

Econoline^{LP}® Columns

Low Pressure, Low Price



HIGHLIGHTS:

- Biocompatible
- Universal Application
- Height Adjustment Plunger at One End
- Ease of Use
- Jacketed Version Available

Advantages of EconolineLP® Columns

1 LINEAR MOTION OF PISTON

Due to true linear motion of piston there is no torsional load imposed on the packed bed assuring true linear compression.

2 TRUE FRITS

True frit without the use of sock design employed in less expensive columns assure even flow distribution across the bed.

3 ROBUST INLET AND OUTLET CONNECTIONS

Inlet and outlet connections are made at the exterior of the column, which provide a more reliable and visible connection.

4 FINE THREAD ADJUSTMENT

Columns are provided with fine thread adjustment of piston to allow for precise control of piston placement.

5 EASY OPEN ENDS

Design features our unique easily removable threaded end fittings making column disassembly effortless.

6 SEAL ADJUSTMENT

Easily adjustable O-ring seals.

7 PACKING ADAPTER

User-friendly packing adapters available.

Pressure Limits:
10 - 30 Bars

Connections:
ID 10mm - 25mm
Two (2) 1/4"-28 fittings
for 1/16" capillaries

ID 32mm - 50 mm
Two (2) 1/4"-28 fittings
for 1/8" capillaries

Height Adjustment:
Multi-variable plunger
at one end, 120 mm
adjustment. Double
piston configuration
available upon request.

Accessories:
User-friendly
packing adapters

EconolineLP® Columns

MATERIALS OF CONSTRUCTION

- Tube: Borosilicate Glass
- Temperature Range: 4° - 40° C
- Plunger: POM (Delrin®)
- Seal: Viton O-ring
- Frit: Porous Glass

INCLUDED SPARE PARTS

I.D. 10-25mm:

- 2 pcs. Nut 1/16"
- 2 pcs. Ferrule 1/16"
- 0.5mm FEP-Tubing I.D. 0.8mm, O.D. 1.6mm
- 1 pc. frit removal tool

I.D. 32-50mm:

- 2 pcs. Nut 1/8"
- 2 pcs. Ferrule 1/8"
- 0.5mm FEP-Tubing I.D. 1.6mm, O.D. 3.2 mm
- 1 pc. frit removal tool

Econoline^{LP}® Columns

Econoline^{LP}® Column
with piston removed.



ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (µm)	ELS Part No
10	0-120	0-9.42	30	16-40	ECO10/120V3V
10	80-200	6.28-15.71	30	16-40	ECO10/200V3V
10	330-450	25.92-35.34	30	16-40	ECO10/450V3V
10	630-750	49.48-58.90	30	16-40	ECO10/750V3V
10	880-1000	69.11-78.54	30	16-40	ECO10/999V3V
10	0-120	0-9.42	30	40-100	ECO10/120V4V
10	80-200	6.28-15.71	30	40-100	ECO10/200V4V
10	330-450	25.92-35.34	30	40-100	ECO10/450V4V
10	630-750	49.48-58.90	30	40-100	ECO10/750V4V
10	880-1000	69.11-78.54	30	40-100	ECO10/999V4V
15	0-120	0-21.21	25	16-40	ECO15/120V3V
15	80-200	14.14-35.34	25	16-40	ECO15/200V3V
15	330-450	58.32-79.52	25	16-40	ECO15/450V3V
15	630-750	111.33-132.54	25	16-40	ECO15/750V3V
15	880-1000	155.51-176.71	25	16-40	ECO15/999V3V
15	0-120	0-21.21	25	40-100	ECO15/120V4V
15	80-200	14.14-35.34	25	40-100	ECO15/200V4V
15	330-450	58.32-79.52	25	40-100	ECO15/450V4V
15	630-750	111.33-132.54	25	40-100	ECO15/750V4V
15	880-1000	155.51-176.71	25	40-100	ECO15/999V4V
20	0-120	0-37.70	20	16-40	ECO20/120V3V
20	80-200	25.13-62.83	20	16-40	ECO20/200V3V
20	330-450	103.67-141.37	20	16-40	ECO20/450V3V
20	630-750	197.92-235.62	20	16-40	ECO20/750V3V
20	880-1000	276.46-314.16	20	16-40	ECO20/999V3V
20	0-120	0-37.70	20	40-100	ECO20/120V4V
20	80-200	25.13-62.83	20	40-100	ECO20/200V4V
20	330-450	103.67-141.37	20	40-100	ECO20/450V4V
20	630-750	197.92-235.62	20	40-100	ECO20/750V4V
20	880-1000	276.46-314.16	20	40-100	ECO20/999V4V

