Technical
User Guide

MAbsorbent® A2P HF & A2P HF LL

Product Codes: 3903 & 3906

INTRODUCTION

MAbsorbents® were developed to mimic the Phe-132, Tyr-133 dipeptide binding site in the hydrophobic core structure of Protein A. The resultant synthetic bifunctional ligands exhibit high affinities for human immunoglobulin G. Hence, MAbsorbent® A2P and A2P LL provide excellent purification performance.

MAbsorbents® effectively bind a wide variety of human and mammalian polyclonal antibodies (including bovine, mouse, sheep, goat, horse and rabbit) as well as whole monoclonal antibodies, humanized antibody chimeras and antibody fragments.

Properties of MAbsorbent® A2P HF & A2P HF LL:

LIGAND:	Synthetic, aromatic triazine derivative
ADSORBENT APPEARANCE:	Pink
MEAN PARTICLE SIZE (µm):	90 µm ± 10µm
MATRIX:	PuraBead® 6HF (6% cross-linked near monodisperse agarose)
DYNAMIC BINDING CAPACITY:	≥ 35 g/L Human IgG*
RECOMMENDED PACKING CONDITIONS:	Packing pressure – 1.5 bar Packing solution - 0.1 M NaCl
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 500 cm/h
OPERATING PH:	pH 2.5 to pH 14.0
PH STABILITY:	Long term: pH 3.0 – pH 12.0
CHEMICAL STABILITY:	All commonly used aqueous buffers and solutions
CLEANING / SANITIZATION:	0.5 to 1.0 M NaOH, 25 °C
STORAGE:	20% ethanol, 2 - 30 °C

* Purified IgG

COLUMN PACKING

MAbsorbent® A2P HF & A2P HF LL are supplied in a 20% ethanol solution. Before commencing the column pack, consult the relevant manufacturer's instructions for the selected column. The method below describes the packing of MAbsorbent® A2P HF & A2P HF LL 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. 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 desired flow to the be for (~ 2CV). The recommended packing condition (to obtain a uniform pack) is at a constant pressure up to 1.5 bar (~ 22 psi). 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 and close the column outlet and stop the liquid flow through the bed.

7. 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).

   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 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 / 0.2M 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 performance is not affected by this procedure.

COLUMN EFFICIENCY TEST

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

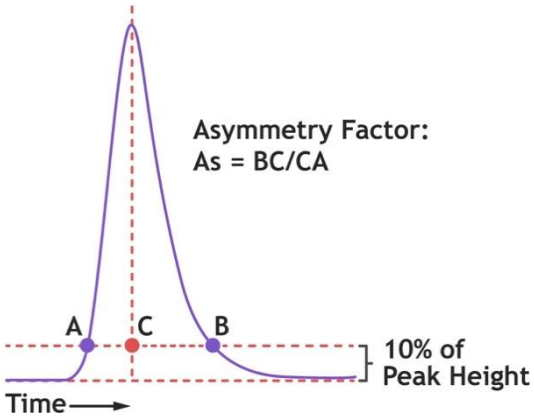
2. Attach the column to an equilibrated workstation.

3. Commence flow for at least 1 CV (i.e. to equilibrate and obtain a baseline) using equilibration buffer (low ionic strength buffer) or saline solution as the mobile phase.

4. Inject a tracer solution of 2% to 5% CV of a 2 M NaCl solution or 2% acetone solution.

5. Continue flow until a UV or conductivity (dependent on tracer solution used) peak is observed and the trace has returned to baseline (~ 1.5 CV).

6. End run and determine the asymmetry factor:



7. MAbsorbent® A2P HF and A2P HF LL are affinity adsorbents, therefore an asymmetry factor for an acceptable packed is between 0.8 to 1.6. The recommended plate count for an acceptable pack is ≥ 1800 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. MAbsorbent® A2P HF & A2P HF LL 1 mL and 5 mL column kits are also available for scouting experiments. The following method is recommended (as a starting point) with an initial flow rate of 100 cm/h for all the column chromatography steps. Subsequent increases / decreases in the flow rate can be investigated to improve binding capacity/resolution or decrease processing times.

1. Sample treatment:

   IgGs from a variety of sources can be purified with MAbsorbent® adsorbents. As with conventional Protein A media, capacity / performance is dependent on the concentration of IgG in the feedstock. Ideally, the antibody concentration should be 1 g/L or higher. For samples containing lower amounts of the antibody, a concentration step is recommended. Appropriate filtration / clarification of the feedstock is recommended.

   Note: For monoclonal feedstocks, feedstock components such as phenol red (pH indicator) or Pluronic F-68 (cyto-protective agent) may affect the performance of the adsorbent, and sample dialysis/diafiltration in these cases is recommended. Alternatively, the additives can be reduced by an initial purification step using ion exchange chromatography. If you have any questions, please contact us at techsupport@astrea-bio.com

2. Equilibrate the column with ~ 5 CV of equilibration buffer (Note: ensure the equilibration buffer of the column is comparable to the protein feedstock - i.e. 25 – 100 mM sodium phosphate buffer at pH 7.0 with the addition of NaCl to increase the conductivity of the equilibration buffer to match that of the feedstock).
3. Apply the protein feedstock onto the equilibrated column using a recommended residence time of ≥ 3 minutes.
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 target protein using up to 5 CV of a low pH low ionic strength buffer (e.g 50 mM citric acid, pH 3.0- or 50 mM glycine-HCl, pH 3.0).
6. For clean-in-place (CIP), the adsorbents are caustic stable, pass through the column 5 CV of 1 M NaOH solution.

   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.
7. Re-equilibrate column with at least 5 CV of equilibration buffer or until the pH of the eluent is comparable to the equilibration buffer entering the column.
8. For long-term storage, place the column into 20% ethanol (3 CV minimum) and store at 2 – 30 °C.

ORDER INFORMATION

Gel Slurry

Code	Description	Pack Size
3903-00025	MAbsorbent® A2P HF	25 mL
3903-00100	MAbsorbent® A2P HF	100 mL
3903-00500	MAbsorbent® A2P HF	500 mL
3903-01000	MAbsorbent® A2P HF	1000 mL
3906-00025	MAbsorbent® A2P HF LL	25 mL
3906-00100	MAbsorbent® A2P HF LL	100 mL
3906-00500	MAbsorbent® A2P HF LL	500 mL
3906-01000	MAbsorbent® A2P HF LL	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

Prepacked column kits (4 x 1 mL and 4 x 5 mL) for small scale experiments are also available for MAbsorbent® A2P HF LL.

Prepacked Column Kits

Code	Description	Pack Size
6630	MAbsorbent® A2P HF LL Column Kit	4 x 1 mL columns
6631	MAbsorbent® A2P HF LL Column Kit	4 x 5 mL columns

If you require any further information on this or have any other supply questions, please contact us at sales@astrea-bio.com

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