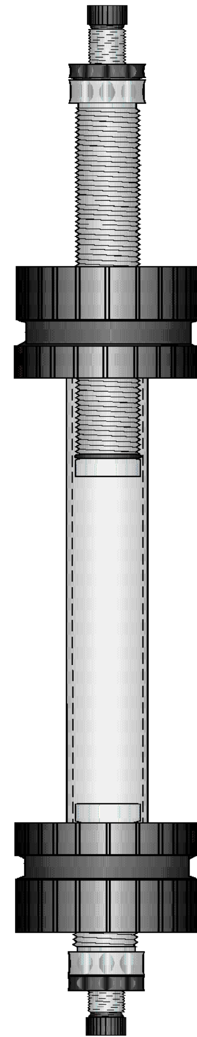

essentialLife Solutions

Econoline LP® Column Manual

Rev 4.0

171 Tosca Drive, Stoughton, MA 02072-1505, United States of America
(781) 341-7240 phone, (781) 341-7241 fax

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171 Tosca Drive
Stoughton, MA 02072-1505 USA
(781)341-7240 phone,(781)341-7241 fax

INTRODUCTION

Essential Life Solutions Econoline LP columns are suited for semipreparative and preparative pressure chromatography. Available with 6 diameters (10,15,20,25,32and50mm) and 5 maximum bed lengths (120, 200, 450, 750, and 999 mm). Econoline LP columns are resistant to back pressures up to 10 bars. Using the hight adjustable pistons dead volumes due to shrinkage of the column bed can easily be removed.

EXTENT OF SUPPLY AND SPECIFICATION

	Name	Material
1	column body	glass
2	piston, variable/fixed	PTFE/Viton PVDF/Kalrez® (SR)
2	counter nuts / screws	Delrin
2	frits (pressed in)	PE or glass
2	fixing screws	Delrin
2	locking rings	Tefzel
1	frit ejector	Delrin/steel
2	connecting tubings	FEP/Tefzel

RECOMMENDED ACCESSORIES

Number	Name	Material	P/N
1 pk./10 pcs.	stoppers	Tefzel	KP311
1	coupling unit	Tefzel	KP630

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The current general conditions and the Essential Life Solutions Ltd. warranty conditions are valid. All information in our manual leaflet is without obligation insofar as it is subject to technical changes serving product improvement.

Revision 4.0
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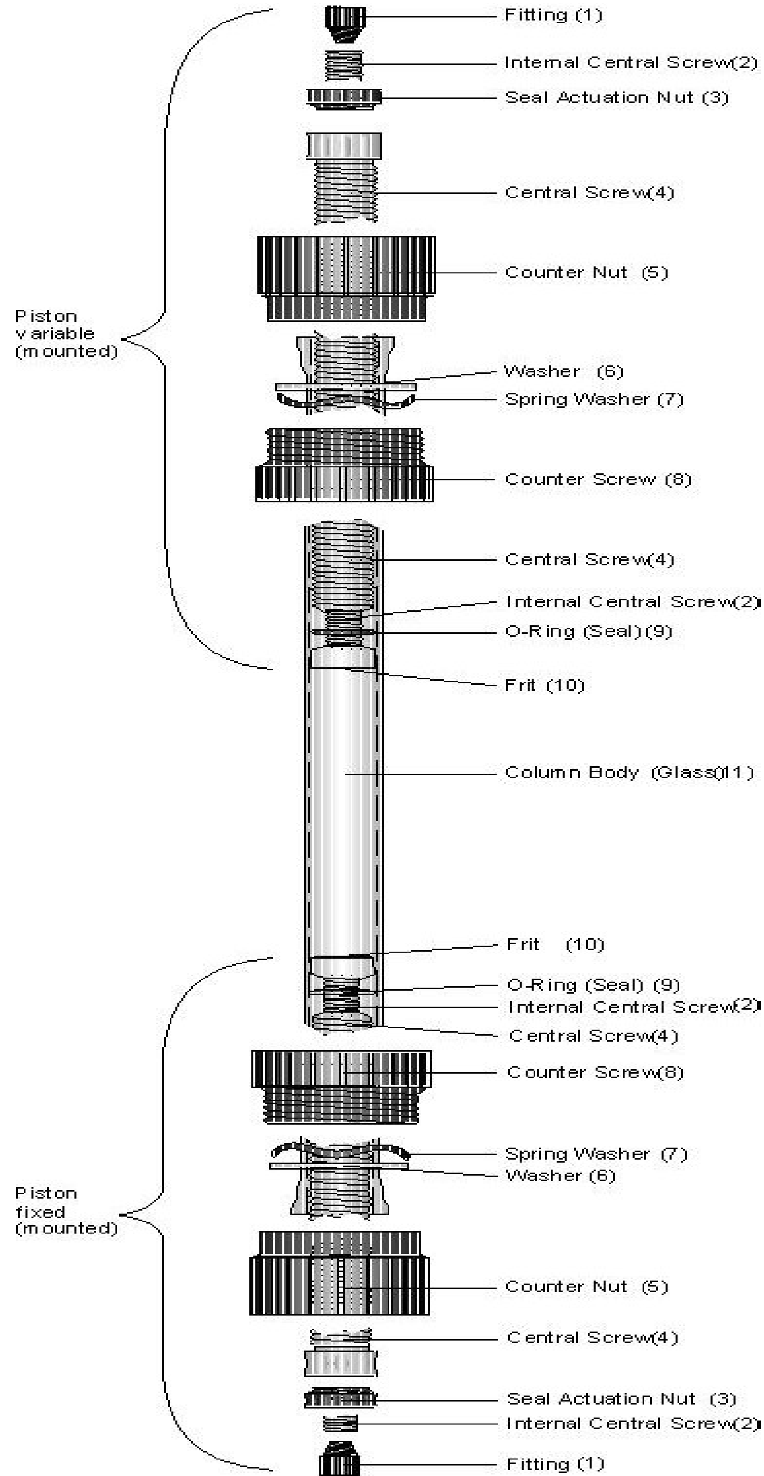
171 Tosca Drive
Stoughton, MA 02072-1505 USA
(781)341-7240 phone,(781)341-7241 fax

