Technical
User Guide

1 mL and 5 mL HT Column Kits

INTRODUCTION

The 1 mL and 5 mL columns kits supplied by Astrea Bioseparations are constructed using biocompatible polypropylene. The column kits are comprised of 4 inexpensive, re-usable, pre-packed columns, each containing either 1 mL or 5 mL of adsorbent. The column kits allow for rapid, accurate and cost-effective method development, small scale purification runs or as single-use disposable columns for contaminant removal conditions.

This technical user guide is applicable for all 1 mL and 5 mL column kits and is designed to be used in conjunction with the corresponding gel slurry technical user guide supplied. The guide shows how to operate the 1 mL and 5 mL columns using either a chromatography workstation or a syringe.

Note: The accompanying gel slurry technical user guide(s) will provide all the relevant chromatography conditions (i.e. equilibration, wash, elution, Clean-in-Place and storage conditions) for the adsorbent(s).

Properties of the 1 mL HT columns:

COLUMN:	1 mL axial flow column (ID: 0.7 cm diameter x 2.5 cm bed height)
COLUMN MATERIAL:	Polypropylene
MAXIMUM RECOMMENDED OPERATING PRESSURE (DELTA COLUMN PRESSURE):	Not exceeding 5 bar (~72 psi) *
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 1 mL/min
OPERATING PH:	See accompanying gel slurry technical user guide for specific information
PH STABILITY:	
CHEMICAL STABILITY:	
CLEANING/SANITIZATION:	
STORAGE:	

* - Delta pressure across the column (not the system pressure)

** - To minimize the risk of the columns drying out.

Properties of the 5 mL HT columns:

COLUMN:	5 mL axial flow column (ID: 1.6 cm diameter x 2.5 cm bed height)
COLUMN MATERIAL:	Polypropylene
MAXIMUM RECOMMENDED OPERATING PRESSURE (DELTA COLUMN PRESSURE):	Not exceeding 5 bar (~72 psi) *
RECOMMENDED OPERATIONAL FLOW RATE:	Up to 5 mL/min
OPERATING PH:	See accompanying gel slurry technical user guide for specific information
PH STABILITY:	
CHEMICAL STABILITY:	
CLEANING/SANITIZATION:	
STORAGE:	

* - Delta pressure across the column (not the system pressure)

** - To minimize the risk of the columns drying out.

OPERATING INSTRUCTIONS

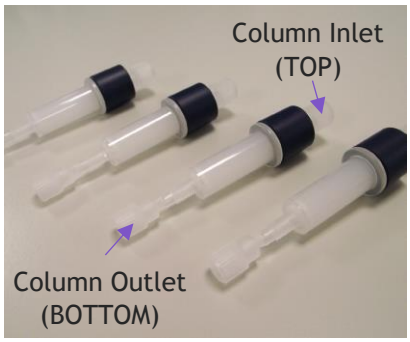
1. The preferred option is to use the 1mL and 5 mL columns with a liquid chromatography system or automated workstation. **Note:** The column can also be operated manually using a peristaltic pump or even a syringe.

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

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

4. Remove the inlet (Top) stopper of the column and attach directly to the liquid chromatography system using a standard 10-32 threaded male for 1/16'' OD tubing connector, ensuring that the tubing connecting the workstation to the column is primed with equilibration buffer.

5. Snap off the outlet (Bottom) plug of the column and attach to the workstation as in step 4. **Note:** The reverse of the bottom plug is threaded and can be used as a stopper.



6. Equilibrate each 1 mL or 5 mL column with 10 CV (10 mL or 50 mL respectively) of an appropriate equilibration buffer.

7. Apply the clarified / filtered feedstock at a flowrate of 0.5 mL/ min (1 mL columns) or 2.5 mL/min (5 mL column), equivalent to a 2 min residence time.

8. Remove any non-bound material from 1mL or 5 mL column with 5 – 10 CV of the equilibration buffer or until the UV trace returns to baseline.

9. Elute the bound protein using up to 10 CV of an appropriate elution strategy.

10. If required, clean the 1mL or 5 mL column with 5 – 10 CV of the recommended clean -in-place solution

11. Re-equilibrate column with 10 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.

12. For storage place the 1 mL or 5 mL column into preservative solution (with up to 10 CV) and disconnect from the chromatography workstation. Connect the end pieces and store appropriately.

Note: The columns cannot be opened or re-packed.

OPERATING INSTRUCTIONS FOR USING A SYRINGE

1. Fill the syringe with binding buffer. Remove the stopper and connect the column to the syringe (using a 1/16” to Luer connector – not supplied) “drop to drop” to avoid introducing air into the column.

2. Remove the snap-off end at the column outlet.

3. Equilibrate the column with 5 column volumes of binding buffer.

4. Apply the pre-treated sample using a syringe fitted to the Luer connector on the column. For optimal results, use a flow rate of 0.2 to 1 mL/min (1 mL column) and 0.5 to 5 mL/min (5 mL column) during sample application*.

5. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent. Maintain a flow rate of 1 to 2 mL/min (1 mL column) and 5 to 10 mL/min (5 mL column) for washing. Optional: collect the flow through (in 1 mL fractions for the 1 mL column and 2 mL fractions for the 5 mL column) and reserve until the procedure has been successfully completed. Retain a sample for analysis by SDS-PAGE to measure the efficiency of protein binding to the medium.

