Technical
User Guide

Mimetic Blue® AP HL P6HF

Product Code: 3115

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PURITY by DESIGN

INTRODUCTION

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase [E.C. 3.1.3.1]) is a widely used reagent in biochemistry, molecular biology and immunodiagnostics.

The enzyme has a broad specificity for phosphate esters of alcohols, amines, pyrophosphate, and phenols. It is routinely used to dephosphorylate proteins (e.g. casein) as well as nucleic acids. Alkaline phosphatase has also been shown to have anti-inflammatory effects and can reduce the severity of septic shock by detoxifying lipid A[1].

Mimetic Blue® AP HL P6HF is an affinity chromatography adsorbent that has a specifically designed Mimetic Ligand™ with a phosphonate group for the purification of alkaline phosphatases.

Properties of Mimetic Blue® AP HL P6HF:

LIGAND:	Synthetic anthraquinone with a phosphonic group
ADSORBENT APPEARANCE:	Blue microspheres
MEAN PARTICLE SIZE (µm):	90 ± 10 µm
MATRIX:	Highly cross-linked 6% near monodisperse agarose (PuraBead® 6HF)
LIGAND DENSITY:	18 – 27 µmol/g moist gel
RECOMMENDED PACKING CONDITIONS:	Packing pressure – 1.5 bar Packing solution - 0.1 M NaCl solution
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 500 cm/h (up to 1 bar)
OPERATING PH:	pH 2 to pH 14 (intermittent)
PH STABILITY:	Long term (3 months) pH 3 to pH 12
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents
CLEANING / SANITIZATION:	0.5 - 1.0 M NaOH, 25 °C
RECOMMENDED STORAGE CONDITION†:	2 – 30 °C, 20% ethanol, 0.1 M NaCl (v/v)

COLUMN PACKING

Mimetic Blue® AP HL P6HF is supplied in 20% ethanol, 80% 0.1 M NaCl (v/v) solution. Due to the presence of ethanol, there may initially be an increased back pressure during the pack, however, this should reduce after ~1 column volume (CV). There is no requirement to remove the preservative 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 Mimetic Blue® AP HL P6HF 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. 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.

4. Allow the adsorbent slurry to settle until a dead space of packing solution has formed (~ 2 cm).

5. Attach the (open) top adaptor to the top of the column and adjust the adaptor into the packing solution above the adsorbent, tighten the adaptor and attach to the workstation. Open the column outlet and apply the packing flow to the bed. The recommended packing condition is at a constant pressure of 1.5 bar (~22 psi). **Note:** The flow rate will be dependent on column dimensions.

6. 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.

7. Lower the top adaptor to the position of the marked bed height, see below for notes:

    - 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.

    - Once the flow is paused the bed may relax and rise.

    - The seal of the top adaptor may need to be loosened to allow the adaptor to be lowered.

    - Depending on the achieved back pressure during the pack, the adaptor may be required to compress the adsorbent further by up to 0.5 cm past the marked packed bed height.

8. Once complete re-tighten the top adaptor (if loosened) and attach back to the workstation (or switch valve back in-line).

9. 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

To check the quality of the column pack, the height equivalent to a theoretical plate (HETP) and peak asymmetry need to be determined. This can be achieved by quantifying the geometry of an isocratic peak, which can be produced by injecting a marker detectable by UV or conductivity using saline or equilibration buffer as the mobile phase.

1. Test the column at a flow rate of 100 cm/h.

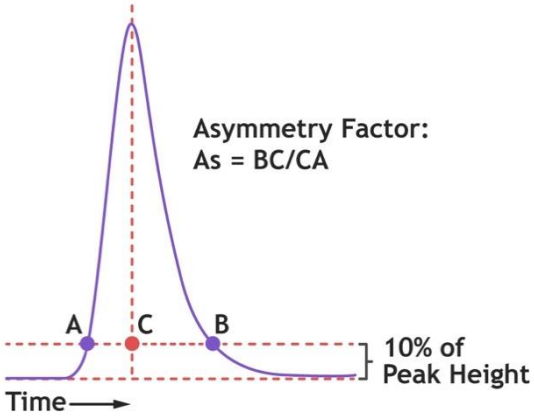
2. Attach the column to an equilibrated workstation.

3. Commence the flow for 1 column volume (CV) to equilibrate and obtain a 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® AP HL P6HF is an affinity adsorbent, therefore an asymmetry factor for an acceptable pack is between 0.8 and 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® AP HL P6HF 1 mL column kits (product code: 6640) are available for screening experiments.

The following instructions are recommended (as a starting point). 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 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).

   Recommended equilibration buffers are 10 mM Tris.HCl, 1 mM $MgCl_2$ buffer, pH 8.0 or 10 mM Tricine-NaOH buffer, pH 8.5.

2. Apply the protein feedstock onto the equilibrated column. A residence time of 5 minutes (or greater) is recommended.

   Note: Clarify the feedstock/protein solution using an appropriate filter and adjust the pH and or conductivity of the solution if required.

   If the target protein is temperature sensitive, perform chromatography at 4 °C

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. It is recommended to carry out a 20 CV linear gradient from 0 to 1 M KCl in equilibration buffer to determine the most appropriate salt concentration to elute the bound protein, and potentially allow for separation of loosely bound non-target materials.

   - Once identified, elute the bound protein using up to 5 CV of an appropriate elution buffer:

   - Desorption of alkaline phosphatase from the column is carried out via a step elution using up to 1 M KCl.

   Alternative specific eluents include 5 mM KH_2PO_4, 5 mM α-napthyl phosphate or 5 mM phenylphosphate in equilibration buffer.

5. If a clean-in-place is required, use 5 CV of a sodium hydroxide solution.

   Removal of any residual adsorbed material including micro-organisms, viruses and endotoxins can be achieved by washing the column with 0.5 M 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.

6. Re-equilibrate column with 5 CV of equilibration buffer (to remove the CIP solution) and check the pH and conductivity of the column eluate is equal to that of the buffer entering the column before storage or re-use.

7. It is recommended to store the column in 20% ethanol, 0.1 M NaCl solution at 2 - 30°C.

   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.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3115-00025	Mimetic Blue® AP HL P6HF	25 mL
3115-00100	Mimetic Blue® AP HL P6HF	100 mL
3115-00500	Mimetic Blue® AP HL P6HF	500 mL
3115-01000	Mimetic Blue® AP HL P6HF	1000 mL

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

Prepacked column kits are also available (4 x 1 mL and 4 x 5 mL) for small scale experiments.

Prepacked Column Formats

Code	Description
6640	Mimetic Blue® AP HL P6HF 4 x 1 mL Column Kit
6641	Mimetic Blue® AP HL P6HF 4 x 5 mL Column Kit

For more information on this or any other supply related matters please contact us at sales@astrea-bio.com

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