Chromabolt® Prepacked Chromatography Columns
Chromabolt® Basic Chromatography Columns

Ion Exchange Resins
Affinity Resins

User Guide

Notice

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Contents

Introduction

For specifications on the Chromabolt® Column, refer to the Certificate of Analysis supplied with the media and the Certificate of Quality supplied with the Chromabolt® Column.

Operator and Equipment Safety

Anyone operating or near the Chromabolt® Column must comply with the following:

- Read and understand this user guide and the media user guide before using the column. Failure to follow instructions could result in user injury or system damage.
- Read and understand all maintenance instructions in this user guide before performing maintenance on the system. Failure to follow instructions could result in user injury or system damage.
- Use appropriate personal protective equipment when operating the system.
- Follow the unpacking instructions on the outside of the crate when removing the system from packaging. Failure to follow these instructions could result in operator injury or equipment damage.
- Do not lift the 20 or 32 cm columns without the proper equipment. Follow local regulations regarding lifting limits.
- Any alteration of the column from factory specification may cause unsafe conditions and void the product warranty.
- Engage the wheel locks when the column is stationary.
- Ensure there are no kinks or bends in any tubing.
- Do not lift 20 and 32 cm units by the push handle as this may damage the column.
- Do not spray IPA directly on the column.
- Prevent 20 and 32 cm ID columns from tipping as this may harm the unit.

Unpacking

Refer to the Unpacking Instructions on the outside of the packing crate for uncrating instructions. Once the outer bag has been removed from the column, follow the instructions below.

10 cm ID Column

1. A 50 mL sample of resin is included with each Chromabolt® Column. The resin sample is taped to the inside of the crate.
2. Lift the column in the inner bag and move it into the Clean Room prep area. The column may be transported on a cart. The column weighs approximately 5.4 kg (12 lbs).
3. Remove the zip tie from the inner bag and remove the bag from the column.
4. Follow Standard Operating Procedures (SOP) to wipe down the column using 70% IPA wipes.

NOTE Do not spray IPA directly on the column.

5. Move column to the final location.

20 and 32 cm ID Columns

1. A 50 mL sample of resin is included with each Chromabolt® Column. The resin sample is wrapped onto the foam in the front of the column.

1. Unlock the wheels on the column.
2. Wheel the column out of the shipping crate using the ramp into the the Clean Room prep area.

NOTE A resin sample is provided with the 20 and 32 cm columns for QC testing.

Do not lift the column without the proper equipment. Follow local regulations regarding lifting limits.

Do not lift the unit by the handle as this may cause damage to the column.

3. Remove the zip tie from the inner bag and remove the bag from the column.
4. Follow Standard Operating Procedures (SOP) to wipe down the column using 70% IPA wipes.

NOTE Do not spray IPA directly on the column.

Prevent 20 and 32 cm ID columns from tipping as this may harm the unit.

5. Move column to the final location.

User Supplied Materials

The following materials, supplied by the user, are required to set up the columns:

Table 1: User Supplied Materials

Item	Quantity
Chromatography system	1
0.75 m (2 ft) Tubing with sanitary fittings: ¼ in. ID tubing for 10 cm ID column ⅜ in. ID tubing for 20 cm ID column ½ in. ID tubing for 32 cm ID column	2
Sanitary clamps	as required
Sanitary gaskets	as required
Sanitary three way valves	2
Sanitary Tee connectors	2
Pressure gauges	2
Water for injection (WFI) or de-ionized water (DI) water	6 Column Volumes (CV)
160 mM sodium chloride (NaCl) solution	6 CV
400 mM NaCl solution	5 CV
1M NaCl solution	5% CV

NOTE The volumes specified in the table above are the minimum volumes required for the device. Prepare enough solution to compensate for system dead volume, priming, purging, etc.

Table 2: Typical Column Volumes

Column Diameter (cm)	Column Volume (L)
10	1.6
20	6.5
32	16.1

Table 3: % Compression Table

Media	% Compression
Fractogel® TMAE HiCap	25
Fractogel® SE HiCap	25
Fractogel® SO3	30
Fractogel® COO	30
Fractogel® TMAE	30
Fractogel® DEAE	28
Eshmuno® A	13
Eshmuno® Q	12
Eshmuno® S	10

% compression = (SBV - PBV)/SBV
where: PBV = Packed Bed Volume
SBV = Gravity Settled Bed volume

Contact your local representative for % Compression of resins in Basic Columns.