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Importers & Manufacturers
www.chromtech.net.au

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Glass Tempering **Jacket** / Double **Piston** Configuration

User-friendly
**PACKING
 ADAPTER**
 Available



ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (μ m)	ELS Part No
25	0-120	0-58.90	15	16-40	ECO25/120M3V-K
25	0-200	0-98.17	15	16-40	ECO25/200M3V-K
25	210-450	103.08-220.89	15	16-40	ECO25/450M3V-K
25	510-750	250.35-368.16	15	16-40	ECO25/750M3V-K
25	760-1000	373.06-490.87	15	16-40	ECO25/999M3V-K
25	0-120	0-58.90	15	40-100	ECO25/120M4V-K
25	0-200	0-98.17	15	40-100	ECO25/200M4V-K
25	210-450	103.08-220.89	15	40-100	ECO25/450M4V-K
25	510-750	250.35-368.16	15	40-100	ECO25/750M4V-K
25	760-1000	373.06-490.87	15	40-100	ECO25/999M4V-K
32	0-120	0-96.51	10	16-40	ECO32/120M3V-K
32	0-200	0-160.85	10	16-40	ECO32/200M3V-K
32	210-450	168.89-361.91	10	16-40	ECO32/450M3V-K
32	510-750	410.17-603.19	10	16-40	ECO32/750M3V-K
32	760-1000	611.23-804.25	10	16-40	ECO32/999M3V-K
32	0-120	0-96.51	10	40-100	ECO32/120M4V-K
32	0-200	0-160.85	10	40-100	ECO32/200M4V-K
32	210-450	168.89-361.91	10	40-100	ECO32/450M4V-K
32	510-750	410.17-603.19	10	40-100	ECO32/750M4V-K
32	760-1000	611.23-804.25	10	40-100	ECO32/999M4V-K
50	0-120	0-235.62	10	16-40	ECO50/120M3V-K
50	0-200	0-392.70	10	16-40	ECO50/200M3V-K
50	210-450	412.33-883.57	10	16-40	ECO50/450M3V-K
50	510-750	1001.38-1472.62	10	16-40	ECO50/750M3V-K
50	760-1000	1492.26-1963.49	10	16-40	ECO50/999M3V-K
50	0-120	0-235.62	10	40-100	ECO50/120M4V-K
50	0-200	0-392.70	10	40-100	ECO50/200M4V-K
50	210-450	412.33-883.57	10	40-100	ECO50/450M4V-K
50	510-750	1001.38-1472.62	10	40-100	ECO50/750M4V-K
50	760-1000	1001.38-1472.62	10	40-100	ECO50/999M4V-K

Glass Tempering **Jacket** / Double **Piston** Configuration

Double Piston
Econoline^{LP}® **Column** with
Glass Tempering Jacket.

