Technical
User Guide

Fabsorbent™ F1P HF

Product Code: 3904

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PURITY by DESIGN

INTRODUCTION

In response to the growth of antibody fragments as research tools and biotherapeutic products, Astrea Bioseparations Ltd and its affiliates (Astrea Bioseparations) developed a new affinity adsorbent for the capture and purification of a wide range of antibody fragments (Figure 1).

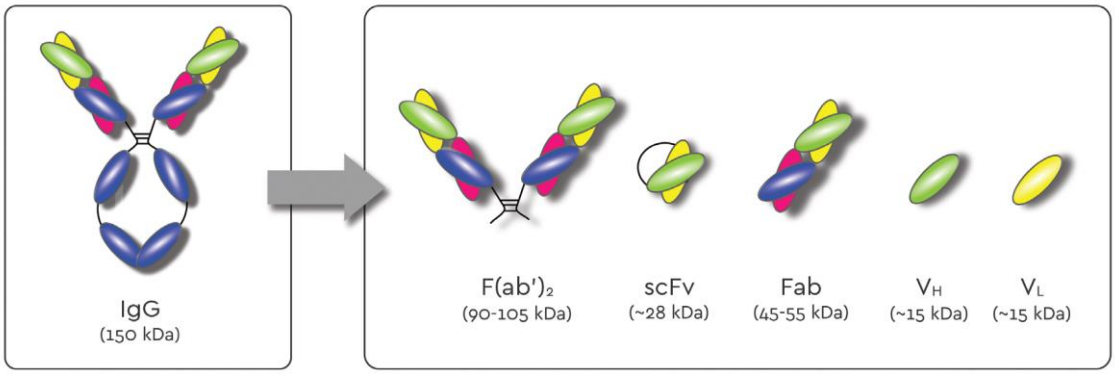


Figure 1: Schematic representation of antibody fragments

Fabsorbent™ F1P HF is an adsorbent with a synthetic ligand, which can be used as a superior alternative to Protein L for the capture and purification of antibody fragments including monovalent antibody fragments (e.g. Fab, scFv), engineered antibody variants and single domain antibodies.

The robustness and broad applicability of Fabsorbent™ F1P HF makes this adsorbent the ideal choice for both early stage research and development applications and also for larger-scale manufacturing uses where the full benefit of the high-flow/low pressure PuraBead® 6HF support matrix can be utilized. Given its broad selectivity, Fabsorbent™ F1P HF provides a "platform" technology for the purification of antibody fragments.

Properties of Fabsorbent™ F1P HF

LIGAND:	F1P synthetic triazine ligand
MEAN PARTICLE SIZE (µm):	90 ± 10 µm
MATRIX:	PuraBead® 6HF (6% cross-linked near monodisperse agarose)
BINDING CAPACITY:	Human Fab: Up to 20 g/L Human IgG: ≥ 40 g/L (depending on antibody / fragment type and source)
RECOMMENDED PACKING CONDITIONS:	At a constant pressure of 1.5 bar using 0.1 M NaCl solution
RECOMMENDED OPERATING PRESSURE:	Up to 1 bar
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 500 cm/h
OPERATING PH:	pH 1.5 to pH 14.0 (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 - 1.0 M NaOH, 25 °C
RECOMMENDED STORAGE CONDITION†:	2 – 30 °C, 20% ethanol

COLUMN PACKING

Fabsorbent™ FIP HF is supplied in 20% ethanol. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of Fabsorbent™ F1P 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 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 to settle in the column leaving a dead volume of packing solution above the adsorbent bed.

5. 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 packing flow to the bed (for ~ 2 CV). The recommended packing conditions (to obtain a uniform pack) is at a constant pressure of 1.5 bar (~ 22 psi) for larger columns. Flow rate will be dependent on column dimensions, however, will be around 600 cm/h.

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 (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).

   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.

8. 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 steps 6 and 7. If no space forms the column is packed and ready to use.

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.

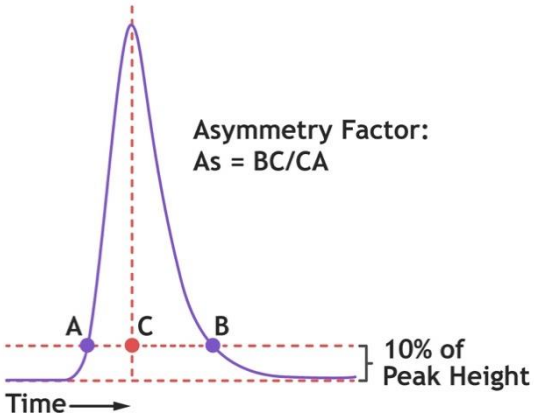
2. Attach the column to an equilibrated workstation.

