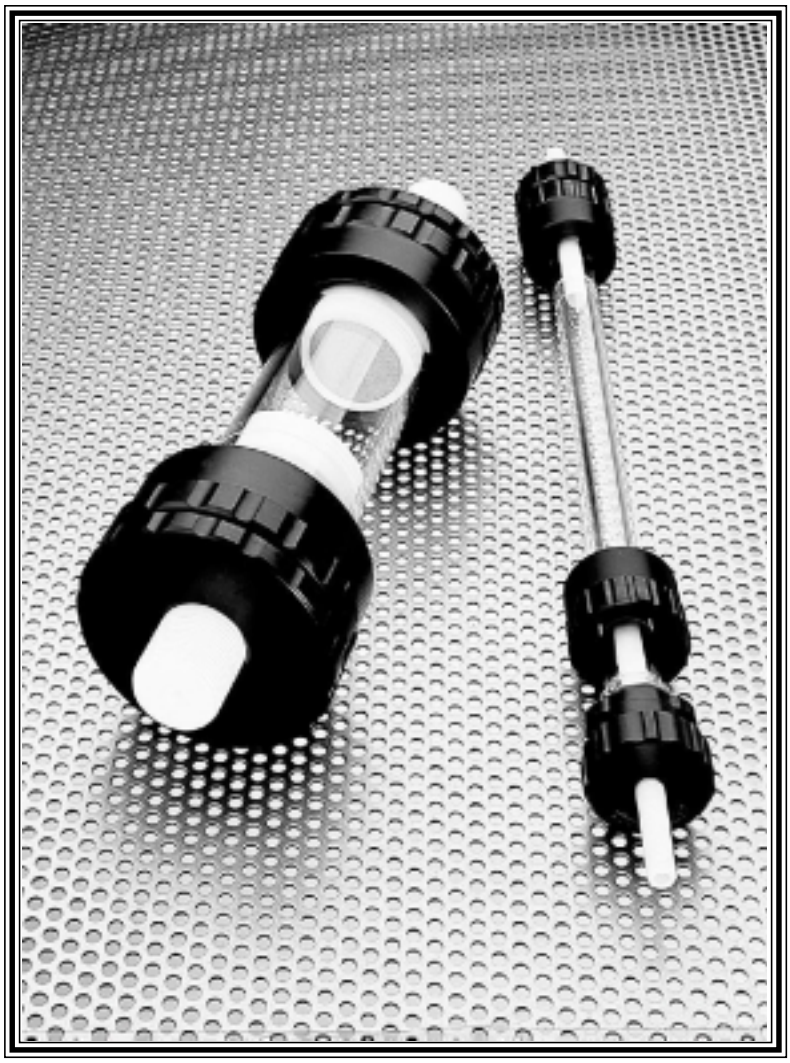


essentialLife Solutions

Econoline® Column Manual

essentialLife Solutions

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

INTRODUCTION

ECONOLINE® is a multipurpose column for almost all types of liquid chromatography application. Adjustable length plungers at both ends and a totally inert triple chevron sealing system support the complete range of applications from classical normal phase and reversed phase chromatography right through to biochromatography.

The Quick-Lock™ connection system makes it possible to open and reseal the column simply and quickly.
ECONOLINE® glass columns are available in two versions. The solvent resistant (SR) version is equipped with the triple chevron sealing system, whereas the aqueous buffer (AB) version is provided with ethylene polymer elastomer sealing rings, which ensure that the column will work perfectly even at low temperatures.

EXTENT OF SUPPLY AND SPECIFICATION

Number	Name	Material
1	column body	borosilicate glass
2	variable pistons	Teflon (SR) or polyethylene (AB)
2	bayonet system locks	Delrin
2	frits (pressed into column piston body)	glass or steel (SR); polyethylene (AB)
2 x 2	locking rings 1/16“ + 1/8“	Tefzel
2 x 2	fixing screws 1/16“ + 1/8“	Delrin
2	coupling units 1/4“-28–>M6	Tefzel
2 x 2	connecting tubes 1/16“ + 1/8“	Tefzel
1	frit ejector	steel / Delrin

