TECHNICAL MANUAL

Revision 06-15

ZetaSep FlashFlow™

Glass Columns

for high-performance preparative chromatography

emp BIOTECH

excellence - made possible

Warnings

WARNING

Please read and fully understand the operating instructions for this equipment prior to use. Improper use of this equipment could result in serious injury or death!

WARNING

Glass ZetaSep FlashFlow™ columns are intended for use in a liquid environment. NEVER use this column with compressed gas. Serious injury or death can result!

WARNING

NEVER exceed the pressure limits stated on the label. Serious injury or death may occur!

WARNING

Inspect components before each use and after any disassembly or cleaning for scratches, chips or defects, particularly on the glass surfaces. DO NOT use column if ANY defect is present. Please notify *emp* Biotech if ANY defect or abnormality is detected.

Maximum Pressure Rating

Column ID (mm)	Pressure (bars)	Pressure (PSI)
10	40	580
15	35	508
25	24	348
35	18	261
50	13	188

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Introduction and Intended Use

Liquid preparative chromatography is a widely used downstream purification technique that can be used on a wide range of compounds. The common targeted molecules includes include proteins, peptides and/or nucleic acids. With the emergence of smaller particle, higher performance chromatography media, there has been identified a need for higher pressure column hardware that can handle the increased back pressure loading in a safe column configuration. Traditionally, this has been addressed with stainless steel hardware, which does not allow the scientist visibility of the column contents.

emp Biotech designed a new column line to address these evolving demands. Drawing on its many years of experience, *emp* Biotech is pleased to announce the most effective and user friendly preparative column on the market.

ZetaSep FlashFlow™ columns have been designed to exceed what is currently available to the scientist for laboratory use. *emp* Biotech understands the value that the scientist places on his or her results, and we have strived to help them achieve those goals.

Careful choice in materials of construction, combined with customer feedback, has driven the design so that biocompatibility can be achieved in virtually any circumstance. *emp* Biotech proudly offers this new column hardware line and appreciates your interest.

Description of Components

Carefully remove the ZetaSep FlashFlow™ Column and/or adaptor from the packaging and check the contents against packing list supplied. Inspect for any missing components or damage (particularly the glass body) that may have occurred during transportation. If any parts are missing or damaged, contact *emp* Biotech Customer Service department immediately at +49 30 9489 2201.

1. GLASS BODY

a. Type 1, Class A Borosilicate, 3.3 Expansion Conforming to US Federal Specification DD-G-5416 and ASTM E-438, also meets the US Pharmacopoeia specifications for Type 1 borosilicate glass.

b. Graduations in mm are fused onto glass surface and will not come off from exposure to organic solvents or autoclaving.

c. Glass is redrawn precision bore tubing.

2. PISTONS

a. ZetaSep FlashFlow™ Columns are configured with various piston combinations which include:
   i. Long, Short / Short, Short / Long, Long

b. Piston material will vary according to order specifications and intended use.

c. Piston Connections are ¼-28 flat bottom HPLC. Adapters have been furnished with the column to convert to M6 Standard flat bottom HPLC.

3. O-RINGS

a. Each Piston will be provided with an elastomeric O-Ring.

b. O-Ring material will vary with order specifications.

c. O-Rings are ALWAYS shipped loose as they will become stuck to the glass wall over long term storage. *emp* Biotech recommends to install O-Rings just prior to use.

d. If O-Rings have been stored for long periods of time they should be inspected prior to use for dryness and cracking.

4. FRITS

a. Each Piston will be provided with a porous frit.

b. Material and frit porosity will vary with order specifications.

