Technical
User Guide

Mimetic Blue® SA HL P6XL

Product Code: 3125

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PURITY by DESIGN

INTRODUCTION

Mimetic Blue® SA HL ("high ligand") P6XL affinity chromatography adsorbent is manufactured exclusively by Astrea Bioseparations Ltd and its affiliates (Astrea Bioseparations).

It comprises Mimetic Blue® ligand covalently attached to a near monodisperse 6% cross-linked agarose bead (PuraBead® 6XL) 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 HL P6XL:

LIGAND:	Synthetic anthraquinone
ADSORBENT APPEARANCE:	Blue microspheres
MEAN PARTICLE SIZE (MM):	100 ± 10 µm
MATRIX:	PuraBead® 6XL (6% cross-linked near monodisperse agarose)
LIGAND DENSITY:	10 ± 1 µmol/g moist gel
DYNAMIC BINDING CAPACITY:	25 to 40 mg HSA/mL of adsorbent
RECOMMENDED PACKING CONDITIONS:	Packing pressure - 2 to 3 bar Packing solution - 0.1 M NaCl solution
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 500 cm/h with column diameter less than 3 cm Up to 200 cm/h with column diameter greater than 5 cm
OPERATING PH:	pH 2 to pH 14 (intermittent)
PH STABILITY:	Long term (3 months) pH 3 to pH 13
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents
CLEANING / SANITIZATION:	0.5 - 1.0M NaOH, 25°C
STERILIZATION:	Autoclavable in 0.1M 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 HL P6XL is supplied in 20% : 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 HL P6XL 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 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.

5. Carefully pour the adsorbent slurry into the column in a single continuous step (use a reservoir if required). 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 just enough to leave a small volume of packing solution above the adsorbent.

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 (to obtain a uniform pack) at a pressure up to but 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.

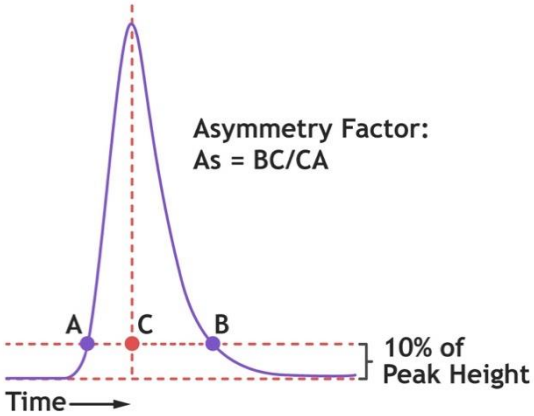
9. Lower the top adaptor by loosening the top adaptor seal (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to the position of the marked bed height (do not push the top adaptor further into the adsorbent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

11. **Please 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. Mimetic Blue® SA HL P6XL 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. Mimetic Blue® SA HL P6XL 1 mL and 5 mL Column Kits (6642 and 6643) are also available for scouting experiments and process optimization.

The following method is recommended (as a starting point) for the purification of albumin and 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 3 CV of equilibration buffer or until the pH/conductivity is at baseline (Note: ensure the equilibration buffer of the column is comparable to the protein feedstock).

   25 mM sodium phosphate buffer at pH 6.0, however other buffers are acceptable with a recommended pH range between pH 4.0 and pH 8.0.

2. Apply the filtered (and pH / conductivity adjusted, if required) protein feedstock onto the equilibrated column. A residence time of 3 minutes (or greater) is recommended.

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

4. If a wash step is required 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 HL P6XL using up to 60 mM sodium octanoate (caprylate) present in the equilibration buffer.

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

6. If a CIP is required, pass 5 CV of 0.5 M NaOH through the column.

   Removal of 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. Once cleaning with NaOH is complete, wash with at least 5 bed volumes of equilibration buffer until the pH and conductivity of the column eluate is equal to that of the buffer entering the column. Complete this process prior to further use or storage in the storage buffer.

8. For later date use, place the column into the storage solution and store at 2 – 30 °C.

Mimetic Blue® SA HL P6XL should be stored in 20% ethanol : 80% 0.1 M NaCl (v/v). For short term storage (< 6 months), Mimetic Blue® SA HL P6XL can also be stored in 0.1 M NaOH

Note: After storage (≥ 1 month) or on a newly packed column, 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
3125-00025	Mimetic Blue® SA HL P6XL	25 mL
3125-00100	Mimetic Blue® SA HL P6XL	100 mL
3125-00500	Mimetic Blue® SA HL P6XL	500 mL
3125-01000	Mimetic Blue® SA HL P6XL	1000 mL

Astrea Bioseparations offers a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes. We also supply Mimetic Blue® SA HL P6XL in 1 mL and 5 mL columns.

Column Format (1 mL and 5mL columns)

Code	Description
6642	Mimetic Blue® SA HL P6XL 1 mL column
6643	Mimetic Blue® SA HL P6XL 5 mL column

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

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