RECOMMENDED ACCESSORIES

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

Revision 2.0
1/1/02

essentialLife Solutions

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

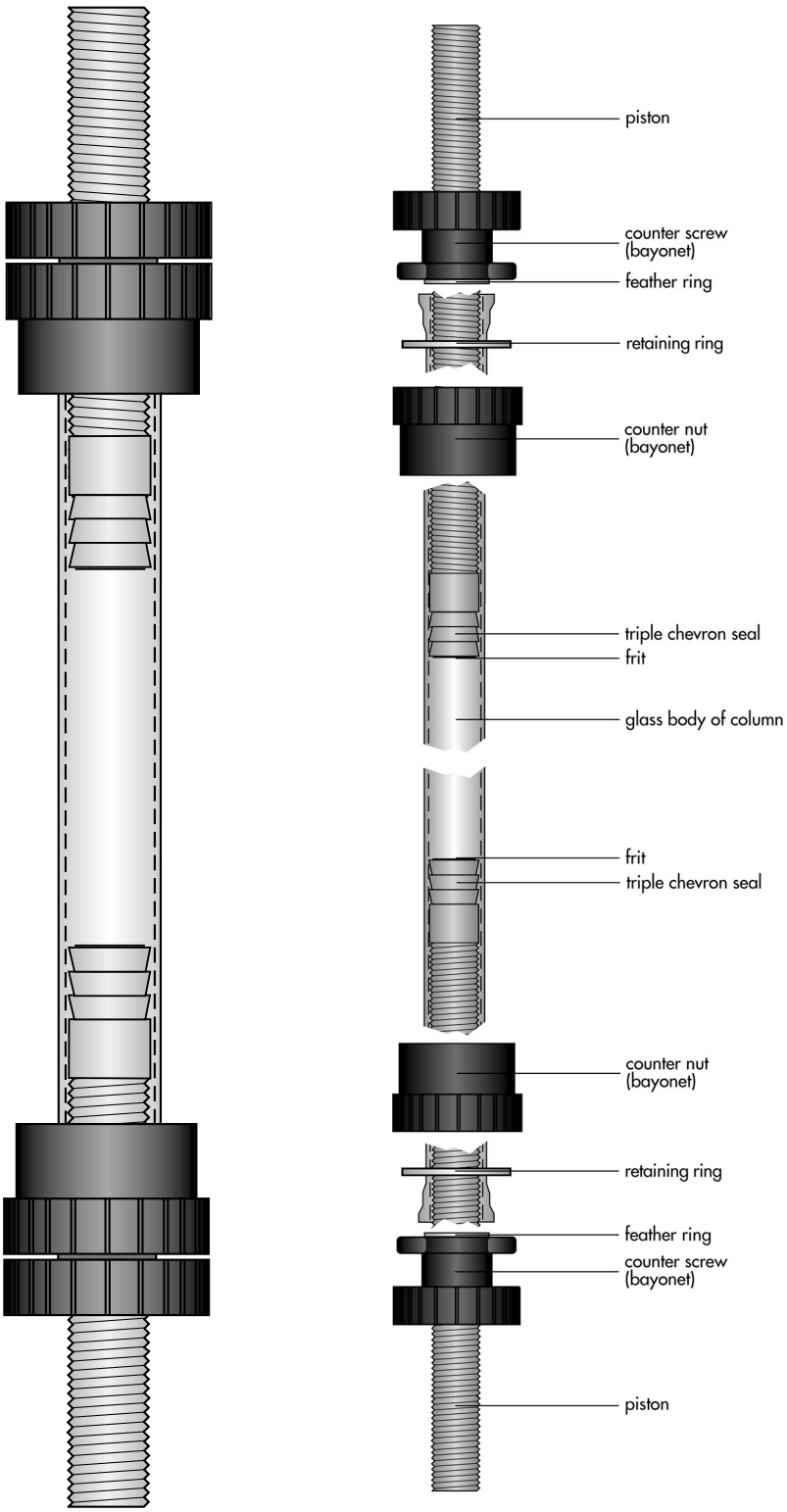
CONTENTS

The general conditions and the Essential Life Solutions Ltd warranty conditions apply in the version that is valid in each case. All information contained within this manual is without obligation, as it is subject to technical changes resulting in product improvement.