ECONOLINE LP COLUMN



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1. DISMOUNTING AND MOUNTING OF THE PISTON

Econoline LP columns are equipped with a screw-lock system which can easily be released by turning the counter nut against the countor screw (2). Before opening the column lock loosen the seal actuation nut until the o-ring seal is released from the column body. Then the counter nut can be extracted from the column end together with the piston.

The system is mounted in the reverse order.

Note: When the piston is mounted care should be taken that the inner glass surface is absolutely particle-free to prevent damage of the piston teflon lips.

2. FRIT REPLACEMENT

1. ***Eject old frit with supplied frit ejector through the drillhole of the piston.***

2. ***Insertion of new frit: The teflon receptacle of the piston is protected when it heated before the new frit is pressed in (max. 121• C).***

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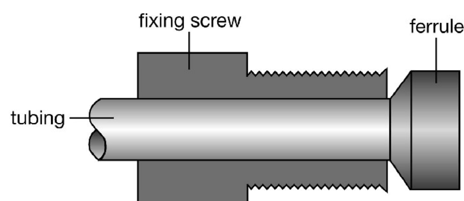
3. MOUNTING OF MPLC-FITTINGS



These mounting instructions are for tubing with 1.6 and 3.2 mm outer diameter.

1. *The end of the tubing is vertically cut with a knife or a tubing cutting device.*

NOTE: Do not cut with scissors to prevent squeezing.
It is essential to cut the tubing vertically, as the cut edge is part of the sealing area!

2. *The fixing screw is drawn over the tubing.*
3. *The locking ring is drawn over the tubing with the conical side against the banjo bolt. If it is not possible to mount the locking ring, the conical end is widened carefully with a suitable arbor (e.g. a scribing iron).*
4. *Now the fitting can be inserted in the wanted position. The seal is pressed onwards, while the screws are fastened until pressure is felt; then they are fastened for another 1/2 turn.*

NOTE: When the fittings are screwed into the teflon thread it should be taken care that the fittings are screwed down straightly and are fastened carefully to prevent damage to the teflon thread.

4. ELIMINATION OF DEAD VOLUME

A dead volume which might occur at the column inlet can be remedied simply without the need to open the column:
1. *Turn off pump.*
2. *Turn the divisible column lock counterclockwise.*

PLEASE NOTE: The piston should only touch the surface of the stationary phase. If it is pressed into the stationary phase, the packing may be destroyed.