Column Set-Up

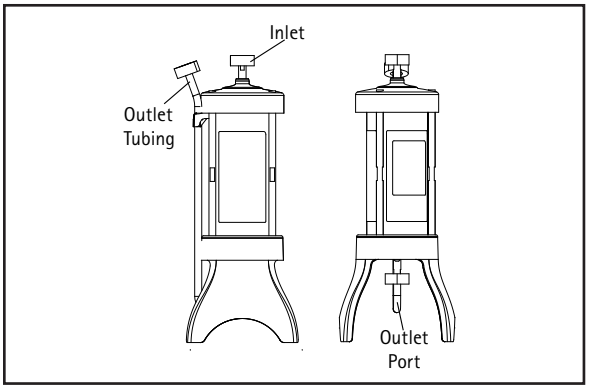

Figure 1: Chromabolt® Prepacked 10 cm Chromatography Column

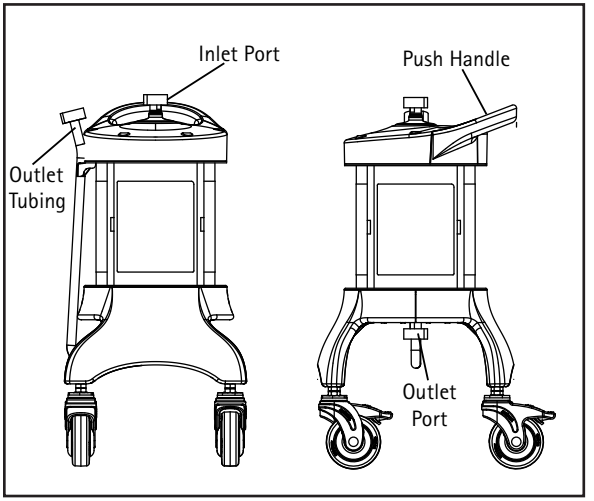

Figure 2: Chromabolt® Prepacked 20 cm Chromatography Column

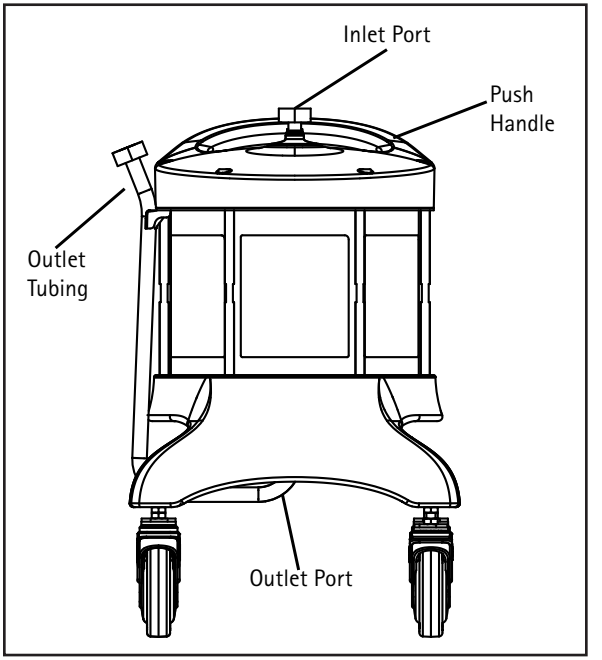

Figure 3: Chromabolt® Prepacked 32 cm Chromatography Column

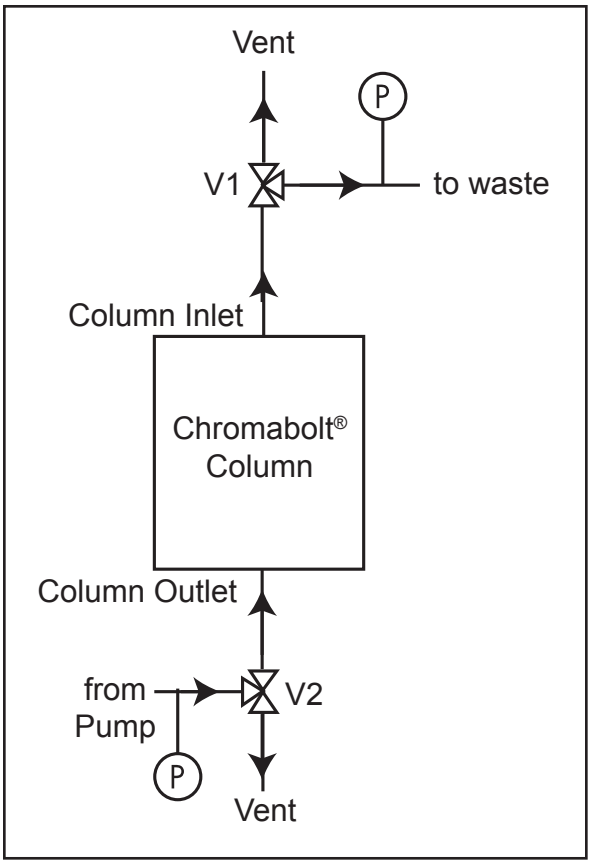

Figure 4: Typical Column Set-up

NOTES Ensure that no air enters the column during set-up.

The outlet tubing should remain in the tubing clip on the column during set-up.

Allow column to equilibrate to room temperature.

1. Remove the end cap from the tubing on the column outlet.
2. Fill the outlet tubing with WFI or DI using a squeeze bottle.
3. Install a three way valve (V2) onto the column outlet tubing.
4. Set V2 to isolate the column outlet.