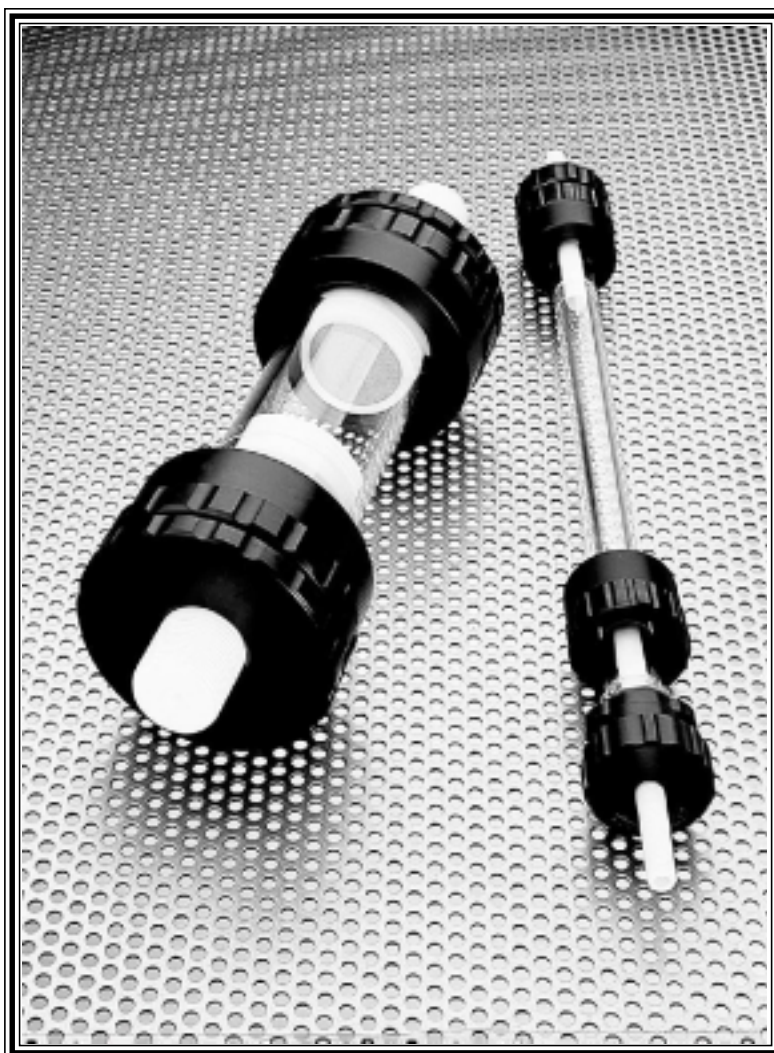


ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (µm)	ELS Part No
10	0-120	0-9.42	30	16-40	ECO10/120M3V-K
10	0-200	0-15.71	30	16-40	ECO10/200M3V-K
10	210-450	16.49-35.34	30	16-40	ECO10/450M3V-K
10	510-750	40.06-58.90	30	16-40	ECO10/750M3V-K
10	760-1000	59.69-78.54	30	16-40	ECO10/999M3V-K
10	0-120	0-9.42	30	40-100	ECO10/120M4V-K
10	0-200	0-15.71	30	40-100	ECO10/200M4V-K
10	210-450	16.49-35.34	30	40-100	ECO10/450M4V-K
10	510-750	40.06-58.90	30	40-100	ECO10/750M4V-K
10	760-1000	59.69-78.54	30	40-100	ECO10/999M4V-K
15	0-120	0-21.21	25	16-40	ECO15/120M3V-K
15	0-200	0-35.34	25	16-40	ECO15/200M3V-K
15	210-450	37.11-79.52	25	16-40	ECO15/450M3V-K
15	510-750	90.12-132.54	25	16-40	ECO15/750M3V-K
15	760-1000	144.30-176.71	25	16-40	ECO15/999M3V-K
15	0-120	0-21.21	25	40-100	ECO15/120M4V-K
15	0-200	0-35.34	25	40-100	ECO15/200M4V-K
15	210-450	37.11-79.52	25	40-100	ECO15/450M4V-K
15	510-750	90.12-132.54	25	40-100	ECO15/750M4V-K
15	760-1000	144.30-176.71	25	40-100	ECO15/999M4V-K
20	0-120	0-37.70	20	16-40	ECO20/120M3V-K
20	0-200	0-62.83	20	16-40	ECO20/200M3V-K
20	210-450	65.97-141.37	20	16-40	ECO20/450M3V-K
20	510-750	160.22-235.362	20	16-40	ECO20/750M3V-K
20	760-1000	238.76-314.16	20	16-40	ECO20/999M3V-K
20	0-120	0-37.70	20	40-100	ECO20/120M4V-K
20	0-200	0-62.83	20	40-100	ECO20/200M4V-K
20	210-450	65.97-141.37	20	40-100	ECO20/450M4V-K
20	510-750	160.22-235.362	20	40-100	ECO20/750M4V-K
20	760-1000	238.76-314.16	20	40-100	ECO20/999M4V-K