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5. OPERATION OF THE COLUMN

Putting into operation

Pistons, frits and column body (11) must be cleaned carefully before first use and before each new packing. In some cases it might be useful to dismount the column and wash these parts in a sonic bath for several minutes. After cleaning all parts must be rinsed with bidestillated water and mounted as described under chapter 1. All parts must be free of dust and particles!
Be sure that the pistons are inserted carfully into the column body (11): If not introduced absolutely axial, the piston seals might be damaged.

For operating the column it is connencted to an appropriate chromatography system or pump. Chose tubing and tubing diameter according to the solvents and flow rates to be applied to the column. The preferred flow direction of the column should be from variable piston to fixed piston. If the column bed shrinks a dead volume can easily be removed by moving down the variable piston (see page 6, chapter 4).

NOTE: Only use degassed and prefiltered solvents for operating the column. Particles may clogg the frits or damage the column packing! Make sure that the particle size of the chosen chromatography packing correlates to the frit porosity of the column!

Hints for operation

1. *Storage of the packed column: Open the sealing stoppers for one turn allowing the compensation of temperature-dependent pressure changes.*

NOTE: Protect moistened column against intensive heat and direct insolation. The heat induces the evaporation of high-volatile solvents, and the resulting pressure can crack the column.

2. *We recommend to operate the column from the bottom (variable end piece) to the top (fixed end piece). The resulting benefit is that air escapes faster from the column so that it is conditioned faster (i.e. with less solvent).*

3. *We recommend to make sure before sample application that no dead volume originated at the column inlet during the conditioning phase (elimination of dead volume see page 6, chapter 4).*

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6. SOLVENT RESISTANCE

Packed columns can be stored either in 20/80 ethanol/water, containing up to 1 M NaCl and neutral buffer or in neutral buffer containing 0,03% sodiumazide. NP and RP columns may be stored in organic solvents as needed for common use. All solvents should be filtered through a 0.45 µm or 0.22 µm filter membrane.

In generell the following solvents and additives can be used in normal operation. However we do not recommend to use any of them for longer periods of time or for storage. For informations please refer to Essential Life Solutions Ltd.

- **With Kalrez®-o-rings resistant to all common solvents as ethanol, methanol, propanol, isopropanol**
- **Resistant to all common aquous buffers**
- **Salts in aquous solution as: NaCl, $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$, etc.**
- **pH 1-14**
- **2 M NaOH**
- **1 M HCl**
- **75 % (v/v) Acetic acid**
- **Detergents (≤ 2% w/v) as SDS, Triton, etc.**
- **6 M Guanidinium-HCl, 8 M Urea**
- **Working temperature range: 4 - 40° C**

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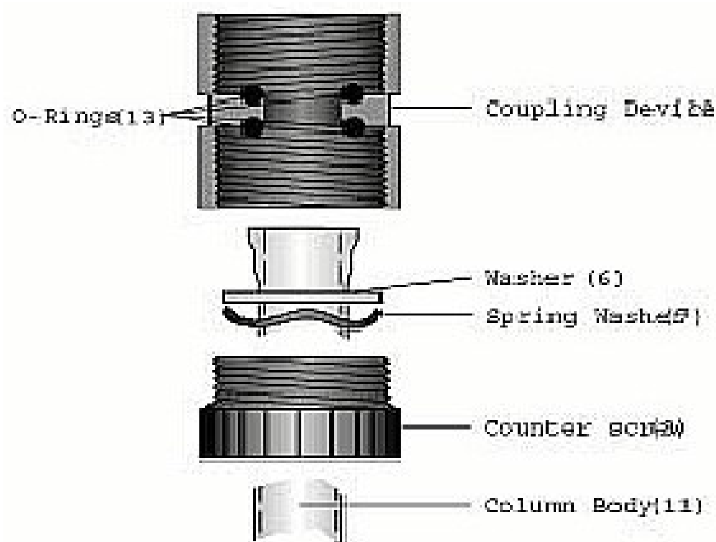
7. PACKING INSTRUCTIONS

These instructions are recommendations. In most cases good results are obtained refering to reproducability and performance. However these methods can be optimized for specific media and needs. To obtain best results regarding packing quality and reproducability it is recommended to use a slurry container for packing Eco glass columns. In some cases, when lower packing height or less quality of packing is required the media can directly be packed into the column tube, also admitting repeated refill of slurry during packing.

Mounting the packing device

Place the slurry container on top of the column tube. Be sure that all seals and frits are correctly in place! The screws of the packing device assembly have to be tightened thoroughly. Make sure there are no particles between glass body and sealings!