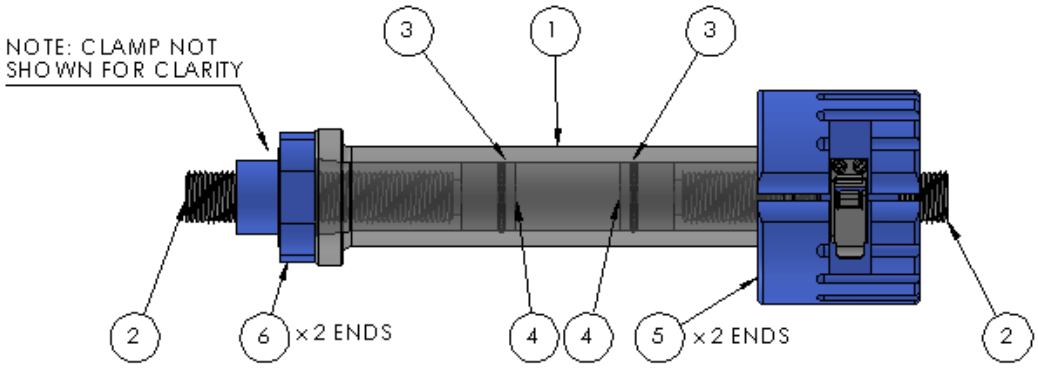
c. Frits can be removed for change out and cleaning. Please confirm that the appropriate frit has been installed in the column for use with mobile and stationary phase. (see pg. 5)

5. Clamp Assembly

a. Each column will be provided with two clamp assemblies produced from cast resin.

6. Piston Adjustment Nut

a. Each column will be provided with two piston adjustment nuts produced from cast resin.

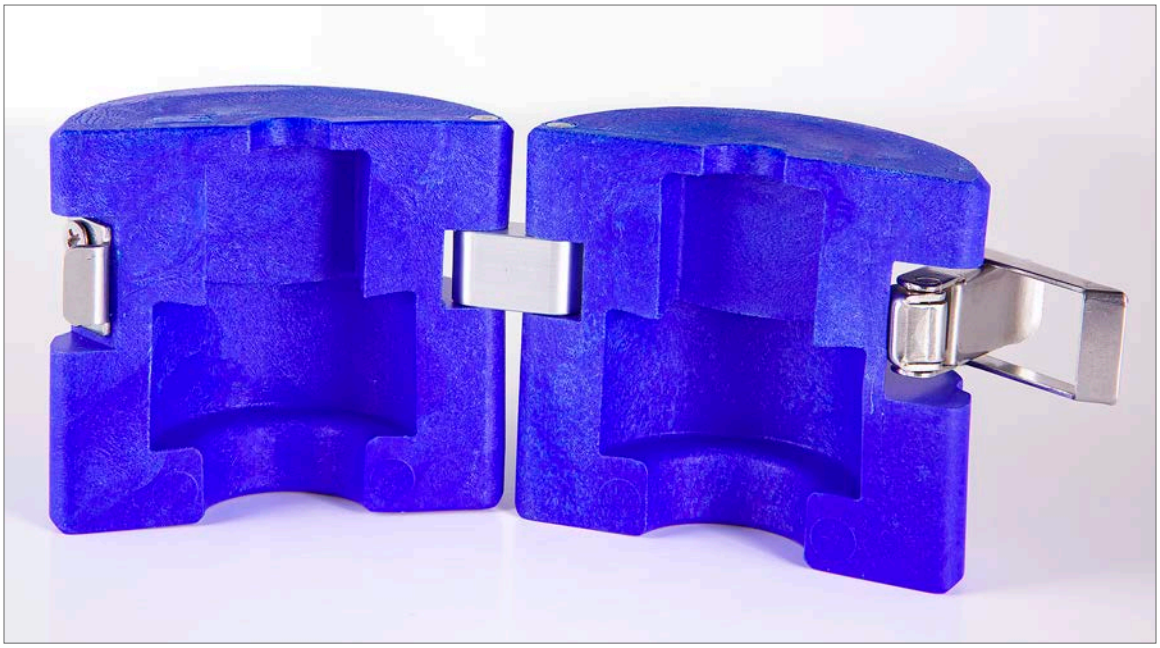


Materials of Construction

Under normal operating conditions, the following items should be considered in contact with the mobile phase. ZetaSep FlashFlow™ Columns come in two standard configurations, which are aqueous buffer (AB) and solvent resistant (SR). Biocompatibility should be verified with material selection of column configuration. If there are any questions regarding the application and biocompatibility, the customer should contact *emp* Biotech's Technical Support.