Econoline^{LP}® Column
with frit removal tool.

ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (µm)	ELS Part No
25	0-120	0.58.90	15	16-40	ECO25/120V3V
25	80-200	39.27-98.17	15	16-40	ECO25/200V3V
25	330-450	161.99-220.89	15	16-40	ECO25/450V3V
25	630-750	309.25-368.16	15	16-40	ECO25/750V3V
25	880-1000	431.97-490.87	15	16-40	ECO25/999V3V
25	0-120	0.58.90	15	40-100	ECO25/120V4V
25	80-200	39.27-98.17	15	40-100	ECO25/200V4V
25	330-450	161.99-220.89	15	40-100	ECO25/450V4V
25	630-750	309.25-368.16	15	40-100	ECO25/750V4V
25	880-1000	431.97-490.87	15	40-100	ECO25/999V4V
32	0-120	0.96.51	10	16-40	ECO32/120V3V
32	80-200	64.34-160.85	10	16-40	ECO32/200V3V
32	330-450	265.40-361.91	10	16-40	ECO32/450V3V
32	630-750	506.68-603.19	10	16-40	ECO32/750V3V
32	880-1000	707.74-804.25	10	16-40	ECO32/999V3V
32	0-120	0.96.51	10	40-100	ECO32/120V4V
32	80-200	64.34-160.85	10	40-100	ECO32/200V4V
32	330-450	265.40-361.91	10	40-100	ECO32/450V4V
32	630-750	506.68-603.19	10	40-100	ECO32/750V4V
32	880-1000	707.74-804.25	10	40-100	ECO32/999V4V
50	0-120	0.235.62	10	16-40	ECO50/120V3V
50	80-200	157.08-392.70	10	16-40	ECO50/200V3V
50	330-450	647.95-883.57	10	16-40	ECO50/450V3V
50	630-750	1237.00-1472.62	10	16-40	ECO50/750V3V
50	880-1000	1727.87-1963.49	10	16-40	ECO50/999V3V
50	0-120	0.235.62	10	40-100	ECO50/120V4V
50	80-200	157.08-392.70	10	40-100	ECO50/200V4V
50	330-450	647.95-883.57	10	40-100	ECO50/450V4V
50	630-750	1237.00-1472.62	10	40-100	ECO50/750V4V
50	880-1000	1727.87-1963.49	10	40-100	ECO50/999V4V



