Technical User Guide

PHENYL PURABEAD® HF

Product Code: 3408

Astrea Bioseparations

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Introduction

Phenyl PuraBead® HF is a hydrophobic interaction chromatography (HIC) adsorbent designed for intermediate purification steps for the purification of mildly hydrophobic biomolecules.

Phenyl PuraBead® HF ligand is an aromatic group (phenyl ring) coupled to highly cross-linked near monodisperse 6% beaded agarose (PuraBead® 6HF) which has excellent flow properties. The adsorbent is stable in up to 1.0 M sodium hydroxide which allows for stringent cleaning and sanitization protocols.

Properties of Phenyl PuraBead® HF:

Ligand:	Phenyl ring
Mean particle size (µm)	90 ± 10 µm
Matrix:	PuraBead® 6HF (Highly cross-linked 6% near monodisperse agarose)
Recommended packing conditions:	Pack at a constant pressure of 1.5 bar (~ 22 psi)
Recommended packing solution:	0.1 M NaCl solution or equilibration buffer
Recommended operational flow rates:	Up to 600 cm/h
Operating pH:	pH 2.0 - pH 14.0
pH stability:	Long term (up to 1 month): pH 3.0 - pH 13.0
Chemical stability:	All commonly used aqueous buffers and co-solvents
Cleaning/sanitization:	0.5 to 1.0 M NaOH
Storage:	2 - 30 °C, 20% ethanol

Column Packing

Phenyl PuraBead® HF is supplied in a preservative containing 20% ethanol. 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 Phenyl PuraBead® HF 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 or equilibration buffer) 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. The compression factor of this adsorbent is 1.15 to 1.20.

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 ~1 cm above the bed, tighten the adaptor and attach to the workstation. Open the column outlet and apply the desired flow to the bed (for ~ 2 CV). The recommended packing condition (to obtain a uniform pack) is at a constant pressure of 1.5 bar (~ 22 psi). The flow rate is dependent on column dimensions, however, will range from 700 to 1000 cm/h.

7. Once the adsorbent has packed (after ~ 2 CV), measure and mark the bed height under packing flow and close the column outlet and stop the liquid flow through the bed.

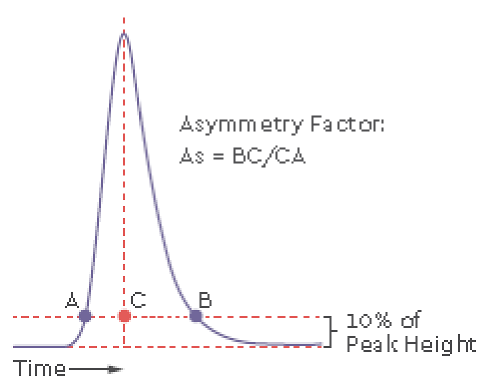
8. Lower the top adaptor (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to up to ~1 cm below the position of the marked bed height.

Note: 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.

9. Re-tighten the top adaptor (if loosened) and attach back to the workstation (or switch valve back in-line). 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.

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. The recommended asymmetry factor for packed Phenyl PuraBead® HF is between 0.8 to 1.2. 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. Phenyl PuraBead® HF column kits are also available for screening experiments.

The following method is recommended (as a starting point), using an initial flow rate of 100 cm/h for 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.

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

Protein adsorption: The binding of protein to Phenyl PuraBead® HF is influenced by:

a) The ionic strength of the equilibration buffer. The salts that cause salting out (i.e. ammonium sulphate) promote the binding of the proteins to HIC adsorbents

b) The binding of proteins to Phenyl PuraBead® HF decreases when temperature decreases.

To ensure that target molecules bind to Phenyl PuraBead® HF, the most common strategy is to apply the sample at high salt concentration in a buffered solution, pH range from pH 4.0 to pH 9.0 and high salt concentrations. The addition of a salting out solution should be added to the protein solution/sample yet ensuring not to precipitate the target protein. The recommended salt solution is ammonium sulphate (up to a final concentration of 2.0 M ammonium sulphate).

The ordering of cations and anions to their respective salting out ability is called the Hoffmeister Series.

The cations are arranged as follows: NH_4+> K+> Na+ >Li+ >Mg_2+ >Ca_2 + where ammonium has the highest ability to precipitate other protein-based solutes.

The order for anions is: SO_4^{2-}> H_2PO_4-> H_3CCOO-> Cl-> NO_3-

Adjust the pH of the feedstock/solution and clarify the feedstock/protein solution using a 0.45 µm filter if required.

1. Sample treatment: In most cases salt is required to be added to the sample. Please take care to avoid protein precipitation. The required amount of salt should be added gradually, either as a solid or concentrated stock solution to the protein solution with continuous stirring. Alternatively, the sample may be buffer exchanged into the high salt equilibration buffer.

2. Equilibrate the column with 5 column volumes (CV) of equilibration buffer (e.g. 20 mM Sodium phosphate, 1 M ammonium sulphate pH 6.0). Allow the column buffers and sample to reach the operational temperature.

Please note that other buffers suitable for use with Phenyl PuraBead® HF to obtain optimal binding include:

Tris, sodium citrate and sodium acetate buffers with a high concentration of salting out salts present (as described above).

3. Apply the clarified / filtered protein feedstock onto the equilibrated column. Recommended residence time of 3 minutes (or greater).

4. 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.

5. Elute the bound material by decreasing the salt concentration either step wise (e.g. 5 CV) or for initial investigations, a linear negative salt gradient (20 CV) from high to no salt is recommended.

   The ionic strength of the elution buffer should be low, with preferably no or minimal salt present. E.g. 20 mM sodium phosphate, pH 6.0

   For tightly bound proteins (i.e. strong hydrophobic) increasing the pH can improve recovery. Additionally, for proteins that are tightly bound to the adsorbent, a positive gradient of polarity-reducing organic solvents (i.e. ethylene glycol, glycerol, ethanol, propan-2-ol at concentrations of up to 50%) can be used.

6. If a CIP is required, use up to 5 CV of 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 column with up to 5 CV of equilibration buffer (to remove sodium hydroxide) and check pH and conductivity of the column eluate is equal to that of the buffer entering the column before storage or re-use.

8. If the column is to be stored for future use, place the column into the storage solution (20% ethanol recommended, 0.1 M NaOH acceptable for short term storage) and store at 2 – 30 °C

Order information

Gel Slurry

Code	Description	Pack Size
3408-00025	Phenyl PuraBead® HF	25 mL
3408-00100	Phenyl PuraBead® HF	100 mL
3408-00500	Phenyl PuraBead® HF	500 mL
3408-01000	Phenyl PuraBead® HF	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

Column Kits

Code	Description	Pack Size
6610	Phenyl PuraBead® HF	4 x 1 mL columns
6611	Phenyl PuraBead® HF	4 x 5 mL columns
6612	Octyl PuraBead® HF	4 x 1 mL columns
6613	Octyl PuraBead® HF	4 x 5 mL columns
6614	HIC selection kit (1 mL columns)	1 x 1 mL Phenyl PuraBead® HF 1 x 1 mL Octyl PuraBead® HF
6615	HIC selection kit (5 mL columns)	1 x 5 mL Phenyl PuraBead® HF 1 x 5 mL Octyl PuraBead® HF

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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

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