Version	Aqueous Buffer	Solvent Resistant
Body	Borosilicate Glass	Borosilicate Glass
Piston	Acetal	PEEK
Frit	Polyethylene	Teflon
O-Ring	EDPM	Viton or Kalrez®
Temperature Range	4°– 40° C	4°– 40° C

The above chart represents standard column configurations for aqueous buffer and solvent resistant. emp Biotech *can provide customized solutions upon request.*



ZetaSep FlashFlow™ Column Clamp

Preparing the Column and Installation

Prior to first use of column, perform the following steps:

1. Disassemble column completely by first unlatching the latches on top and bottom clamps
2. Remove piston/nut assembly by pulling piston straight out
3. Clean all components with soapy water or laboratory detergent. Always finish cleaning by thoroughly rinsing with distilled water.
4. Install supplied O-Ring on piston end
5. Prior to reinserting pistons, frit must be “wetted out” with 20% ethanol to break surface tension and allow for unrestricted flow
6. When reinserting the piston(s), take care to insert straight into the glass body, otherwise the seal risks breakage
7. Rotate the nut block until it contacts the glass
8. For packing purposes, install the bottom clamp only, leaving top open for pouring media

Connecting the Column

Securely fasten the inlet and outlet connections to ensure that leakage does not occur during use.

emp Biotech provides connections for either 1/16" or 1/8" tubing. The connection on the end of the piston is stan-dard ¼-28 HPLC flat bottom. Due to the fact that many systems standardize on M6, *emp* Biotech has provided fittings to accommodate this condition. ***Check your system’s standard threads to properly connect the column and avoid cross threading.***

Tubing should be connected to the ZetaSep FlashFlow™ Columns with the following screw: For tubing with 1/16" outer diameter:

AX-PP207X AX-PP206 AX-PP200NX	M6 Nut (Black), for connecting tube to System 1/4"-28 Nut (Blue), for connecting tube to Column Ferrule, 1/16"	M6 to System (Black) 

For tubing with 1/8" outer diameter:

AX-PP307X AX-PP306 AX-PP300NX	M6 Nut (Black), for connecting tube to System 1/4"-28 Nut (Blue), for connecting tube to Column Ferrule, 1/8"	1/4"-28 to Column (Blue) 