Revision 2.0
1/1/02

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

ECONOLINE COLUMN



Revision 2.0
1/1/02

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

1. REMOVAL/INSERTION OF THE PISTON

Opening the lock

To open the lock, the counter nut and counter screw are pressed gently towards each other and the screw is turned a quarter turn anticlockwise. This frees the piston, so that it and the counter screw can be removed as a single piece from the column.

Resealing the column

Gently insert the piston, with the counter screw attached, into the column body, ensuring that it goes in straight and not at an angle. Bring the counter screw and the counter nut into position by turning them in such a way that the stopper of the bayonet lock fits into the opening of the the counter nut. The column is sealed by turning the counter screw a quarter turn clockwise. The bayonet lock will engage audibly.

PLEASE NOTE: When inserting the piston, it is absolutely essential that it goes straight in and not at an angle. The inner glass surface and the seals must be clean, and the seals should be moistened with solvent Both the O-rings on the AB version and the Teflon triple chevron seals on the SR version can be damaged by foreign particles or by incorrect insertion, rendering the seal useless.
The column can only be used with correctly locked end fittings, otherwise the pistons will be forced out of the column by the pressure inside. Please make sure you hear the bayonet lock click, which means that it is locked correctly.

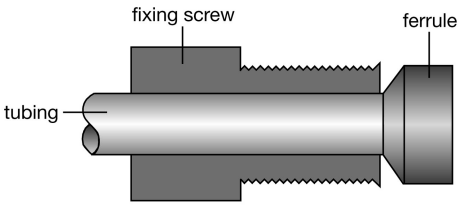
2. FRIT REPLACEMENT

1. ***Eject the old frit with the frit ejector supplied.***

2. ***Insertion of new frit: the Teflon piston end can be protected by heating it (to a maximum of 121°C) before the new frit is pressed into position. Before the piston can be inserted into the column, it must be cooled back down to the temperature of its surroundings.***

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

3. ASSEMBLY OF Essential Life Solutions Ltd. MPLC-FITTINGS



These assembly instructions are for tubing with an outer diameter of 1.6mm or 3.2 mm.

1. ***The end of the tubing is cut square with a knife or a tubing cutter.***

> **PLEASE NOTE: Do not cut with scissors, as the tubing will be squashed. It is important to cut the tubing at 90°, as the cut edge forms part of the sealing area.**

2. ***The fixing screw is pushed onto the tubing.***
3. ***The ferrule is pushed onto the tubing with the conical end towards the fixing screw. If the tubing cannot be inserted into the ferrule, the conical end can be widened slightly with a suitable instrument (e.g. a scriber).***
4. ***The fitting can now be inserted into the desired position. The screws should be tightened until the pressure can be felt, after which they should be tightened by another half turn.***

> **PLEASE NOTE: When the fittings are screwed into the Teflon thread, care should be taken to screw them in straight and fasten them carefully, to avoid damaging the Teflon thread.**

4. ELIMINATION OF DEAD VOLUME

Dead volume which occurs at the column inlet can be remedied simply without needing to open the column:

1. ***Turn off the pump.***
2. ***Turn the column lock anticlockwise.***

> **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.**

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

5. OPERATION OF THE COLUMN

Putting the column into operation

The pistons, frits and glass body must be cleaned thoroughly before the column is used for the first time. In some cases it may be worth dismantling the column and washing the parts in a sonic bath for a few minutes. After cleaning, all parts must be rinsed in double-distilled water and assembled as described in the second part of point 1 (page 5). Care must be taken that the piston is straight when inserted into the column body, because if it is inserted at an angle, the seal might be damaged.

To operate the column, it must be attached to an appropriate chromatography system or pump using the fittings supplied. Take care when selecting the tubing: tubing diameter must be appropriate to the flow rate to be applied, and the tubing itself must not react with the solvent in question. Should the column bed shrink during use, dead volume can easily be eliminated by moving the variable piston downwards.

PLEASE NOTE: only use degassed and pre-filtered solvents. Particles in the solvent may clog the frits or damage the column packing. Make sure that the particle size of the chromatography material in question is considerably greater than the porosity of the frits!