Preparing the slurry

Slurry the required amount of media with a suitable solvent or buffer (see instructions of use for media) to reach the volume of the packing device assembly or the column tube. The slurry has to be shaken gently to get a homogenous solution (never use a magnetic stirrer for mixing the slurry). Degass the slurry right before use.

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7 . PACKING TECHNIQUES (con't)

Precautions

Always use relevant safety equipment when packing glass columns under high pressure. Never use compressed air or gas for packing glass columns.The pressure limits of columns and equipment have to be kept absolutely. Be aware of wearing correct laboratory clothing and safety glasses.

NOTE: A glass column should always be used without gas pressure. Even a small tension in the glass body is sufficient to cause explosion of the glass body, resulting in liberation of the expansion energy of the gas causing the glass pieces to act like projectiles. If the glass body shatters under liquid pressure there is no danger as liquids are only little compressible thus having practically no expansion energy.

Packing the column with rigid media

Only use degassed and filtered solvents for packing of chromatography columns. Make sure that all steps during packing of the column are carried out as quick as possible! Fill a few ml solvent or buffer (see instructions of use for media) into the column tube, so that the bottom frit is covered with liquid. Be sure there are no air bubbles in or on top of the bottom frit. Carefully shake the slurry to homogeneity and fill it into the glass tube without generating air bubbles. The slurry container has to be filled up completely without any air remaining. If needed fill up the packing device with solvent. Close the packing assembly and connect it to a pump. Adjust the pump flow to the pressure limit of the column or pump with the maximum flow rate of the pump (never exceed the column pressure limit!). Keep pumping at least until the column pressure stays constant.
After the packing is finnished, the packing device is disconnectet from the pump and the upper tube (slurry container) is screwed off from the column tube. Make sure that residual system pressure is relaxed by opening the fitting at the column outlet. Be careful when installing the adjustable piston: There shall not be any particles between piston sealing and glass tube; remaining air bubles on top of the gel bed must be pushed out through the piston inlet. Reconnect the column to the pump and start pumping with a low flow rate. Increase the pump flow slowly until the pressure limit of the column is reached. Due to the high flow rate a small dead volume between upper piston and gel bed may occur. Remove this dead volume by moving down the adjustable piston: Make sure the pump is switched off and slowly turn the upper screw counter clockwise until the dead volume is dimineshed. The column can now be equilibrated to the required chromatographic conditions and is ready for use.

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7. PACKING TECHNIQUES (con't)

Packing the column with soft gels

Only use degassed and filtered solvents for packing of chromatography columns. Wetten the bottom frit with packing solvent and allow a small volume of solvent to stand above the frit. Carefully pour the slurry into the column and, at the same time, open the column outlet. During pouring the slurry the solvent can also be soaked from the column outlet with a peristaltic pump. Be sure not to generate air bubles during filling or that the solvent level sinks below the packing horizont! After the slurry has been filled completely into the column, allow the media to settle and the level of solvent to sink roughly 0,5 - 1cm above the gel bed. Close the column outlet or stop the peristaltic pump and carefully place the piston into the column. There must not be any particels between sealing and glass tube! Tighten the piston seal a little and move the piston downwards by turning the top screw until the frit touches the gel bed. Be sure to push out all air bubles on top of the gel bed through the column inlet when moving down the piston. Then the piston seal can be tightened completely. Never compress the gel bed with the piston!
The column can now be equilibrated with the required buffers. If a dead volume on top of the gel bed may occure it can easily be removed by carefully adjusting the piston hight (see above).

Checking the column performance

We strongly recommend to test the column performance with a suitable test substance to obtain HETP and peak symmetry. By repeating this test regularly the deterioration of the column packing can easily be determined.

Number of theoretical plates:

$N = 5{,}54\ 5\ (T_1 / W_{1/2})$ — T_1: *Retention time (sec)*; $W_{1/2}$: *Peak width (sec) at halve peak height*

$HETP = L / N$ — L: *Column length in mm*

Peak symmetry:

$S = W_{1/2,\ re} / W_{1/2,\ li}$ — $W_{1/2,\ re}$: *Peak width, right of peak median*; $W_{1/2,\ li}$: *Peak width, left of peak median*

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8. CLEANING INSTRUCTIONS FOR PACKED COLUMNS (CIP)

A cleaning procedure includes the three following steps: regeneration of the chromatographic support, sterilization and depyrogenation.