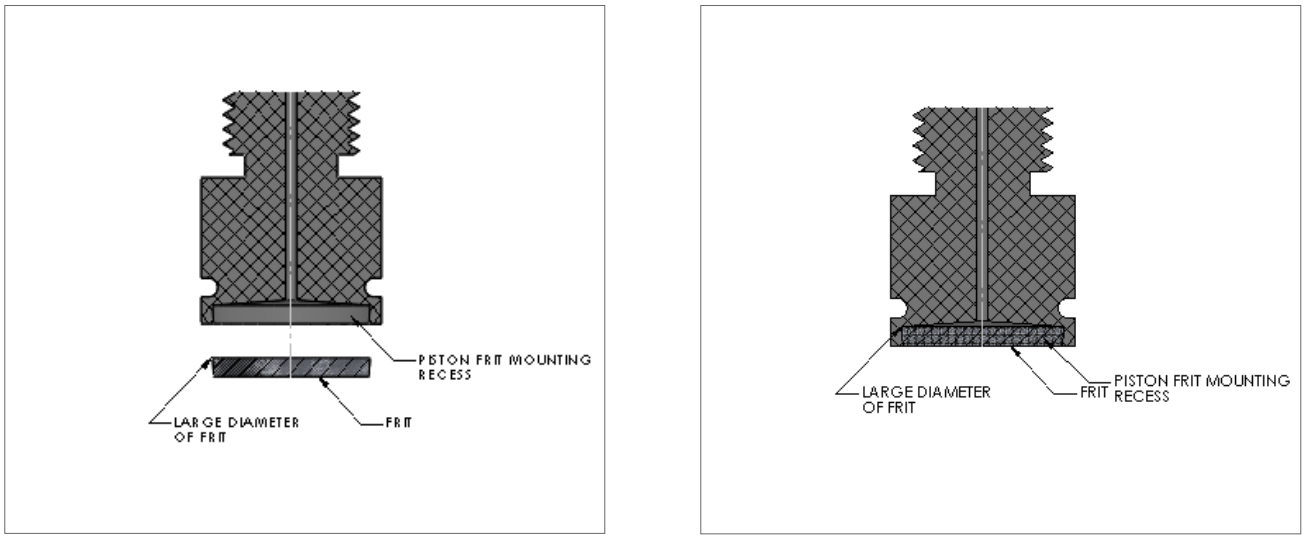
Frit Replacement

Each piston will be supplied with a porous frit, which can be removed for cleaning or change out. It is important to note that there is a specific orientation to the frit. See diagram below.

To remove existing frit, insert frit ejector into inlet end of piston and push frit out gently. If the frit does not dislodge easily, put piston assembly in hot water (maximum of 120°C) to expand plastic and allow for easier frit removal.

NOTE: Stainless Steel frits cannot be changed.

Frit Ejection Method



Frit Installation

emp Biotech *recommends the use of a packing adaptor when the desired bed length is greater than half of the maximum bed length allowed for in the column.*

Operating the Column without the Packing Adaptor

Preparation:

The main column components in contact with the mobile phase must be cleaned thoroughly before the column is used for the first time. *emp* Biotech recommends dismantling the column and washing the components in an appropriate laboratory cleaning solution prior to use. After cleaning, all components should be rinsed in double distilled water and reas-sembled.

emp Biotech assumes that the column will be slurry packed. Slurry packing would involve mixing approximately equal parts mobile phase and stationary phase outside of the column prior to packing. *Consult with your resin supplier for media bulk density.* This will result in a volume approximately double the final packed volume.

$$V = \frac{(\pi R^2 \, H)}{1000}$$

V=volume (mL) R=radius of column (mm) H=desired height (mm)

$$m = V \text{x} BD$$

m=mass (grams) BD=bulk density (grams/mL)

Column operation is initiated by attaching to an appropriate chromatographic system or pump using the supplied fittings. Tubing size should be selected to provide appropriate flow conditions. Tubing material should also be biocompatible with the mobile phase selected. Connect the inlet tubing to the end of the piston and the controller unit. To prevent back pressure, do not connect the outlet tubing to the controller unit and cap the end of the tubing during the packing process.

Packing the Column:

Position the column so that it is perfectly vertical. In an external beaker using proper ventilation, mix the buffer and stationary phase until suspended in solution. Slowly add the slurry to the inside wall of the glass column being careful not to trap air bubbles. Allow resin to sit until approximately one centimeter of buffer sits on top of the bed. Continue to add additional buffer until a meniscus forms on top of the column.

Wet the tip of the piston (Frit and O-Ring) in 20% ethanol to break the surface tension and allow for unrestricted flow. Insert the piston just inside the resin volume. Make sure air bubbles are not present. Position the nut so that it contacts the glass. Attach the clamp and fasten the latch. Remove cap from outlet tubing and direct it to waste.

Turn on the pump to the appropriate flow rate and pressure. Slightly before the resin bed packs half of the desired length, pause the pump. Disconnect inlet tubing and direct to waste. Cap the outlet tubing. Slowly turn the clamp anti-clockwise. As the piston submerges into the buffer, excess buffer will flow up the piston into the waste. Stop piston just above the resin bed. Uncap the outlet tubing directing it to waste and reconnect the inlet tubing to the controller unit. Allow to run for about two more minutes.

Once the bed compresses fully, stop the pump, cap the outlet tubing and unscrew the inlet tubing sending it to waste. Turn the clamp counterclockwise until the frit just contacts the surface of the bed. Reconnect the inlet and outlet tubing to the control unit. The column is ready to use.