Hints for operation

1. ***Storage of the packed column: open the sealing stoppers by one complete turn in order to compensate for temperature-related changes in pressure.***

IMPORTANT: Protect moistened columns from intense heat and direct sunlight. The heat induces evaporation of highly volatile solvents, and the resulting pressure can crack the column.

2. ***We recommend eluting the column from bottom to top so that any air present can escape more quickly. As a result, the column is conditioned more quickly, so less solvent is necessary.***
3. ***Before sample application, please ensure that no dead volume has occurred at the column inlet during the conditioning phase. (For removal of dead volume, see page 6).***

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

6. SOLVENT RESISTANCE

For the storage of packed columns, we recommend 20% ethanol in H_2O, possibly buffered neutrally with up to 1 M NaCl, or a neutral buffer with 0.03% sodium azide. Normal and reversed phase columns should be stored in the solution they are used with, but with a minimum proportion of 10% organic solvent. All solvents used should be filtered through at least 0.45µm, or preferably through 0.22µm filters.

In general the following solvents and additives can be used. However, we do not recommend using any of them for longer periods of time or for storage. For further information please contact Essential Life Solutions Ltd.

- **Resistant to all common organic solvents such as:**
  ethanol, methanol, propanol, isopropanol, acetonitrile
  Please note that the AB version is only resistant to organic solvents to a limited extent
- **Resistant to all common aqueous buffers**
- **Salts in aqueous solutions such as: NaCl, $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$, etc.**
- **pH 1-14**
- **2 M NaOH**
- **1 M HCl**
- **75% acetic acid**
- **Detergents ($\leq$2%) such as SDS, Triton, etc.**
- **6 M guanidinium-HCl, 8 M urea**
- **Working temperature range:**
  with Teflon piston / triple chevron sealing system:
  16-40° C (SR version)
  with polyethylene piston / O-Ring-seals:
  4-40° C (AB version)

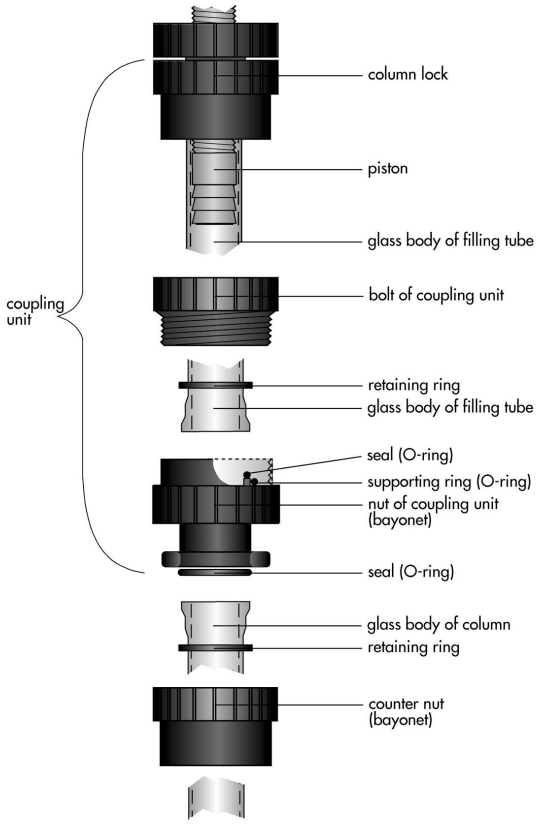
1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

7. PACKING INSTRUCTIONS

This packing method is a general recommendation, which usually gives good results in terms of reproducibility and resolution. Best results can be achieved by using a filling tube when packing the column. If you only require a short bed length or relatively low packing quality, the column can also be packed directly and the slurry can also be topped up during packing if necessary. In individual cases, we advise optimising the methods depending on the specific media or application in question.

Assembling the packing device

The glass column body is sealed at one end with the piston. The filling tube is attached to the column funnel with the coupling unit. Care should be taken to position the O-rings and the frits correctly. The packing device must be screwed on tightly, to prevent foreign bodies from coming between the seals and the glass column body or getting caught inside.



Producing the slurry

A suitable solvent or buffer is added to the appropriate amount of packing material until it reaches the total volume of the packing device or the column (see instructions on packing material). The slurry is gently shaken until it has a uniform consistency (never use a magnetic stirrer) and degassed thoroughly just before packing.