Regeneration eliminates mineral and organic contaminants which are non-specifically fixed on the chromatographic resin. These molecules are most often lipid substances (including pyrogens), protein aggregates, pigments, polyphenols or metal complexes. These substances decrease the performance of the packing material (capacity, resolution, purity and yield). These problems may be overcome by using a regeneration solution (table II) which should be selected according to the nature of the contaminant and the chemical resistance of the packing material.

Sterilization involves the removal of micro-organisms by an appropriate chemical treatment. Sodium hydroxide is commonly used for the sterilization of chromatographic supports. Ethanol based solutions containing acetic acid or sodium hydroxide are also efficient (see technical appendices).

Depyrogenation eliminates endotoxins fixed on the chromatographic support. According to the solution used, this operation may be performed at the same time as sterilization, or in a following step.

Maintaining a column sterile requires a number of precautions:

After disassembling the column to its sub-assembly components : column tube unit, piston and base, wash each part with a sanitizing solution (diluted sodium hypochloride, 0.5 N NaOH). Frits must be immersed in this solution for 30 to 60 min.

Rinse extensively with an apyrogenic sterile solution before re-assembling the column.

Column should be packed (see section 7) following clean room precautions, especially concerning the working environment, which should be clean. Solutions pumped into the column must be sterilized and checked for pyrogens.

The chromatographic support will be decontaminated. It is advisable to include 0.2 µm filters in-line at column inlet and outlet.

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8. CLEANING INSTRUCTIONS FOR PACKED COLUMNS (CIP)

Cleaning procedures according to the nature of the adsorbed material to be eliminated. Please refer also to the instructions for use of the related column packing!

Treatment	Contamination	Sterilization	Depyroge-nation
1-2 M sodium chloride	Highly charged molecules	Ineffective	Ineffective
Buffers pH 3-5	Highly charged molecules	Ineffective	Ineffective
Pronase treatment, neutral pH, calcium ions	Hydroiysis of adsorbed proteins	Ineffective	Ineffective
Pepsin treatments pH 1.5-2	Hydroiysis of adsorbed proteins	Ineffective	Ineffective
Non ionic detergents (e.g. Triton X-100, Tween 80)	Removal of hydrophobic proteins and lipidic substances	Ineffective	Ineffective
Cationic detergents pH 9-11	Removal of hydrophobic proteins and lipidic substances	Ineffective	Possible
Non ionic detergents pH 3 acetic acid)	Removal of hydrophobic proteins and lipidic substances	Ineffective	Possible
Urea 6-8 M	Removal of protein aggregates	Ineffective	Unknown
1-100 mM EDTA in neutral or slightly acidic solution	Removal of metal complexes	Ineffective	Ineffective
2-3 M sodium chloride + 0.1-1 M hydrochloric acid	Removal of various small charged molecules and pigments	Ineffective	Effective
0.1-1 M sodium hydroxide	Removal of bound hydrophobic proteins, lipopoly-saccharides and other unknown contaminants	Effective	Effective
60% ethanol, 0,5-1 M acetic acid	Elimination of lipids, pigments, lipopolys-accharides and other lipophilic substances	Very effective	Effective
50-80% acetic add	Solubilization and elimination of precipitated proteins	Unknown	Unknown
40-60% ethanol	Removal of certain proteins and lipid-like substances	Unknown	Unknown
Isopropanol gradient up to 100%	Removal of non polar lipids	Ineffective	Unknown
0.1-1 M mineral or organic acids	Elimination of various charged molecules and hydrolysis of some bound substances	Unknown	Unknown
0.1 M - 1 M HCl in 60% ethanol	Elimination of various charged molecules and lipids	Unknown	Effective

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9. TROUBLESHOOTING

Problem	Cause	Remedy
1. Peak shape of eluted compounds deteriorates	1. Dead volume at column inlet	1. see page 6, chapter 4: elimination of dead volume
	2. Inlet frit partially obscured	2. Remove and dismaunt piston, replace frit, mount and insert piston again.The recondition column.
	3. Outlet frit partially obscured	3. Remove and dismount fixed piston, replace frit, mount and insert piston again.Then recondition column.
	4. Separation efficiency of stationary phase changed due to contamination	4. Repack column
	5. Stationary phase mechanically destroyed	5. Repack column
2. "Air" in the column	Gas evolution or solvent evaporation during storage	Recondition column
3. Abnormal pressure increase during operation	1. Incorrect valve position	1. Check valve position
	2.Obscured frit	2. see remedy,page 5,1.2
	3. Fittings tightened too strong	3. Replace fittings and ferrules, cut obscured tube.
4. Pressure drop during operation	1. Line or fitting between pump and column leaks	1. Check lines and connections
	2. Solvent supply empty	2. Refill solvent
5. Solvents leaks from column	See diagram on following page	See diagram on following page