*essential*Life **Solutions**

Econoline® Column Manual

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

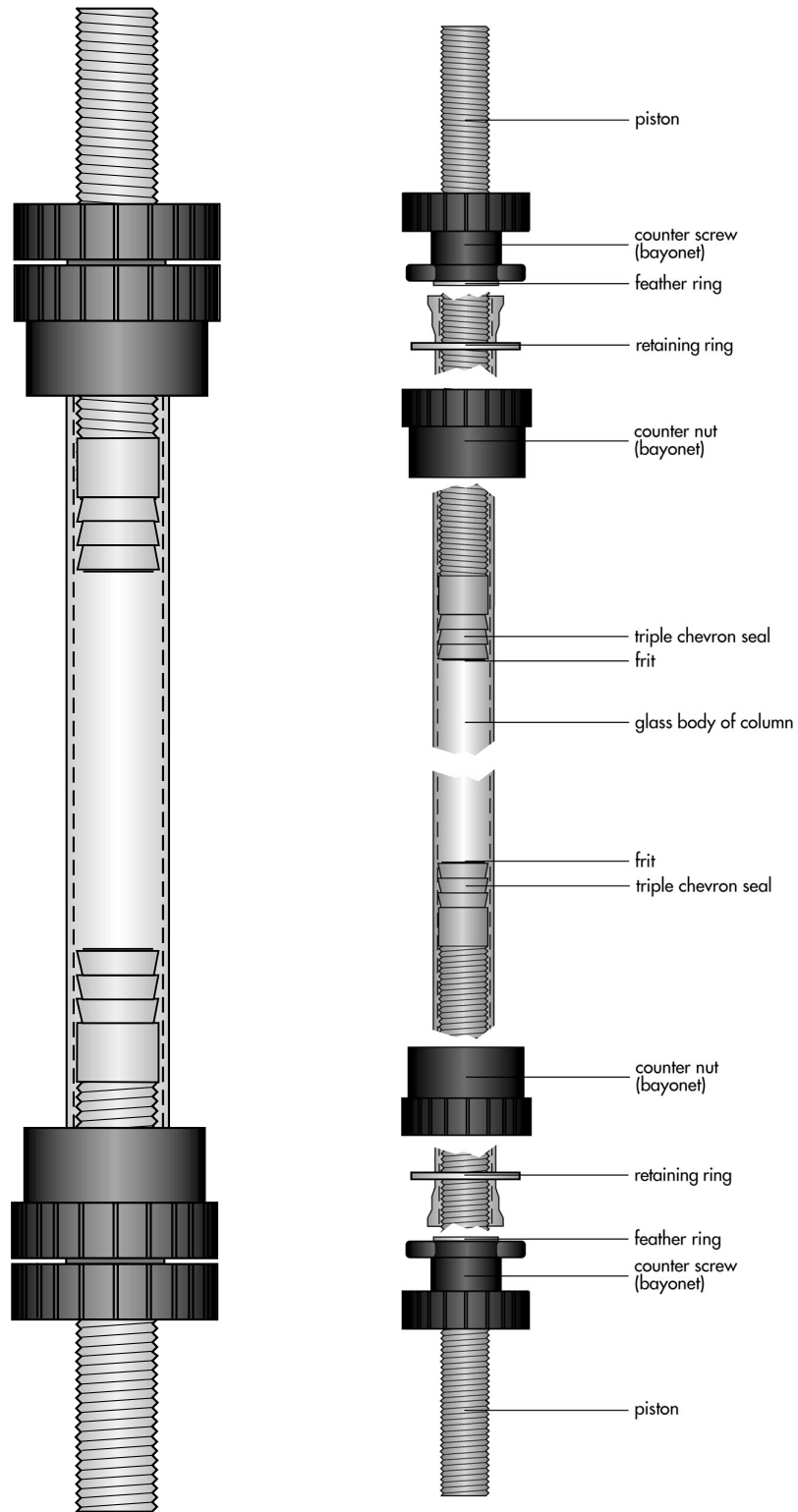
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The general conditions and the Essential Life Solutions Ltd warranty conditions apply in the version that is valid in each case. All information contained within this manual is without obligation, as it is subject to technical changes resulting in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

Revision 2.0
1/1/02

**ECONOLINE
 COLUMN**



Revision 2.0
 1/1/02

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 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.

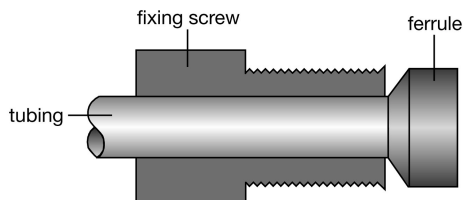
The column can only be used with correctly locked end fittings, otherwise the pistons will be forced out of the column by the pressure inside. Please make sure you hear the bayonet lock click, which means that it is locked correctly.

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.*

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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.

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).*

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6. SOLVENT RESISTANCE

For the storage of packed columns, we recommend 20% ethanol in H₂O, 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.