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

7. PACKING INSTRUCTIONS

Safety tip

Glass columns must never be packed under gas pressure without the appropriate protection equipment. You should always keep within the stated pressure limits for columns and related equipment. Appropriate clothing for the laboratory and safety glasses are essential.

PLEASE NOTE: A glass column should never be used under gas pressure. Even small stresses in the body of the column are sufficient to cause the column to explode, thus freeing the expansion energy of the gas and causing the shards of glass to act like projectiles. If the glass body shatters under the pressure of a liquid, on the other hand, there is no danger, since liquids are much less compressible and have virtually no expansion energy.

Packing the column with rigid media

Introduce a few ml of solvent or buffer (see instructions on packing material) into the packing device, so that the lower frit is moistened and free from air bubbles. Next the slurry is shaken carefully until it has a uniform consistency and quickly poured into the packing device without introducing any air bubbles. The slurry container must not have any air bubbles at all in it. If necessary, it can be topped up with solvent. The packing device is then sealed and packed as quickly as possible using a pump: this means that the flow rate should be set at the pressure limit of the column, so if necessary packing is carried out at the pump's maximum flow rate. A narrow PEEK capillary at the column outlet may improve packing quality, as it will act like a back-pressure regulator and prevent the slurry entering the column too quickly at first. Pumping must continue at least until a constant pressure is reached. The flow rate during packing should always be considerably higher (>20%) than the flow rate needed for later use. After packing, the filling tube is unscrewed. Care must be taken when opening the column outlet so that any remaining pressure is released completely.

The piston is introduced carefully, without allowing any particles to get between the glass and the piston seal. The column is now re-attached to the pump, the pump is started at low pressure and the flow gradually increased to the pressure limit of the column. At this point dead volume may occur between the variable piston and the column bed, which can be removed by moving the variable piston towards the column bed. For this, the column must not be under pressure, i.e. the pump must be turned off and the column inlet opened. Next the column is conditioned with the relevant eluents and is ready to be used.

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

7. PACKING INSTRUCTIONS

Packing the column with soft gels

Only degassed and filtered solvents or buffers may be used when packing chromatography columns.
The lower frit is dampened and covered with approx. 1cm solvent. Next the slurry is introduced carefully and quickly, ensuring that no air bubbles occur. The column outlet should be open while the column is being filled; the solvent can also be sucked from the column outlet with a peristaltic pump at the same time. When the slurry has all been poured in, the gel must be allowed to settle and the solvent sink to approx. 0.5 – 1cm above the packing level in the gel bed. The gel bed must not be allowed to run dry. The column outlet is closed or the peristaltic pump stopped. Next the variable piston is inserted, without allowing particles to come between the seal and the column body. By turning the lock slowly, the piston can be moved towards the gel bed. At the same time, all the air above the gel bed should be forced out of the column inlet. It is essential that the gel bed is not compressed when moving the piston towards it. Now the column can be equilibrated with the appropriate buffer or solvent.
Dead volume can occur between the gel bed and the piston during normal use, but this can be removed by moving the piston inwards.

Quality control

We recommend that you determine plate count and peak symmetry with a suitable (non-adsorbent) test substance after packing the column. By repeating this test frequently, the quality and durability of the packing material can be recorded efficiently.

Amount of theoretical plates (N):

$N = 5.54 \times (T_1 / W_{1/2})$	T_1:	retention time (sec)
	$W_{1/2}$:	peak width (sec) at half peak height
$HETP = L / N$	L:	column length in mm

Peak symmetry (S):

$S = W_{1/2,r} / W$	$W_{1/2,r}$:	peak width to the right of the peak median
	$W_{1/2,l}$:	peak width to the left of the peak median

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

8. CLEANING INSTRUCTIONS FOR PACKED COLUMNS (CIP)

The cleaning of a chromatography column involves the following three stages: regeneration of the column packing, sterilisation and depyrogenation.

Regeneration removes chemical and organic contamination that becomes non-specifically attached to the chromatography material, considerably reducing the capacity and resolution of the column. This kind of contamination is usually caused by lipids and pyrogens, protein aggregates, pigments, polyphenols and metal complexes.