5. Remove the end cap from the column inlet port and install a three way valve (V1) onto the column inlet.
6. Connect a tee with a pressure gauge to V1 (Figure 4).
7. Connect a tee with pressure gauge to V2 (Figure 4).

The column is now ready to connect to a chromatography system.

Column Flushing

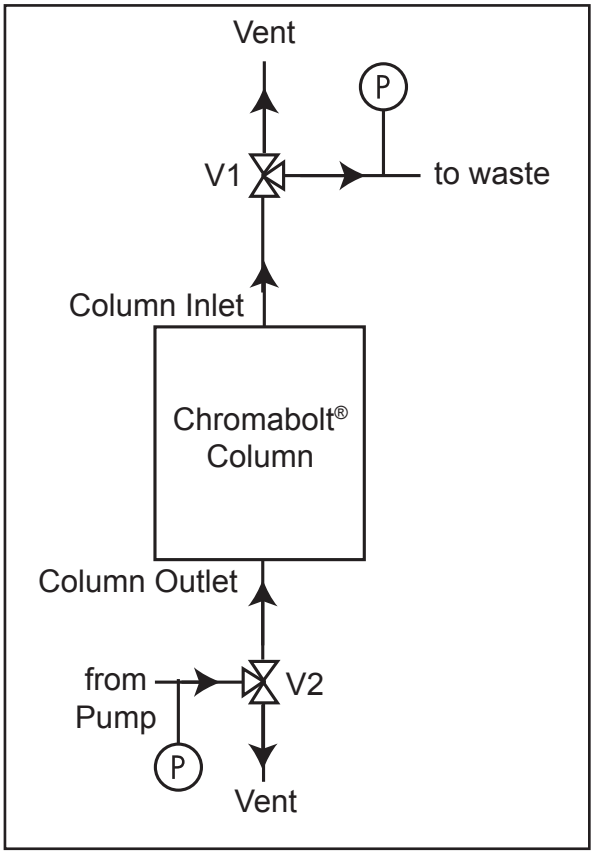

Figure 5: Column Flushing Set-up

NOTE Follow the sequence below as presented to prevent over-pressurizing the column which may damage the column.

If the chromatography system allows reversal of flow direction through column:

1. Connect V1 to the system column top/ column in port using the tubing described in Table 1.
2. Connect V2 to the system column bottom/ column outlet port.
3. Set the system inlet to WFI/DI.
4. Direct the system waste port to appropriate collection vessel/drain.
5. Set the flow direction to reverse (flow from column outlet to column inlet).

