



Technical User Guide

IsoClear B™

Product Code: 9302



Introduction

Properties of IsoClear B™:

Ligand:	Trisaccharide
Function:	For the capture of anti-A isoagglutinin
Adsorbent appearance:	White microspheres
Mean particle size (µm)	65
Mean pore diameter:	1000 Ångström
Matrix:	Beaded polymethacrylate resin
Recommended packing conditions:	Pack at flow rates up to 850 cm/h (pressure up to 2 bar) using 0.1 M NaCl solution
Recommended operational flow rate:	Up to 500 cm/h
Recommended operating pressure:	Up to 1 bar
Storage*:	2 - 30°C*, 20% ethanol

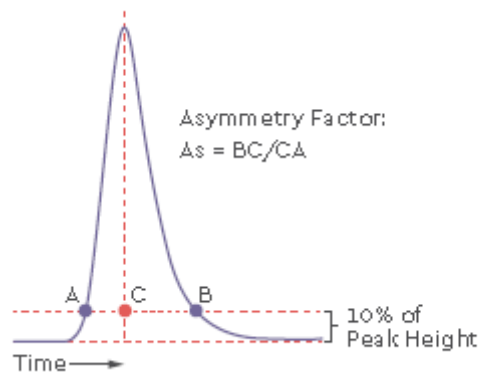
* Note: do not store the resin in buffers of extreme pH or at elevated temperatures.

Column Packing

1. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column.
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. Slurry the resin and pour the appropriate amount into the column in a single continuous step (Note: the material has a compression ratio of ~ 1.15). Pouring the adsorbent down the side of the column helps to prevent air becoming trapped within the adsorbent bed. Ensure that the bottom outlet is not attached to the workstation (into waste vessel) but is closed off.
5. Allow the adsorbent to settle (Note: preferably overnight).
6. Once the adsorbent has settled, ensure the top adaptor is free from air by pumping the packing solution (0.1 M NaCl solution) through it and then place into the top of the column to just above the settled resin.
7. Open the bottom outlet and commence the pack under flow.
8. Increase the flow rate up to 850 cm/h (i.e. packing flow rate) or to a maximum pressure of 2 bar (dependant on column dimensions) and pack for 2 CV. Measure and mark the top of the bed. Stop the flow, close the bottom outlet and lower the top adaptor up to 1 cm below the packed bed mark (dependant on bed height), allowing the packing solution out of the top outlet.
9. Open the bottom outlet and recommence packing flow rate for a further 1 CV. There should be no further bed compression (if there is repeat step 8). Slow the flow to operational flow rate and attach the bottom adaptor to the workstation.
10. IsoClear B™ 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. IsoClear B™ 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

The following buffers/method is recommended as a starting point for the removal of anti-B isoagglutinins.

An initial flow rate of 100 cm/h is recommended. In order to optimise the resin performance (i.e. residence time and column bed height) further optimization may be required.

The preferred option is to use IsoClear B™ with a liquid chromatography system or automated workstation. Note: the resin can also be operated manually using peristaltic pumps or even a syringe.

Filter all buffers and feedstock through an appropriate filter, prior to running the column.

1. Equilibrate the column with 5 CV of equilibration solution/buffer (e.g. a near neutral buffer such as PBS - to match the condition of the feedstock). Allow the column buffers and sample to reach the operational temperature.
2. Apply filtered sample onto the column at a flow rate of 100 cm/h. A minimum residence time of 1.5 minutes is required (Note: ≥ 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:** elution of the bound protein may be possible by lowering the pH with up to 5CV of elution buffer (e.g. 50 mM sodium citrate, pH 3.0).
5. It is possible to remove residual adsorbed material by washing the resin with 20% ethanol, M acetic acid as a Clean-in-Place (CIP) step. Alternatively, 6.0 M guanidine HCl can be used.
6. Note: avoid alkali buffers/solutions and **DO NOT** use sodium hydroxide (NaOH).
7. Note: prolonged contact with the CIP solution may affect the resin performance. Do not store the resin in CIP. Remove the CIP solution with at least 5 bed volumes of equilibration solution/ buffer until the pH and conductivity of the column eluate is equal to that of the buffer at the column inlet.
8. Re-equilibrate the column with 5 CV of equilibration solution/buffer (to remove the CIP solution) and check pH and conductivity before re-use.
9. If required later, store the column in 20% ethanol (2 - 30 °C).

Order information

Gel Slurry

Code	Description	Pack Size
9301-00025	IsoClear A™	25 mL
9301-00100	IsoClear A™	100 mL
9301-00500	IsoClear A™	500 mL
9301-01000	IsoClear A™	1000 mL
9302-00025	IsoClear B™	25 mL
9302-00100	IsoClear B™	100 mL
9302-00500	IsoClear B™	500 mL
9302-01000	IsoClear B™	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

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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