Operating the Column with the Packing Adaptor

Preparation:

The main column components in contact with the mobile phase must be cleaned thoroughly before the column is used for the first time. *emp* Biotech recommends dismantling the column and washing the components in an appropriate laboratory cleaning solution prior to use. After cleaning, all components should be rinsed in double distilled water and reassembled.

emp Biotech assumes that the column will be slurry packed. Slurry packing would involve mixing approximately equal parts mobile phase and stationary phase outside of the column prior to packing. *Consult with your resin supplier for media's bulk density.* This will result in a volume approximately double the final packed volume.

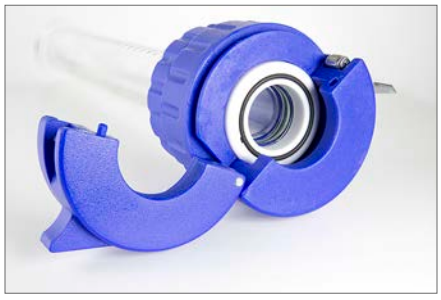
$$V = \frac{(\pi R^2 H)}{1000}$$

V=volume (mL) R=radius of column (mm) H=desired height (mm)

$$m = V x BD$$

m=mass (grams) BD=bulk density (grams/mL)

Assemble the packing adaptor on top of the processing column. Clip split ring over glass column at 0 mm end. Install the compression nut so that the split ring is positioned partially inside. Insert seal ring with the small face down. Partially thread the glass column/ compression nut assembly into the body of the packing adaptor. Open the jaw and place onto the column's bare glass tube. Fasten the latch and fully tighten the compression nut. ***See diagram below.***



Column operation is initiated by attaching to an appropriate chromatographic system or pump using the supplied fittings. Tubing size should be selected to provide appropriate flow conditions. Tubing material should also be biocompatible with the mobile phase selected. Connect the inlet tubing to the end of the piston and the controller unit. To prevent back pressure, do not connect the outlet tubing to the controller unit and cap the end of the tubing.

Operating the Column with the Packing Adaptor *(continued)*

Packing the Column:

In an external beaker using proper ventilation, mix the buffer and stationary phase until suspended in solution. Slowly add the slurry to the inside wall of the glass column being careful not to trap air bubbles. Allow resin to sit until approximately a centimeter of buffer sits on top of the bed. Position the packing adaptor and column so that they are perfectly vertical.

Wet the tip of the piston (Frit and O-Ring) in 20% ethanol to break the surface tension and allow for unrestricted flow. Insert the piston just inside the resin volume. Make sure air bubble are not present. Position the nut so that it contacts the glass. Attach the clamp and fasten the latch. Remove cap from outlet tubing and direct it to waste.

Turn on the pump to the appropriate flow rate and pressure. Slightly before the resin bed packs half of the desired length, stop the pump. Disconnect inlet tubing and direct to waste. Cap the outlet tubing. Slowly turn the clamp counterclockwise. As the piston submerges into the buffer, excess buffer will flow up the piston into the waste. Stop piston just above the resin bed. If the bed has compressed to exist in only the column and not the packing adaptor, the packing adaptor can be removed by opening the jaw and dismounted from the column. Uncap the outlet tubing directing it to waste. Insert the piston like stated earlier and reconnect the inlet tubing to the controller unit

Once the bed compresses fully, stop the pump, cap the outlet tubing and unscrew the inlet tubing sending it to waste. Turn the clamp anti-clockwise until the frit just contacts the surface of the bed. Reconnect the inlet and outlet tubing to the control unit. The column is ready to use.

