Technical
User Guide

Hydrophobic Interaction Chromatography Media (PuraBead® HF)

Phenyl PuraBead® HF
Product Code: 3408

Butyl PuraBead® HF
Product Code: 3416

Hexyl PuraBead® HF
Product Code: 3426

Octyl PuraBead® HF
Product Code: 3455

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PURITY by DESIGN

INTRODUCTION

Hydrophobic Interaction Chromatography (HIC) is used to separate proteins based on their relative hydrophobicity. Separations on HIC adsorbents are carried out in aqueous salt solutions under non-denaturing conditions. Samples are applied to the adsorbent in buffers containing high concentration of salt and elution is achieved by a descending salt gradient. Consequently, HIC is particularly useful for the purification of proteins from high ionic strength biological extracts since binding is performed in the presence of salt and elution in the absence of salt and can be used for the capture or intermediate purification steps for the purification of mildly hydrophobic biomolecules.

Astrea Bioseparations Ltd and its affiliates (Astrea Bioseparations) HIC adsorbents comprise of a range of four ligands of increasing hydrophobicity; phenyl, butyl, hexyl and octyl, coupled to our highly cross-linked near monodisperse 6% beaded agarose (PuraBead® 6HF) which has excellent flow properties. The adsorbent is compatible with most biological buffers and commonly encountered buffer additives and is stable in up to 1.0 M sodium hydroxide which allows for stringent cleaning and sanitization protocols.

This technical user guide describes the use of the Astrea Bioseparations' HIC PuraBead® HF adsorbents:

- Phenyl PuraBead® HF Product Code: 3408
- Butyl PuraBead® HF Product Code: 3416
- Hexyl PuraBead® HF Product Code: 3426
- Octyl PuraBead® HF Product Code: 3455

Properties of HIC PuraBead® HF adsorbents:

ADSORBENT:	Phenyl PuraBead® HF	Butyl PuraBead® HF	Hexyl PuraBead® HF	Octyl PuraBead® HF
LIGAND:	Aromatic Phenyl ring	Aliphatic 4 carbon chain	Aliphatic 6 carbon chain	Aliphatic 8 carbon chain
ADSORBENT APPEARANCE:	White microspheres			
MEAN PARTICLE SIZE:	90 ± 10 µm			
MATRIX:	PuraBead® 6HF (Highly cross-linked 6% near monodisperse agarose)			
RECOMMENDED PACKING CONDITIONS:	0.1 M NaCl solution at a constant pressure of 1.5 bar (~ 22 psi)			
RECOMMENDED OPERATIONAL FLOW RATES:	Up to 600 cm/h			
OPERATING PH:	pH 2.0 – pH 14.0			
PH STABILITY (LONG TERM):	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

The HIC adsorbents are supplied in 20% ethanol 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 the HIC PuraBead® HF adsorbents 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) 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/measure a sample of the complete slurry, drain away the preservative and re-weigh/measure the adsorbent. The final measurement over the total measurement will determine the slurry percentage. The compression factor of this adsorbent is 1.20 to 1.25 (dependant on column size/dimensions).

4. 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. Ensure the complete transferral of the known volume of adsorbent into the column.

5. Allow the adsorbent to settle in the column just long enough to leaving a small 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 lower slightly until packing solution extruded from the top 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 600 to 1000 cm/h.

7. Once the adsorbent has packed (after 2 to 5 CV), measure and record 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 ~1 cm below the position of the recorded 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.

   If a predetermined volume has been added, lower the adaptor to the required bed height. No further adaptor adjustment should be required.

COLUMN EFFICIENCY TEST

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

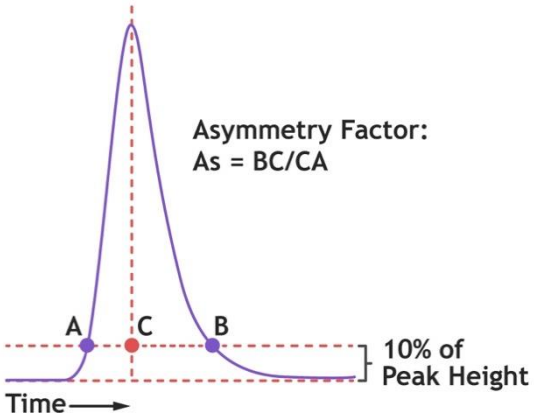
2. Attach the column to an equilibrated workstation.