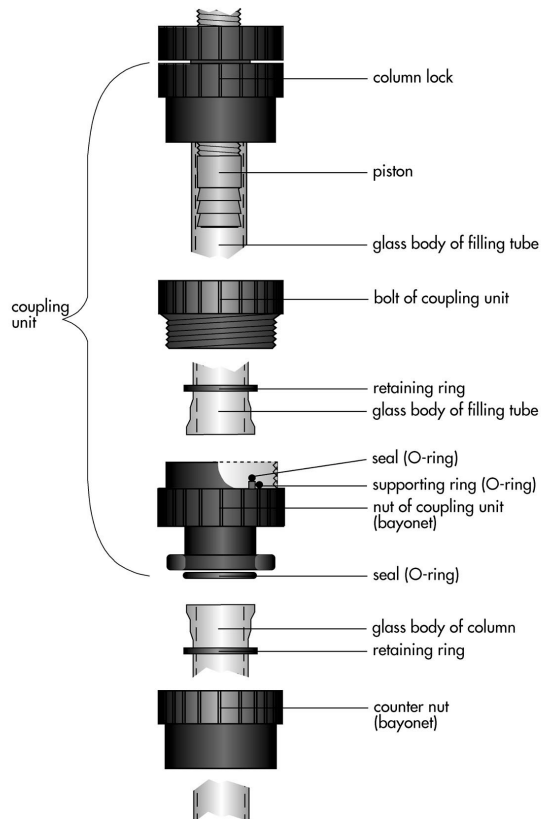
- W** Resistant to all common organic solvents such as:
ethanol, methanol, propanol, isopropanol, acetonitrile
- U** *Please note that the AB version is only resistant to organic solvents to a limited extent*
- W** Resistant to all common aqueous buffers
- W** Salts in aqueous solutions such as: NaCl, (NH₄)₂SO₄, MgCl₂, CaCl₂, etc.
- W** pH 1-14
- W** 2 M NaOH
- W** 1 M HCl
- W** 75% acetic acid
- W** Detergents (≤ 2%) such as SDS, Triton, etc.
- W** 6 M guanidinium-HCl, 8 M urea
- W** 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)

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.

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.

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):

$$S = W_{1/2,r} / W_{1/2,l}$$

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

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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 hypochlorite (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.

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!