CAUTION

Confirm that V2 is in the vent position.

6. Prime the chromatography system with all solutions in Table 1.
7. When a steady stream of liquid exits V2, set the system flow rate to 100cm/hr.
8. Once the system flow has stabilized, open V1, then open V2, to allow flow through the column.
9. After 6 CV of WFI/DI has been flushed through column, stop the flow and set the flow direction on the system to forward.
10. Proceed to the Equilibration and Pressure Test.

If the chromatography system does not allow reversal of flow direction through column:

1. Connect V1 to the system column bottom/ column out port using the tubing described in Table 1.
2. Connect V2 to the system column top/ column in port.
3. Set the system inlet to WFI/DI.
4. Direct the system waste port to an appropriate collection vessel or drain.

CAUTION

Confirm that V2 is in the vent position.

5. Prime the chromatography system.
6. When a steady stream of liquid exits V2, set the system flow rate to 100cm/hr.
7. Once the system flow has stabilized, open V1, then open V2, to allow flow through the column.
8. After 6 CV of WFI/DU has been flushed through column, stop the flow and isolate the column from the system using V1 and V2.
9. Connect V1 to the system column top/ column in port using the tubing described in Table 1.
10. Connect V2 to the system column bottom/ column out port. Confirm that tV1 and V2 are directed to vent.
11. Set the linear velocity to 100 cm/hr (or the corresponding flow rate) and start the pump.
12. When liquid is seen exiting V1, tap the tee and tubing to ensure that all air is removed.
13. When no more air is visible exiting V1, stop the flow and proceed to the Equilibration and Pressure Test.

Equilibration and Pressure Test

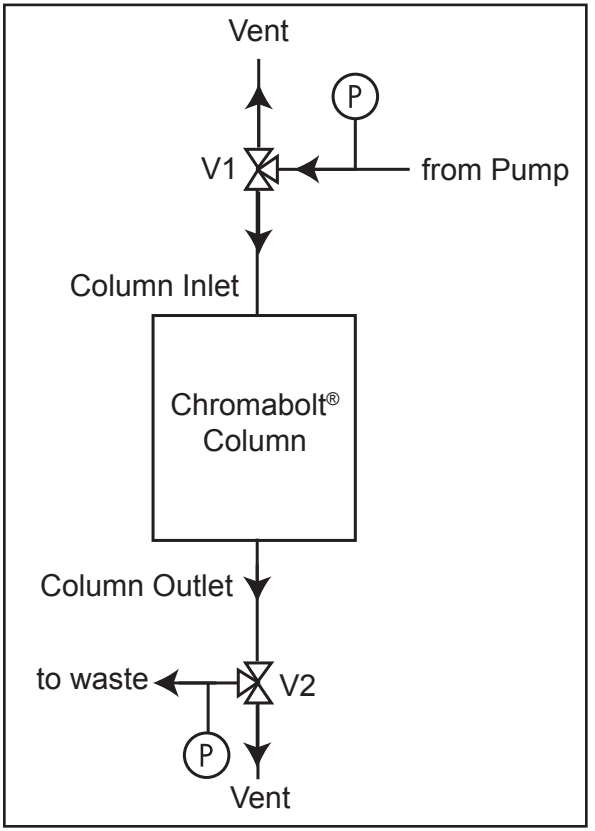

Figure 6: Equilibration and Pressure Test Set-up

1. Set the system inlet to the 160 mM NaCl solution source.
2. Direct the waste to an appropriate collection vessel or drain.
3. Set the system flow rate to 100 cm/hr.
4. Confirm that V1 and V2 are directed to vent.
5. Once the system flow has stabilized, open V2, then open V1 to allow flow through the column.
6. After 6 CV of 160 mM NaCl has been flushed through column, gradually increase pump flow rate until the differential pressure across the column is 15 psi.
7. Measure the flow rate at the outlet of the column using a graduated cylinder.
8. Record the temperature of the liquid at the outlet of the column and verify that it is equal to the inlet liquid temperature.
9. If the temperature is 25 °C, record the measured flow rate as the column flow rate at a differential pressure of 15 psi. If temperature is not 25 °C, multiply the measured flow rate by the compensation factor (F) listed in the Table below.