Tips for Successful Column Packing and Storage

- Use only degassed and pre-filtered solvents as particulate in solvent may clog the frits or damage the column packing.
- Make sure that frits are properly sized for chromatographic media. *emp* Biotech recommends that the frit porosity be at least one half of the average media particle diameter.
- Columns should always be sealed with appropriate stoppers when not in use to avoid bed degradation and drying out of media.
- *emp* Biotech recommends eluting the column from bottom to top so that any air present can more readily be released. The result will be that the column will condition more quickly requiring less solvent.
- Before sample injection, ensure that no dead volume is present at the column inlet during the conditioning phase.
- Before attempting to pack the column, please consult with the media supplier and/or *emp* Biotech for media-specific instructions.

Quality Control

emp Biotech recommends determining plate count and peak symmetry with an appropriate (non-adsorbent) test substance after packing the column. Repeating this test frequently will ensure that the quality and durability of the packing material is recorded efficiently.

Calculating Amount of theoretical Plates (N):

$$N = 5.54 \times \left(\frac{t^1}{W_{1/2}} \right)$$

T1: retention time(s) $W_{1/2}$: peak width (s) at half peak height

$$\text{HETP} = \frac{L}{N}$$

L: column length (mm)

Peak Symmetry (S):

$$S = \frac{W_{1/2,r}}{W}$$

$W_{1/2,r}$: peak width to right of peak median

$W_{1/2,l}$: peak width to left of peak median

Cleaning Instructions for Packed Columns (CIP)

Cleaning a ZetaSep FlashFlow™ Column involves three Stages:

1. Regeneration of the column packing
2. Sterilization
3. Depyrogenation

Regeneration removes chemical and organic contamination that binds to the chromatography material, which significantly reduces the capacity and resolution of the column. Contamination is usually caused by lipids and pyrogens, protein aggregates, pigments, polyphenols and metal complexes.

Sterilization removes and/or denatures microorganisms and spores with chemical treatment, otherwise they could contaminate the purified product. Ethanol solutions containing sodium hydroxide or acetic acid are the most common methods for sterilization.

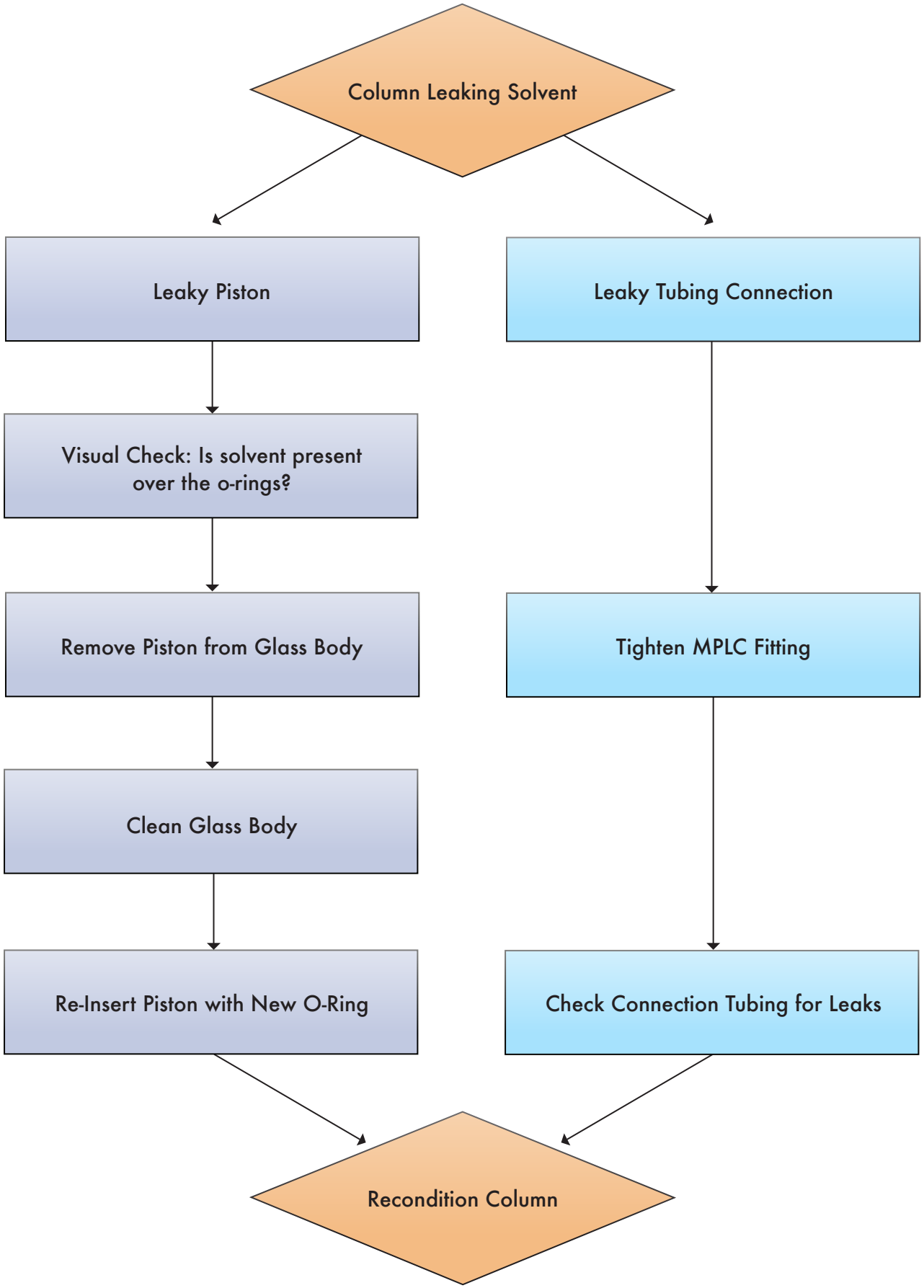
Depyrogenation involves the breaking down of endotoxins that have become attached to the chromatography material or the column hardware. (frits, tubing, etc.) Endotoxins can contaminate the target compounds in question by being washed gradually through the column. Some methods used to sterilize equipment will break down pyrogens.