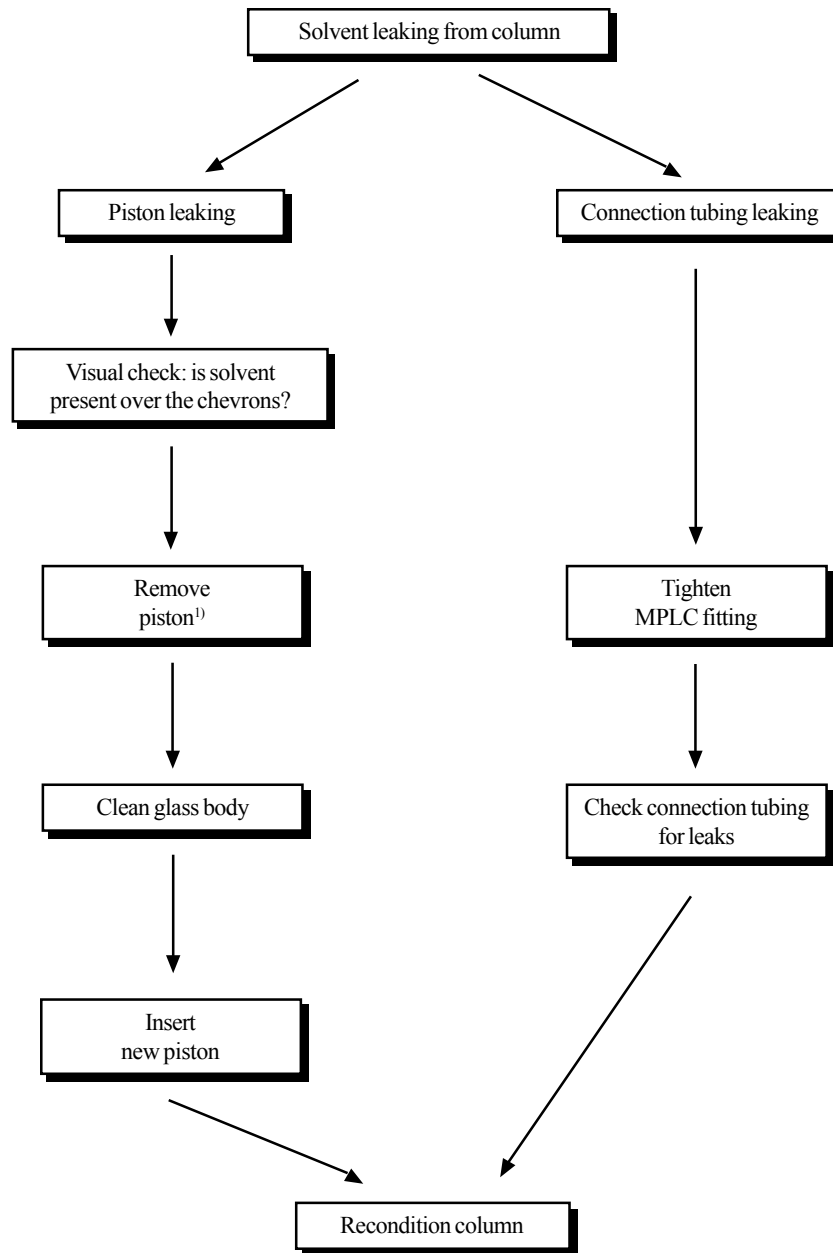
Treatment	Contamination	Sterilization	Depyrogenation
1-2 M NaCl	Highly charged molecules	Ineffective	Ineffective
Buffer pH 3-5	Highly charged molecules	Ineffective	Ineffective
Treatment with pronase at a neutral pH, calcium ions	Hydrolysis of adsorbed proteins	Ineffective	Ineffective
Treatment with pepsin, pH 1.5-2	Hydrolysis of adsorbed proteins	Ineffective	Ineffective
Non-ionic detergents (e.g. Triton X-100, Tween 80)	Removal of hydrophobic proteins and lipids	Ineffective	Ineffective
Cationic detergents pH 9-11	Removal of hydrophobic proteins and lipids	Ineffective	Partial
Non-ionic detergents pH 3 (acetic acid)	Removal of hydrophobic proteins and lipids	Ineffective	Partial
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 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 hydrophobic proteins and lipopolysaccharides	Effective	Effective
0.5-1 M acetic acid in 60% ethanol	Removal of lipids, pigments, lipopolysaccharides and other lipophilic substances	Very effective	Effective
1500 ppm peracetic acid in 0.5 M sodium acetate, pH 5	Removal/denaturing of spores, viruses and bacteria	Very effective	Unknown
50-80% acetic acid	Dissolving and removal of precipitated proteins	Unknown	Unknown
40-60% ethanol	Removal of various proteins and lipids	Unknown	Unknown
Isopropanol-gradient up to 100% in water	Removal of non polar lipids	Ineffective	Unknown
0.1-1 M mineral or organic acids	Removal of various charged molecules and hydrolysis of bonded substances	Unknown	Unknown
0.1 M - 1 M HCl in 60% ethanol	Removal of various charged molecules and lipids	Unknown	Effective

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9. TROUBLESHOOTING

Problem	Cause	Remedy
1. Peak shape of eluted substances deteriorates	1. Dead volume at column inlet 2. Inlet frit partially blocked 3. Outlet frit partially blocked 4. Separation efficiency of stationary phase affected by contamination 5. Stationary phase damaged mechanically	1. see point 4 on page 6: elimination of dead volume 2. Remove and dismantle piston, replace frit, reassemble and re-insert piston. The column will need to be reconditioned. 3. Remove piston, replace frit, reassemble and re-insert piston. The column will need to be reconditioned. 4. Repack column 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 2. Blocked frit 3. Fittings tightened too much	1. Check valve position 2. see above, 1. 2. 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 2. Solvent supply has run out	1. Check tubing and connections 2. Refill solvent
5. Solvents leaking 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.

