capacity:	Up to 25 g/L of Adsorbent (dependent on target protein and feedstock)
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
Operating pH:	pH 2.5 to pH 8.0
pH Stability:	Long term (3 months) pH 3.0 to pH 13.0
Chemical stability:	Stable in all commonly used buffers and solutions
Cleaning / sanitization:	0.5 – 1.0 M NaOH
Recommended storage condition†:	Store in the dark, 20% ethanol, 2 – 30 °C*

* Please do not store the Insulin Adsorbent in buffers of extreme pH or elevated temperature

Column Packing

Insulin Adsorbent is supplied in 20% ethanol solution. Due to the presence of ethanol, there may initially be an increased back pressure however, this should reduce after ~1 column volume (CV) during the pack. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of Insulin Adsorbent 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).

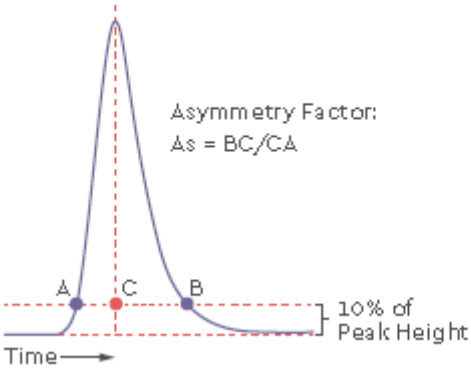
   Note: Once the flow is paused the bed may relax and rise.

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.

   Note: it is recommended that either before first use or after prolonged storage in the preservative solution, the packed column is washed with 30% iso-propanol/0.2 M NaOH (2 CV) to dislodge loosely bound agarose chains and attached ligand which may arise from the very low-level hydrolysis of the agarose polymer chains.

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. Insulin Adsorbent 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. Insulin Adsorbent 1 mL Column Kits (code: 4444-00001) 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.

   Recommended equilibration buffer: 0.2 M sodium acetate buffer, pH 5.5.

2. Apply filtered sample onto the column at a flow rate of 100 cm/h. 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 solution/ 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: Wash buffers can be used containing from 0.01 to 0.4 M sodium acetate (from pH 5.0 to pH 5.6) to improve target protein purity or recovery if required.

5. Elute the bound protein using 5 CV of elution buffer. It is recommended to use an acidic solution of 0.1 M acetic acid (increasing the molarity up to 1.0 M of the acetic acid can improve target protein recovery)

   Note: 20% ethanol, 1.0 M acetic acid can be used as a strip post elution to remove any tightly bound target and non-target material if required.

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 before re-use.

8. For long term storage, it is recommended to store Insulin adsorbent in the dark, in 20% ethanol at 2 – 30 °C.

Order information

Gel Slurry

Code	Description	Pack Size
3160-00025	Insulin Adsorbent	25 mL
3160-00100	Insulin Adsorbent	100 mL
3160-00500	Insulin Adsorbent	500 mL
3160-01000	Insulin Adsorbent	1000 mL
4444-00001	Insulin Adsorbent 1 mL Column Kit	4 x 1mL columns

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**

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.

Horizon Park, Barton Road,
Comberton, Cambridge,
CB23 7AJ, UK

Tel: +44 (0) 1223 433 800

Sales:	sales@astrea-bio.com
Technical support:	techsupport@astrea-bio.com
Quality support:	quality@astrea-bio.com
Website:	www.astreabioseparations.com

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