Sterilize and Purify the column with the following steps:

1. Disassemble the column into individual parts
2. Wash in a dilute solution of caustic soda or sodium hypochloride (leave frits in solution for 30-60 minutes)
3. Wash parts in a sterile, pyrogen-free solution and reassemble
4. Pack column in sterile environment
5. Solvents and solutions must also be sterile and pyrogen-free. *emp* Biotech recommends in-line filtration through a 0.22 µm filter.

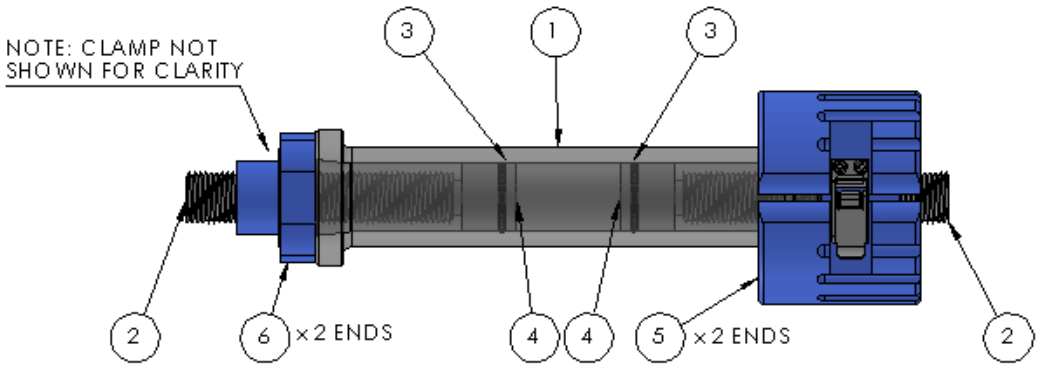
Troubleshooting

Problem	Cause	Solution
1. Air Pockets	Solvent Evaporation or gas evolution during storage	Recondition the column
2. Abnormal pressure fluctuations during operation	1. Incorrect valve position 2. Blocked frit 3. Fitting tightened too much	1. Check valve position 2. Remove and dismantle piston, replace frit, reassemble and re-insert piston. 3. Recondition column. Replace fittings and ferrules, re-cut the end of the tubing
3. Column leaking solvents	See Diagram on Pg. 12	See Diagram on Pg. 12
4. Deteriorated Peak shape of elut-ed substance	1. Bead bed mechanically damaged 2. Inlet frit partially blocked 3. Outlet frit partially blocked 4. Dead volume at column inlet 5. Contamination affecting separati-on efficiency of stationary phase	1. Repack column 2. See 2.2 above 3. See 2.2 above 4. Rotate the clamp anti-clockwise until piston just contacts bed 5. Repack column in sterile environment
5. Pressure drop during operation	1. Tubing or fitting leak between pump and column 2. Solvent supply is dry	1. Check tubing/connections 2. Refill Solvent


Column Leaking Solvent
Leaky Piston
Visual Check: Is solvent present over the o-rings?
Remove Piston from Glass Body
Clean Glass Body
Re-Insert Piston with New O-Ring
Leaky Tubing Connection
Tighten MPLC Fitting
Check Connection Tubing for Leaks
Recondition Column

Column Components

When contacting *emp* Biotech's Customer Service, please refer to the following part numbers:



1. Glass Column Body
2. Pistons
3. O-Rings
4. Frits
5. Clamp Assembly
6. Piston Adjustment Nut

The following are Standard Consumable Parts:

Frits

Column ID	AB		SR	
	Polyethylene		Teflon	
Porosity	5 µm	10 µm	2 µm	10 µm
10 mm	AX-P10FRPE05	AX-P10FRPE10	AX-P10FRT02	AX-P10FRT10
15 mm	AX-P15FRPE05	AX-P15FRPE10	AX-P15FRT02	AX-P15FRT10
25 mm	AX-P25FRPE05	AX-P25FRPE10	AX-P25FRT02	AX-P25FRT10
35 mm	AX-P35FRPE05	AX-P35FRPE10	AX-P35FRT02	AX-P35FRT10
50 mm	AX-P50FRPE05	AX-P50FRPE10	AX-P50FRT02	AX-P50FRT10

Column Components *(continued)*

Piston O-Rings

Column ID	AB	AB/SR	SR
	EPDM	Viton	Kalrez
10 mm	AX-PR010E	AX-PR010V	AX-PR010K
15 mm	AX-PR013E	AX-PR013V	AX-PR013K
25 mm	AX-PR117E	AX-PR117V	AX-PR117K
35 mm	AX-PR123E	AX-PR123V	AX-PR123K
50 mm	AX-PR132E	AX-PR132V	AX-PR132K

Spare Parts Kit (Included with Column)

INCLUDED SPARE PARTS KITS

Part No.	Tubing OD
AX-P16KIT	1/16"
AX-P8KIT	1/8"

Spare 1/16" Parts Kit Includes:

AX-PP200NX	4 units	Ferrule, 1/16"
AX-PP206	2 units	1/4"-28 Nut (Blue), for connecting tube to Column
AX-PP207X	2 units	M6 Nut (Black), for connecting tube to System
AX-PPFRT1	1 unit	Frit Removal Tool

Spare 1/8" Parts Kit Includes:

AX-PP300NX	4 units	Ferrule, 1/8"
AX-PP306	2 units	1/4"-28 Nut (Blue), for connecting tube to Column
AX-PP307X	2 units	M6 Nut (Black), for connecting tube to System
AX-PFRT1	1 unit	Frit Removal Tool

WARRANTY

emp Biotech offers a one-year limited guarantee on these columns covering defects in material and workmanship if used in accordance with the conditions detailed in the instruction manual provided with the column. This guarantee does not include frits, O-rings and glass tube.

emp Biotech makes no other representation or warranty, express or implied with respect to these columns, and it disclaims all implied warranties of merchantability or fitness for a particular purpose. In no event shall *emp* Biotech be liable for any special, indirect or consequential damages.

ZetaSep FlashFlow™ Glass Columns
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