3. Commence the flow for 1 column volume (CV) to equilibrate using a 0.1 M NaCl solution and obtain a baseline.

4. Inject 2% to 5% CV of a 2% acetone or 1 M to 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. Fabsorbent™ F1P HF 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

Recommended Chromatography Buffers for using Fabsorbent™ F1P HF

The following method is recommended (as a starting point). 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.

The range of antibodies and antibody fragments that can be purified using Fabsorbent™ F1P HF is extensive. To achieve optimal capture and recovery for the different protein molecules, it is important to carry out an initial scouting experiment to identify suitable binding and elution conditions (STEP 1).

Once the elution condition of the target molecule has been identified, the chromatography process can be further optimized (STEP 2). This may be required if excessive non-specific binding is observed, depending on feedstock.

The preferred option is to use Fabsorbent™ F1P HF with a liquid chromatography system or automated workstation. **Note:** The adsorbent can also be evaluated using a peristaltic pump or even a syringe.

Allow the column, buffers and sample to reach the operational temperature.

STEP 1 - Binding conditions:

- Antibodies and antibody fragments can be loaded onto the adsorbent between pH 6.0 and pH 8.0. It is recommended that the initial evaluation is performed using 50 mM sodium phosphate, pH 8.0.

- Apply the filtered/clarified protein solution/sample onto the column and collect the flow through. A residence time of ≥ 3 minutes is recommended.

- If required, wash the column with equilibration buffer to flush out the remaining protein solution with at least 1 CV or until a baseline is achieved.

- To determine the optimum elution condition and wash strategy (if required) for the target molecule, it is recommended that the initial elution evaluation is carried out using a McIlvaine's buffer (citrate/phosphate buffer) pH gradient from pH 7.6 to pH 3.0:

  - 1 L McIlvaine's buffer pH 7.6 = 63.50 mL of a 0.1 M solution of citric acid mixed with 936.50 mL of a 0.2 M solution of Na_2HPO_4

  - 1 L McIlvaine's buffer pH 3.0 = 794.50 mL of a 0.1 M solution of citric acid mixed with 205.50 mL of a 0.2 M solution of Na_2HPO_4

- Carry out a 20 CV gradient using the McIlvaine's buffers from pH 7.6 to pH 3.0.

- **Note**: Typically, Fab fragments will elute off the Fabsorbent™ F1P HF adsorbent at around pH 4.0, whereas other impurities including whole IgG will elute at pH 3.0 or below.

STEP 2 – Wash / Elution Optimization:

- **Note:** For rapid elution optimization and scouting experiments, Fabsorbent™ F1P HF is available in 1 mL Column Kits (Product code: 6632).

- When using Fabsorbent™ F1P HF to purify genetically engineered antibody fragments from complex feedstocks, some non-specific binding can occur and compromise the purity of the target material. To overcome this issue certain proven wash strategies can be used:

  - Glycine buffer, pH 9.0

  - Elution buffer additives such as:

    - IPA (5, 10 or 20%)
    - PEG (up to 15%)
    - Detergents (Triton X-100)
    - Propylene glycol (up to 50%)

- Other elution buffers can also be used to elute target material from Fabsorbent™ F1P HF, such as:

    - Sodium acetate
    - Sodium citrate
    - Glycine-HCl

Note: These elution buffers will have differing buffer values than that of McIlvaine's and therefore further optimization may be required to identify the optimal pH once again for target elution.

Clean-in-Place (CIP) and Sanitization:

Removal of any residual adsorbed material can be achieved by washing the column with 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.

Once cleaning with NaOH is complete, wash with at least 3 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.

Note: Column performance is not affected by this procedure.

Storage:

For long-term storage, it is recommended that Fabsorbent™ F1P HF is stored in 20% ethanol at 2 – 30 ºC.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3904-00025	Fabsorbent™ F1P HF	25 mL
3904-00100	Fabsorbent™ F1P HF	100 mL
3904-00500	Fabsorbent™ F1P HF	500 mL
3904-01000	Fabsorbent™ F1P HF	1000 mL

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

Pre-packed Column Format

Code	Description
6632	Fabsorbent™ F1P HF column kit (4 x 1 mL)
6633	Fabsorbent™ F1P HF column kit (4 x 5 mL)

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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