NOTE The temperature must be 25° C ±2° when calculating temperature/viscosity compensation to get accurate readings.

10. If the temperature corrected flow rate is ≥150 cm/hr, the column is within specification.
11. Set V1 and V2 in the positions to isolate the column.

Table 4: Temperature Correction Factor (F)*

Temperature		F
°F	°C	
86.0	30	0.896
84.2	29	0.915
82.4	28	0.935
80.6	27	0.956
78.8	26	0.978
77.0	25	1.000
75.2	24	1.023
73.4	23	1.047
71.6	22	1.072
69.8	21	1.098
68.0	20	1.125
66.2	19	1.152
64.4	18	1.181
62.6	17	1.212
60.8	16	1.243
59.0	15	1.276
57.2	14	1.310
55.4	13	1.346
53.6	12	1.383
51.8	11	1.422
50.0	10	1.463
48.2	9	1.506
46.4	8	1.551
44.6	7	1.598
42.8	6	1.648
41.0	5	1.699
39.2	4	1.751

Based on Water Fluidity Relative to 25 °C (77 °F)
Fluidity Value $F = (\mu_{T\,^{\circ}C} / \mu_{25\,^{\circ}C})$ or $(\mu_{T\,^{\circ}F} / \mu_{77\,^{\circ}F})$.

HETP/Asymmetry Test

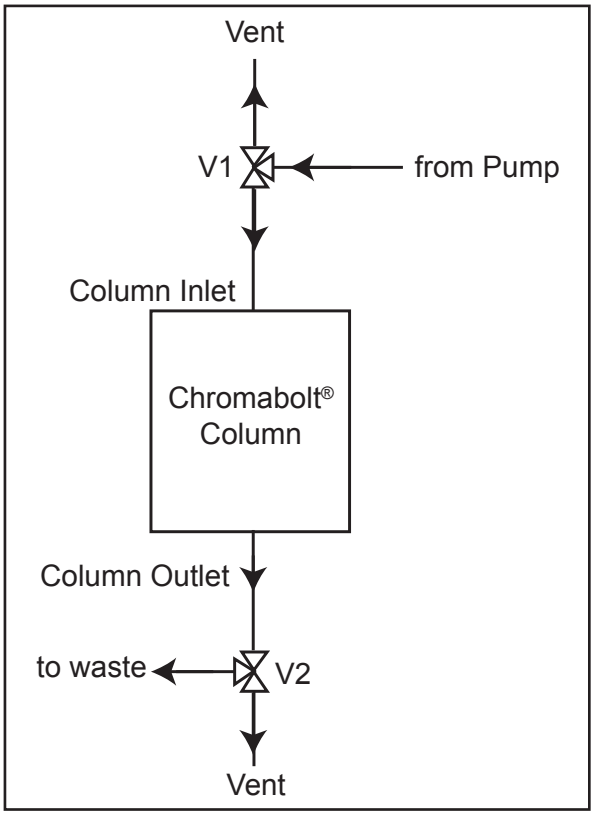

Figure 7: HETP/Assymetry Test Set-up

NOTE The HETP and Asymmetry acceptance criteria have been developed based on set up with a conductivity sensor at the column outlet. If this set up cannot be reproduced and acceptance criteria cannot be met as a result, contact your local representative.

Remove any additional tubing, T connectors or instruments such as pressure gauges while performing Asymmetry and HETP measurement to minimize dead spaces which can adversely affect the readings.

