Technical
User Guide

Mimetic Blue® SA P6HF

Product Code: 3135

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PURITY by DESIGN

INTRODUCTION

Mimetic Blue® SA P6HF affinity chromatography adsorbent is manufactured exclusively by Astrea Bioseparations Ltd.

It comprises Mimetic Blue® SA ligand covalently attached to a near monodisperse 6% cross-linked agarose bead (PuraBead® 6HF) and is immobilised using a defined and highly stable spacer-arm linkage which promotes optimal interaction with the target protein.

Properties of Mimetic Blue® SA P6HF:

LIGAND:	Synthetic anthraquinone
FUNCTION:	For the purification of Serum Albumin
ADSORBENT APPEARANCE:	Blue
MEAN PARTICLE SIZE (µM)	90 ± 10 µm
MATRIX:	PuraBead® 6HF (6% cross-linked near monodisperse agarose)
BINDING CAPACITY:	>17mg HSA/mL of adsorbent
RECOMMENDED PACKING CONDITIONS:	Packing pressure: up to 3 bar
RECOMMENDED PACKING SOLUTION:	0.1 M NaCl solution (saline)
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 650 cm/hr
OPERATING PH:	pH 2 to pH 14 (intermittent)
PH STABILITY:	Long term (3 months) pH 3 to pH 13
CHEMICAL STABILITY:	Stable in all commonly used buffers and solutions
CLEANING / SANITIZATION:	0.5 - 1.0 M NaOH, 25 °C
STERILIZATION:	Autoclavable in 0.1 M saline at 121 °C for 30 minutes
RECOMMENDED STORAGE CONDITION†:	2 – 30 °C, 20% ethanol : 80% 0.1 M NaCl (v/v)

COLUMN PACKING

Mimetic Blue® SA P6HF is supplied in 20% ethanol : 80% 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 Mimetic Blue® SA P6HF into axial columns:

1. Decant off the shipping preservative and prepare a 50% slurry of the adsorbent with 0.1 M NaCl solution.

2. 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.

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 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 the column (to obtain a uniform pack) at a pressure not exceeding 3 bar (~ 45 psi).

8. 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.

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).

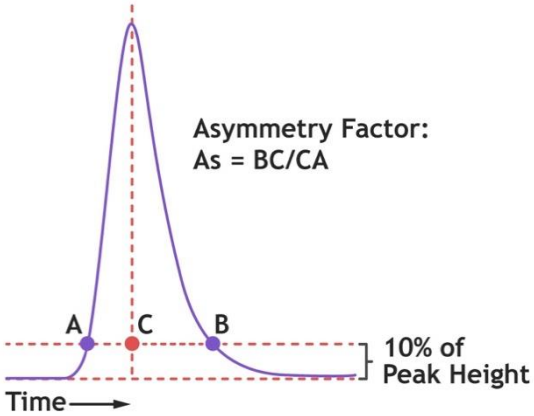
   Note: Once the flow is paused the bed may relax and rise.

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.

    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 / 0.2M NaOH (2CV) to dislodge loosely bound agarose chains and attached ligand which may arise from the very low-level hydrolysis of the agarose polymer chains.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. Mimetic Blue® SA P6HF 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. Mimetic Blue® SA P6HF. 1 mL and 5 mL Column Kits (code: 6620 & 6621) are available for scouting experiments.

The following instructions are recommended (as a starting point) for the purification of albumin and albumin related 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 3 CV of equilibration buffer (e.g. 25 mM sodium phosphate buffer at pH 6.0) or until the pH/conductivity is at baseline. (Note: ensure the equilibration buffer of the column is comparable to the protein feedstock). Other buffers (e.g. Tris and citrate) are acceptable with a recommended pH range between pH 4.0 and pH 8.0.

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:** The use of a wash buffer may be employed to remove any loosely bound non-target material, initially a moderate amount of salt (up to 0.2 M NaCl) can be added to the equilibration buffer to remove non-specifically bound proteins. Alternatively, additional washes of buffers with increasing pH can also have the same desired effect.

5. Elute the bound protein using up to 5 CV of an appropriate elution buffer. Albumin and albumin related proteins are selectively eluted from Mimetic Blue® SA P6HF using up to 60 mM sodium octanoate (caprylate) present in the equilibration buffer.

6. **Note:** Increasing the concentration of salt (up to 2 M NaCl) will non-selectively promote elution from the adsorbent

7. 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.

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. For later date use, place the column into the storage solution and store at 2 – 30 °C. Mimetic Blue® SA P6HF should be stored in 20% ethanol : 80% 0.1 M NaCl (v/v).

   Note: After long term storage (≥ 3 months), it is recommended to wash the adsorbent with 2 bed volumes of 30% isopropanol / 0.2 M NaOH solution at a linear flow rate equivalent to 50% of the operational flow rate. Once completed re-equilibrate the adsorbent prior to use.

   If you have any questions, please contact us on techsupport@astrea-bio.com

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3135-00025	Mimetic Blue® SA P6HF	25 mL
3135-00100	Mimetic Blue® SA P6HF	100 mL
3135-00500	Mimetic Blue® SA P6HF	500 mL
3135-01000	Mimetic Blue® SA P6HF	1000 mL

Astrea Bioseparations offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes.

Small column kits are also available for screening experiments.

Code	Description	Pack Size
6620	Mimetic Blue® SA P6HF 1 mL Column Kit	4 x 1 mL columns
6621	Mimetic Blue® SA P6HF 5 mL Column Kit	4 x 5 mL columns

Astrea Bioseparations 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

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