3. Commence flow for at least 1 CV (i.e., to equilibrate and obtain baseline) using a low ionic strength solution e.g., 0.1 M NaCl solution.

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 hydrophobic interaction chromatographic adsorbents have an ideal recommended asymmetry factor for the packed adsorbent of 0.8 to 1.2, however the asymmetry factor range should be established to the purposes of the packed column. 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.

1 mL and 5 mL pre-packed columns are available for scouting experiments.

The preferred option is to use HIC adsorbents with a liquid chromatography system or automated workstation. Note: the adsorbents can also be operated manually using a peristaltic pump or even a syringe.

An initial flow rate of 100 cm/h for the column chromatography steps is recommended. Subsequent changes to the flow rate can be investigated to improve binding capacity / resolution or decrease processing times.

Protein adsorption: The binding of protein to any of the HIC adsorbents 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 HIC adsorbents decreases when temperature decreases.

The most common strategy to ensure that target molecules bind to HIC adsorbents, is to apply the sample/feedstock at high salt concentration in a buffered solution, pH range from pH 4.0 to pH 9.0. The addition of a salting out solution should be added to the protein solution/sample, however ensuring not to precipitate the target protein.

Ammonium sulphate (up to a final concentration of 2.0 M ammonium sulphate) is recommended as a suitable starting salting out solution for binding target biomolecules to the HIC adsorbents.

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 protein-based solutes.

The order for anions is: $SO_4^{2-} > PO_4- > H_3CCOO- > Cl- > NO_3-$.

Between cations and anions in solution the concentration of the anion typically has the greatest effect on protein precipitation.

Adjust the pH of the feedstock/solution (if required). It is recommended to clarify/filter the feedstock/protein solution using a 0.45 µm filter.

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. Other equilibration buffer components suitable for use with the HIC adsorbents include tris, sodium citrate and sodium acetate buffers (in each case with a high concentration of salting out salts present as described previously).

3. Apply the clarified feedstock / sample onto the column. A minimum residence time of ≥ 3 minutes is recommended.

4. Remove any non-bound material in the column with 5 CV of equilibration 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., strongly 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 Clean-in-Place (CIP) is required, use 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.

   For a stringent CIP, 25% IPA/1.0M NaOH solution has been shown to remove very tightly bound materials.

7. Post CIP, wash the column with at least 5 CV of elution buffer (i.e., low salt buffer) to remove the CIP solution and check pH and conductivity of the column eluate is equal to that of the buffer entering the column before re-use or storage.

8. If required at a later date, store the column into the storage solution at 2 – 30 ºC. The recommended preservative is 20% ethanol.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3408	Phenyl PuraBead® HF	25 mL , 100 mL , 500 mL , 1000 mL
3416	Butyl PuraBead® HF	
3426	Hexyl PuraBead® HF	
3455	Octyl PuraBead® HF	

For ordering the above quantities as well as other information, please visit our web shop at: https://www.astreabioseparations.com/product-category/general-purification/hydrophobic-interaction/. We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes We also offer a range of larger pack sizes for supply of bulk resins into cGMP development and manufacturing scale processes.

Astrea Bioseparations also supply small scale pre-packed columns for scouting experiments.

Code	Description	Pack Size
6610	Phenyl PuraBead® HF	4 x 1 mL columns
6611	Phenyl PuraBead® HF	4 x 5 mL columns
6634	Butyl PuraBead® HF	4 x 1 mL columns
6635	Butyl PuraBead® HF	4 x 5 mL columns
6636	Hexyl PuraBead® HF	4 x 1 mL columns
6637	Hexyl 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 Butyl PuraBead® HF 1 x 1 mL Hexyl 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 Butyl PuraBead® HF 1 x 5 mL Hexyl PuraBead® HF 1 x 5 mL Octyl PuraBead® HF

Astrea Bioseparations can also offer column packing services. For more information on this or any other supply related matters please contact us at sales@astrea-bio.com

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