1. Remove the tee and the pressure gauge from column top and bottom.
2. Connect the top of the column to the system column top/column in port using the tubing described in Table 1.
3. Connect V2 to the system column bottom/column out port.
4. Ensure that the column is in the forward position if the system allows for flow reversal.
5. Set the system inlet to 400 mM NaCl.
6. Set the system waste to an appropriate collection vessel or drain.
7. Confirm that V1 and V2 are set to the vent positions.
8. Set the linear velocity to 150 cm/hr (or the corresponding flow rate) and start the pump.
9. When liquid is seen exiting the V1, tap the tubing to ensure that all air is removed.
10. Once the system flow has stabilized, open V2, then open V1, to allow flow through the column.
11. Flow 4CV of 400 mM NaCl solution at 150cm/hr through the column to equilibrate the column.
12. Inject 1 M NaCl solution, 1.25% of the CV into the column. The pulse injection method may vary depending on the system being used.
13. Measure and record the effluent conductivity.
14. Assuming a Gaussian peak distribution of the salt conductivity measured at the column outlet, calculate the HETP and Asymmetry of the column using the equations below

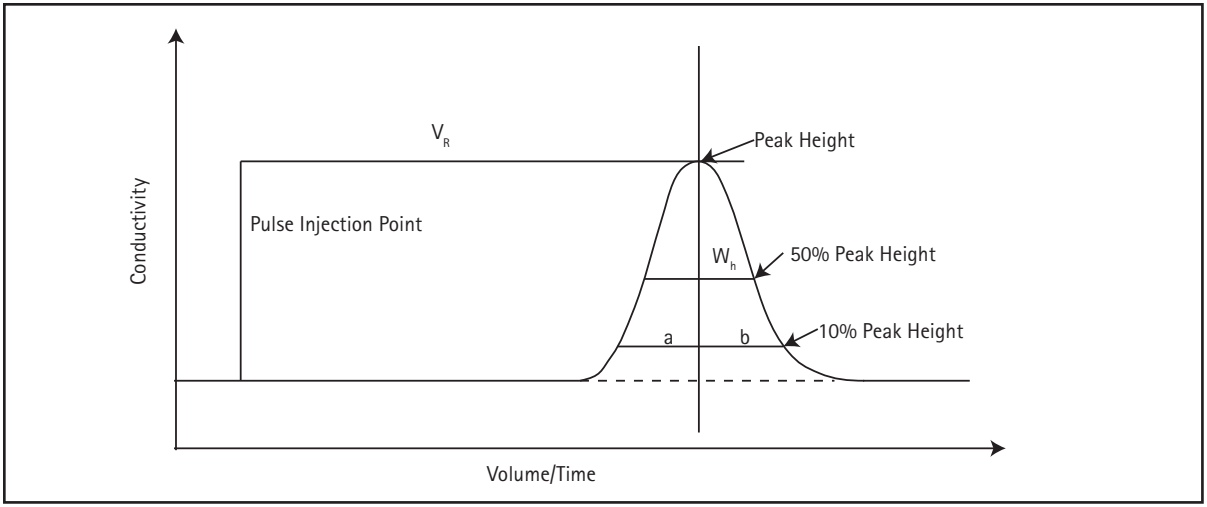

Figure 8: Gaussian Peak

$$N = 5.54 \times (V_R/W_h)^2$$

$$HETP = L/N$$

$$As = b/a$$

Where:

N	Theoretical number of plates
L	Height of the resin bed
HETP	Height equivalent theoretical plates
As	Asymmetry factor
V_R	Peak retention (elution) volume or time
W_h	Peak width at half height expressed in the same units as VR
a	Partial peak width, measured at 10% of the peak height for the leading part of the peak to the center of the peak
b	Partial peak width, measured at 10% of the peak height for the tailing part of the peak to the center of the peak

The Chromabolt® Column is now ready for use.

Sanitization

1. Refer to the table below for storage solution selection for Chromabolt® Prepacked Chromatography Columns and Chromabolt® Basic Chromatography Columns. Flow 4 CV of sanitization solution through column at 100 cm/hr.

Resin	Solution
Fractogel® COO Fractogel® DEAE Fractogel® TMAE Fractogel® TMAE HiCap Fractogel® SE HiCap Fractogel® SO3 Eshmuno® Q Eshmuno® S	0.5N sodium hydroxide (NaOH)
Eshmuno® A	PAB solution (120mM phosphoric acid, 167mM acetic acid, 2.2% benzyl alcohol)

2. Stop the pump and close the inlet and outlet valves on the column.
3. Incubate the column in the sanitization solution for three hours.
4. Connect the pump to a WFI/DI water source and flow through system.
5. Wash the column by pumping WFI/DI water volume listed above at 100 cm/hr through the system.