Sterilisation is the removal and/or denaturing of micro-organisms and spores, which could contaminate the purified product, by chemical treatment. The most frequently used sterilisation method is treatment with sodium hydroxide, acetic acid or ethanol solutions containing sodium hydroxide or acetic acid.

Depyrogenation includes the breaking-down of endotoxins that have become attached to the chromatography material or the column hardware (frits, tubing etc.) and can soil the target compounds in question by being washed gradually through the column. Often the methods used to sterilise equipment will also break down pyrogens.

Chromatography columns can be purified and sterilised by taking the following steps:

The column is dismantled and the individual parts (column body, pistons, end fittings, frits) are washed in a dilute solution of caustic soda or sodium hypochloride (0.5 N NaOH or dilute NaOCl); the frits should be left in the same solution for 30-60 minutes. Before the column is re-assembled, all parts should be washed in a sterile, pyrogen-free solution.

The column must be packed in a sterile environment. All solvents and solutions used for the column must be sterile and pyrogen-free. We recommend in-line filtration through a 0.22 µm filter.

PLEASE NOTE: Check carefully that all moistened parts of the column are stable with all reagents used. If in doubt, contact Essential Life.

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

8. CLEANING INSTRUCTIONS FOR PACKED COLUMNS (CIP)

There is a range of cleaning methods, according to the nature of the substance to be removed. Please refer to the instructions given for the column packing in question!

