Technical
User Guide

HCPure™

Product Code: 3260

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PURITY by DESIGN

INTRODUCTION

HCPure™ is an HCP clearance chromatography adsorbent developed and manufactured exclusively by Astrea Bioseparations Ltd and its affiliates. Composed of a proprietary multi-mode ligand attached to a beaded agarose support.

The adsorbent selectively binds host cell proteins, light chain IgG as well as high molecular weight aggregates from a variety of sources, including feedstock derived from CHO, HEK and E. coli cell cultures, to yield highly purified biological products.

Despite considerable advances in downstream purification for mAbs and other recombinant proteins from various expression systems, there remains a need for effective removal of process and product related impurities that could impact product safety, stability and efficacy. To solve this problem, Astrea Bioseparations has designed and developed an adsorbent specifically to remove host cell proteins from mixtures using different starting materials, HCPure™. Utilizing mixed-mode purification techniques, Astrea Bioseparations delivers a solution that has a number of advantages over traditional workflows.

HCPure™ clearance adsorbent selectively removes HCPs and other impurities from a feed stock without detrimentally affecting yield of the target protein. Removing such impurities from systems expressing various recombinant proteins is possible due to its highly selective nature and ability to be tuned to meet process requirements.

The Astrea Bioseparations HCPure™ clearance adsorbent may be used to remove impurities from expression systems including but not limited to E. Coli, HEK 293, Pichia Pastoris, Saccharomyces Cerevisiae and Spodoptera frugiperda (Sf9 and Sf21).

Properties of HCPure™:

LIGAND:	Synthetic chemical ligand (proprietary)
ADSORBENT APPEARANCE:	White
MEAN PARTICLE SIZE (µM):	90 ± 10 µm
MATRIX:	Highly cross-linked 6% near monodisperse agarose (PuraBead® 6HF)
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 600 cm/h
CHEMICAL STABILITY:	All commonly used aqueous buffers and co-solvents
OPERATING PH:	pH 4.0 to pH 8.0 (intermittent)
PH STABILITY:	Long term (3 months) pH 3 to pH 12
CLEANING / SANITIZATION:	0.5 - 1.0 M NaOH, 25°C
RECOMMENDED STORAGE CONDITION:	2-30°C, 20% ethanol (v/v)

Important Notes

- It is good practice to filter all protein samples and chromatography buffers prior to use.
- Removal of residual HCP from the final protein product solution may require process optimization to identify optimal buffer conditions.
- HCPure™ can be operated across a wide pH range (pH 4.0 – pH 9.0) for HCP binding applications.
- If required, HCP clearance can be optimized by decreasing the flow rate (increasing residence time) used during sample loading.
- For optimum performance of HCPure™ it is recommended that a minimum residence time of 3 minutes are used.

COLUMN PACKING

HCPure™ is supplied in 20% ethanol (v/v) solution. There is no requirement to remove the storage solution prior to packing. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method described below describes the packing of HCPure™ 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 1.25.

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 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 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.

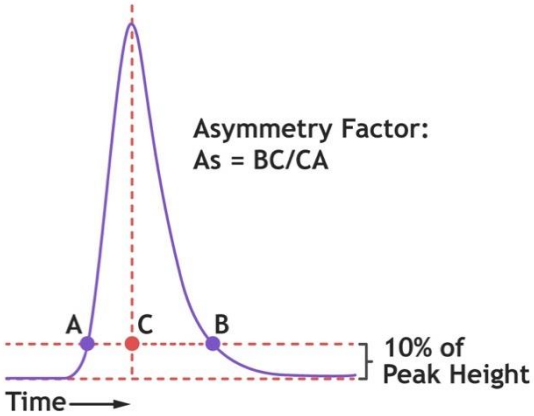
8. Lower the top adaptor (the top adaptor must allow free flow from the workstation either by loosening the top adaptor connection or if present switching the top valve to waste) to ~1 mm below the position of the recorded bed height. Note: once the flow is paused the bed may relax and rise. The seal 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.

10. 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 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. HCPure™ 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 is recommended for process optimisation, including flow rate optimisation to improve binding capacity/ resolution or decrease processing times.

1. Attach the column directly to the liquid chromatography system, ensuring that the tubing connecting the system to the column is primed with equilibration buffer.

2. The optimum equilibration buffer (pH and conductivity) is dependent on the HCP Impurity profile of a given expression system and on the target protein to avoid target binding. It is recommended to screen for these equilibration conditions including a pH range (e.g pH 4 to 9) and a range of conductivities (e.g 6 to 18 mS/cm).

3. In lieu of a screen for optimum equilibration conditions, the following conditions can be used as a starting point for the stated expression systems:

    - E.Coli – 25 mM sodium citrate, 25 mM Tris base pH 6.0 adjusted to 6 mS/cm conductivity with 3 M NaCl

    - Chinese Hamster Ovary (CHO) - 25 mM sodium acetate, 25 mM Tris base pH 8 at conductivity ~3 mS/cm

4. Apply the protein solution/sample onto the column at the recommended operational flow rate of 120 cm/hr (≥ 3 minute residence time is recommended) and collect the flow through in endotoxin free collection vessels.

5. If required, wash the column with equilibration buffer at 120 cm/h to flush out and recover the remaining target protein with at least 1 CV or until a UV baseline is achieved.

6. The HCPure™ column can be regenerated by passing 2 CV of 0.5 M NaOH through the column, followed by an overnight hold in 0.5 M NaOH (> 16 hours). Note: HCPure™ is stable to exposure of sodium hydroxide 0.5 M to 1 M NaOH, though regeneration and re-use of the adsorbent is dependent on the nature of the sample applied to the column and will require validation by the end-user.

7. Post overnight hold, flush the column with a further 1 CV of 0.5 M NaOH.

8. Wash out the sodium hydroxide solution with equilibration buffer (50 mM sodium phosphate, 0.1 M NaCl, pH 7.2) until the eluent matches the pH of the equilibration buffer entering the column.

9. For storage, place the column into 20% ethanol solution and store at 2 –30 °C.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3260-00025	HCPure™	25 mL
3260-00100	HCPure™	100 mL
3260-00500	HCPure™	500 mL
3260-01000	HCPure™	1000 mL

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

HCPure™ is available in a carefully selected range of disposable columns sizes with bed volumes of 5 mL and 50 mL both with a standard bed height of 10 cm, suitable for process development applications and polishing steps.

Disposable Column Format

Code	Description
6650	HCPure™ Pre-packed Columns, 4 x 1 mL
6651	HCPure™ Pre-packed Columns, 4 x 5 mL

In addition, Astrea Bioseparations can offer column packing services.
For more information on this or any other supply related matters, please do not hesitate to contact us at sales@astrea-bio.com

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