Resin	WFI/DI Volume (CV)
Fractogel® COO Fractogel® DEAE Fractogel® TMAE Fractogel® TMAE HiCap Fractogel® SE HiCap Fractogel® SO3 Eshmuno® Q Eshmuno® S	6
Eshmuno® A	10

6. Flow 6 CV of 20% Ethanol with 150 mM NaCl in DI water through the column at 100 cm/hr to equilibrate the column with storage solution.
7. Stop the pump, close the inlet and outlet valves of the column and disconnect the tubing external to the column valve assembly.

Storage

1. The column can be stored at the recommended temperature for the resin. For Eshmuno® A, store at 2 to 8° C. and refer to the resin user guide for further recommendations.
2. Columns stored long term, before or after use, should be inspected every twelve months for storage buffer level in outlet tube. Top-off the tube with the same storage buffer if the level has dropped. This procedure should be performed in a clean environment.
3. Do not freeze the column.

Troubleshooting

Symptom	Possible Cause	Potential Remedies
High back pressure in column	Flow rate greater than the resin recommended flow rate	Reduce pump flow rate or stop pump
	Column downstream or upstream valve closed	Stop the pump. Open the valve to the vent to relieve the pressure and then switch it to the column
	Tubing is pinched	Look for kinked tubing and adjust the pinched areas.
	Frit is plugged due to dirty feed, particulates in solution.	Reverse flow through column. Use a guard column for future runs.
	Tubing size smaller than recommended	Use appropriate tubing
	System internal tubing and set up not fit for column size being used	Check system pressure drop independent of the column. If high, use a larger system.
Leaks at connectors	Missing or damaged sanitary gasket	Replace sanitary gasket
	Improper connection or damaged connector	Tighten connector or change if damaged
	High back pressure	See previous Symptom
Leaks from Column	High back pressure	Check if operating pressure is greater than 75psi.
	Column sanitary connector damaged	Replace connector
	Column tube damaged	Replace whole column
Bed is compressing during processing	High back pressure	Flow rate greater than the resin recommended flow rate
	High viscosity liquid being flowed through column	Reduce flow rates
	Low temperature liquid being flowed through the column	Reduce flow rates
Column QC fails HETP and Asymmetry specification	Incorrect salt concentration or buffer conductivity. In correct salt solution prepared	Check salt concentration and buffer conductivity. If not as recommended prepare appropriate buffer
	Incorrect flow rate used	Adjust or reduce flow rate to recommended flow rate.

Symptom	Possible Cause	Potential Remedies
Column QC fails flow specification	Buffer used	Verify proper buffer is being run. If not prepare appropriate buffer.
	Buffer temperature	If buffer temperature is low increase the temperature to 25°C.
	Buffer viscosity	If buffer viscosity is high, change to recommended buffer.
Column fails Bioburden and Entotoxin Specification After Use	Test the input fluid being used in the column	If out of specification, change fluid and sanitize column.
	Incomplete sanitization	Re-sanitize column. Flush column with 6 CV (Ion Exchange Resins) or 10 CV (Affinity Resins) each of endotoxin free water and buffer.
	Sanitization time less than three hours	Follow suggestion for incomplete sanitization.
	Sanitant concentration	Verify that the sodium hydroxide or PAB (for Eshmuno® A) used to prepare the sanitant has not expired. Prepare new sanitant solution and resanitize column.
Column appears hazy	Visual effect of the resin bed in the acrylic tube	Verify that the column is within specifications. If out of specification follow, see next Symptom.
	Air in the column	See below.
Introduced air into the column or spot air in the column	Degassing	Verify that the column is within specifications. If column is out of specification, flush 6 CV of DI/WFI through the column in reverse flow mode to purge all the air out of the column, followed by equilibration with 6 CV of salt buffer. Retest column for specifications.
	Leaking connector upstream of column	
	Leaking connector on suction side of pump	
	Column drained due to open outlet	

Standard Product Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the "Terms and Conditions of Sale" applicable to your purchase transaction).

Technical Assistance

For more information, contact the office nearest you or visit the Technical Service page at www.millipore.com/techservice. Worldwide contact information is available at www.millipore.com/offices.