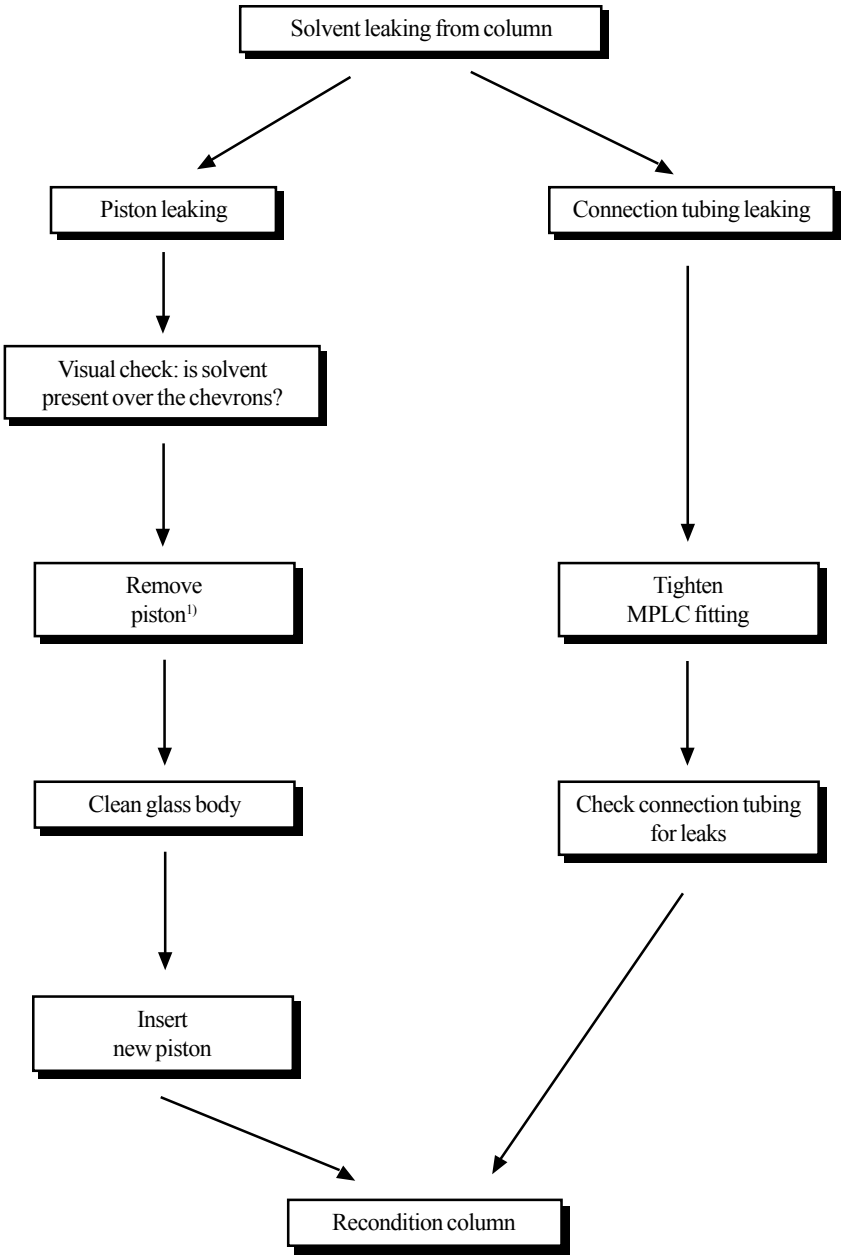
Treatment	Contamination	Sterili-zation	Depyroge-nation
1-2 M NaCl	Highly charged molecules	Ineffective	Ineffective
Buffer pH 3-5	Highly charged molecules	Ineffective	Ineffective
Treatment with pronase at	Hydrolysis of adsorbed proteins	Ineffective	Ineffective
a neutral pH, calcium ions			
Treatment with pepsin,	Hydrolysis of adsorbed proteins	Ineffective	Ineffective
pH l.5-2			
Non-ionic detergents	Removal of hydrophobic proteins and	Ineffective	Ineffective
(e.g. Triton X-100, Tween 80)	lipidis		
Cationic detergents pH 9-11	Removal of hydrophobic proteins and	Ineffective	Partial
	lipidis		
Non-ionic detergents pH 3	Removal of hydrophobic proteins and		
(acetic acid)	lipidis	Ineffective	Partial
Urea 6-8 M	Removal of protein aggregates	Ineffective	Unknown
1-100 mM EDTA in neutral	Removal of metal complexes	Ineffective	Ineffective
or slightly acidic solution			
2-3 M NaCl in	Removal of various small, charged molecules	Ineffective	Effective
0.1-1 M HCl	and pigments		
0.1-1 M NaOH	In particular the removal of bonded	Effective	Effective
	hydrophobic proteins and lipo-polysaccharides		
0.5-1 M acetic acid in	Removal of lipids, pigments, lipo-poly-		
60% ethanol	saccharides and other lipophilic substances	Very effective	Effective
1500 ppm peracetic acid in	Removal/denaturing of spores, viruses		
0.5 M sodium acetate, pH 5	and bacteria	Very effective	Unknown
50-80% acetic add	Dissolving and removal of	Unknown	Unknown
	precipitated proteins		
40-60% ethanol	Removal of various proteins and lipids	Unknown	Unknown
Isopropanol-gradient	Removal of non polar lipids	Ineffective	Unknown
up to 100% in water			
0.1-1 M mineral or	Removal of various charged molecules	Unknown	Unknown
organic acids	and hydrolysis of bonded substances		
0.1 M - 1 M HCl	Removal of various charged molecules	Unknown	Effective
in 60% ethanol	and lipids		

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax

9. TROUBLESHOOTING

Problem	Cause	Remedy
1. Peak shape of eluted substances deteriorates	1. Dead volume at column inlet	1. see point 4 on page 6: elimination of dead volume
	2. Inlet frit partially blocked	2. Remove and dismantle piston, replace frit, reassemble and re-insert piston. The column will need to be reconditioned.
	3. Outlet frit partially blocked	3. Remove piston, replace frit, reassemble and re-insert piston. The column will need to be reconditioned.
	4. Separation efficiency of stationary phase affected by contamination	4. Repack column
	5. Stationary phase damaged mechanically	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. Blocked frit	2. see above, 1. 2.
	3. Fittings tightened too much	3. Replace fittings and ferrules, re-cut the end of the tubing.
4. Pressure drop during operation	1. Leak in tubing or fitting between pump and column	1. Check tubing and connections
	2. Solvent supply has run out	2. Refill solvent
5. Solvents leaking from column	See diagram on following page	See diagram on following page

1415 Hyde Park Avenue
Boston, MA 02136-2699 USA
(617) 326-0444 phone, (617) 326-0443 fax



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