6. Elute with 5 to 10 column volumes of elution buffer. Maintain a flow rate of 0.2 to 1.0 mL/min (1 mL column) and 0.5 to 5 mL/min (5 mL column) for elution.

7. After elution, regenerate the column by washing it with 3 to 5 column volumes of binding buffer. The column is now ready for a new purification.

   * 1 mL/min corresponds to approximately 30 drops/min when using a syringe with a HT 1 mL column; 5 mL/min corresponds to approximately 120 drops/min when using a HT 5 mL column.

ORDER INFORMATION

HT Column Kits

Code	Adsorbent	Pack Size
6600	Q PuraBead® HF	4 x 1 mL
6601	Q PuraBead® HF	4 x 5 mL
6602	DEAE PuraBead® HF	4 x 1 mL
6603	DEAE PuraBead® HF	4 x 5 mL
6604	SP PuraBead® HF	4 x 1 mL
6605	SP PuraBead® HF	4 x 5 mL
6606	CM PuraBead® HF	4 x 1 mL
6607	CM PuraBead® HF	4 x 5 mL
6608	IEX Selection Kit, 1 mL	1 x Q PuraBead® HF 1 mL Column 1 x DEAE PuraBead® HF 1 mL Column 1 x SP PuraBead® HF 1 mL Column 1 x CM PuraBead® HF 1 mL Column
6609	IEX Selection Kit, 5 mL	1 x Q PuraBead® HF 5 mL Column 1 x DEAE PuraBead® HF 5 mL Column 1 x SP PuraBead® HF 5 mL Column 1 x CM PuraBead® HF 5 mL Column
6610	Phenyl PuraBead® HF	4 x 1 mL
6611	Phenyl PuraBead® HF	4 x 5 mL
6612	Octyl PuraBead® HF	4 x 1 mL
6613	Octyl PuraBead® HF	4 x 5 mL
6614	HIC Selection Kit (1mL columns)	1x 1mL Phenyl PuraBead® HF 1x 1mL Octyl PuraBead® HF
6615	HIC Selection Kit (5mL columns)	1x 5mL Phenyl PuraBead® HF 1x 5mL Octyl PuraBead® HF
6616	Aminophenylboronate P6XL	4 x 1 mL
6617	Aminophenylboronate P6XL	4 x 5 mL
6618	p-Aminobenzamidine Agarose 6XL	4 x 1 mL
6619	p-Aminobenzamidine Agarose 6XL	4 x 5 mL

6620	Mimetic Blue® SA P6HF	4 x 1 mL
6621	Mimetic Blue® SA P6HF	4 x 5 mL
6622	Mimetic Blue® 1 P6HF	4 x 1 mL
6623	Mimetic Blue® 1 P6HF	4 x 5 mL
6624	Mimetic Blue® SA HL P6HF	4 x 1 mL
6625	Mimetic Blue® SA HL P6HF	4 x 5 mL
6626	AlbuPure®	4 x 1 mL
6627	AlbuPure®	4 x 5 mL
6628	Albumin purification selection kit, 1 mL columns	1 x Mimetic Blue SA P6HF 1 x Mimetic Blue SA HL P6XL **2 x** AlbuPure
6629	Albumin purification selection kit, 5 mL columns	1 x Mimetic Blue SA P6HF 1 x Mimetic Blue SA HL P6XL **2 x** AlbuPure
6630	MAbsorbent® A2P HF LL	4 x 1 mL
6631	MAbsorbent® A2P HF LL	4 x 5 mL
6632	Fabsorbent™ F1P HF	4 x 1 mL
6633	Fabsorbent™ F1P HF	4 x 5 mL
6634	Butyl PuraBead® HF	4 x 1 mL
6635	Butyl PuraBead® HF	4 x 5 mL
6636	Hexyl PuraBead® HF	4 x 1 mL
6637	Hexyl PuraBead® HF	4 x 5 mL
6638	PE PuraBead® 6HF	4 x 1 mL
6639	PE PuraBead® 6HF	4 x 5 mL
6640	Mimetic Blue® AP HL P6HF	4 x 1 mL
6641	Mimetic Blue® AP HL P6HF	4 x 5 mL
6642	Mimetic Blue® SA HL P6XL	4 x 5 mL
6643	Mimetic Blue® SA HL P6XL	4 x 1 mL
6644	Insulin Adsorbent P6HF	4 x 1 mL
6645	Insulin Adsorbent P6HF	4 x 5 mL

6646	EtoxiClear™	4 x 1 mL
6647	EtoxiClear™	4 x 5 mL

For more information on these products or any other supply related matters, please do not hesitate to contact us at sales@astrea-bio.com

+44 (0) 1223 433 800 | astreabioseparations.com

sales@astrea-bio.com | techsupport@astrea-bio.com | quality@astrea-bio.com

Global bases in North America, Canada and Cambridge UK HQ:
Horizon Park, Barton Road, Comberton, Cambridge, CB23 7AJ, UK

Issue Date: 31 May 2022
CCR Number: CCR-1729
Author Name: R Dodd
QA Reviewer Name: R Hawkins
This product is covered by or for use under one or more patents: www.astreabioseparations.com/patents

Astrea Bioseparations