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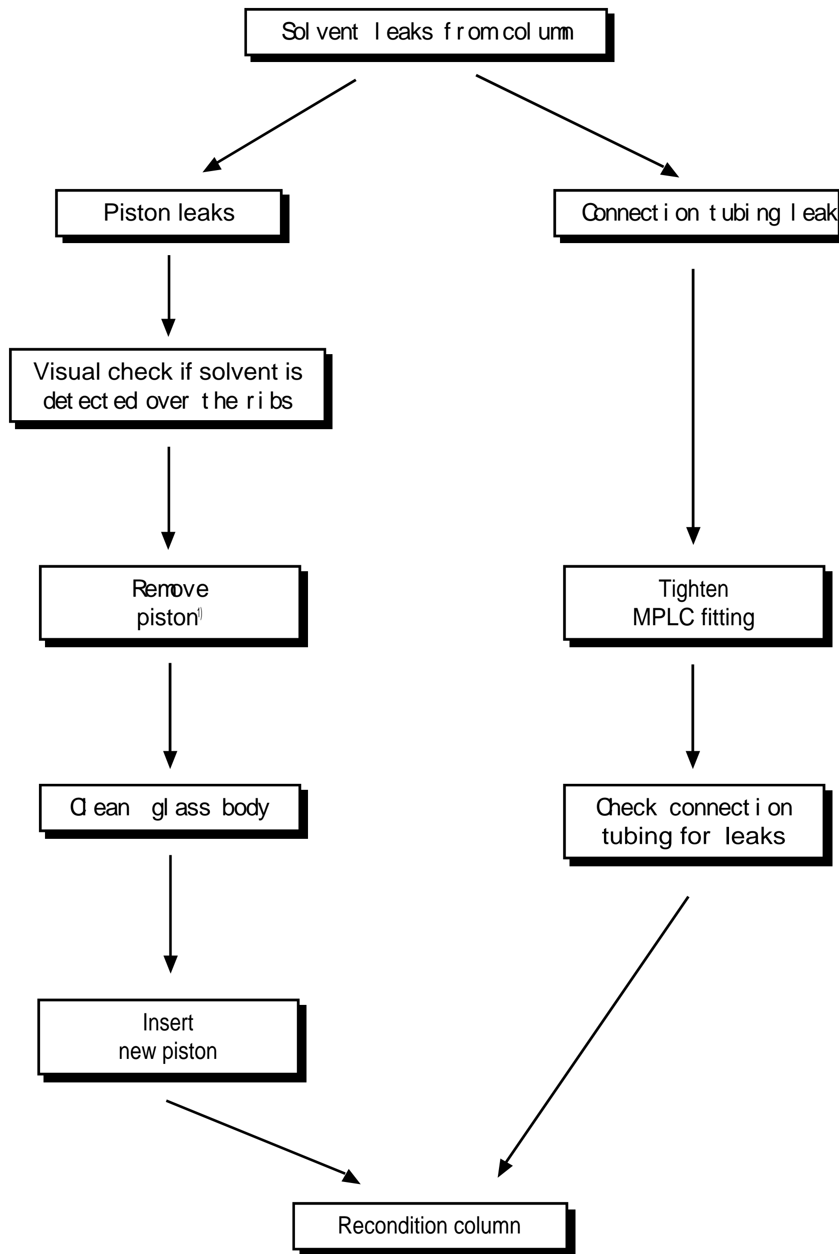

[1] CAUTION: Open connection tubing first to prevent cracking of the packing due to vacuum originated by removal of the piston.

Thank you for your purchase of this column. Please do not hesitate to contact our office with any questions or comments you may have relative to the use and maintenance of your column.

We at Essential Life Solutions Ltd. appreciate your business!

ESSENTIAL LIFE SOLUTIONS LTD.
171 Tosca Drive
Stoughton, MA 02072-1505 USA

PHONE:
(781) 341-7240

FAX:
(781) 341-7241

E-MAIL:
mailstop@essential-life.net

WEB:
www.essential-life.net

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