Media-Flex® Columns

"Quick Change"
Cartridge Lab Columns



HIGHLIGHTS:

- Pressure to 86 bar
- Low Dead Volume Design
- Fully Visible Bed
- Autoclavable
- Reusable Glass Cartridge
- EPDM Seal for Cold Room Applications

Advantages of Media-Flex® Columns

1 HIGHER PRESSURE RATINGS INCORPORATING GLASS CONSTRUCTION

Pressures to 86 Bars (1,247 psig)
Full view of bed unlike stainless steel
Rugged construction for hard lab use

2 LINEAR MOTION OF PISTON

Due to true linear motion of piston there is no torsional load imposed on the packed bed assuring true linear compression.

3 TRUE FRITS

True frit without the use of sock design employed in less expensive columns assure even flow distribution across the bed.

4 ROBUST INLET AND OUTLET CONNECTIONS

Inlet and outlet connections are made at the exterior of the column, which provide a more reliable and visible connection.

5 FINE THREAD ADJUSTMENT

Columns are provided with fine thread adjustment of piston to allow for precise control of piston placement.

6 QUICK RELEASE ENDS

Design features our unique quick-disconnect end fittings making column disassembly and cartridge removal effortless.

7 UNIQUE CARTRIDGE DESIGN

Media-flex® offers the benefit in that the cartridge unit quickly releases from the housing without disturbing the bed or packing allowing for rapid, inexpensive change-out of the column.

Pressure Limits:
28 - 86 Bars

Connections:
Two 1/4" - 28 Fittings
(one for 1/16" and one for 1/8" capillaries).
The column is supplied with an M6 adapter to allow direct connection to any LC system.

Height Adjustment:
Multi-variable plunger,
6 cm/12 cm adjustment.

Accessories:
Packing adapters
Spare cartridges

MEDIA-FLEX® COLUMN MATERIALS OF CONSTRUCTION

SR (solvent resistant)

- Tube: Borosilicate Glass
- Temperature Range: 18° - 60° C
- Plunger: PTFE
- Seal: PTFE triple chevron
- Frit: ID 5 mm - 15 mm: sinter glass frit
ID 25 mm - 50 mm: stainless steel

AB (aqueous buffer)

- Tube: Borosilicate Glass
- Temperature Range: 4° - 40° C
- Plunger: Polyethylene
- Seal: EPDM O-ring
- Frit: ID 5 mm - 50 mm: polyethylene

Media-Flex Lab[®] Columns

ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (μ m)	Frit Type	Column Type (application)	ELS Part No
5	0 - 120	0.00 - 2.36	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-120-L-10-SR
5	60 - 120	1.18 - 2.36	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-120-S-10-SR
5	140 - 260	2.75 - 5.11	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-260-L-10-SR
5	200 - 260	3.93 - 5.11	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-260-S-10-SR
5	380 - 500	7.46 - 9.82	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-500-L-10-SR
5	440 - 500	8.64 - 9.82	86.67	10	Porous Glass	Solvent Resistant (SR)	MF-5-500-S-10-SR
5	0 - 120	0.00 - 2.36	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-120-L-2-SR
5	60 - 120	1.18 - 2.36	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-120-S-2-SR
5	140 - 260	2.75 - 5.11	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-260-L-2-SR
5	200 - 260	3.93 - 5.11	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-260-S-2-SR
5	380 - 500	7.46 - 9.82	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-500-L-2-SR
5	440 - 500	8.64 - 9.82	86.67	2	Porous Glass	Solvent Resistant (SR)	MF-5-500-S-2-SR
10	0 - 120	0.00 - 9.42	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-120-L-10-SR
10	60 - 120	4.71 - 9.42	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-120-S-10-SR
10	140 - 260	11.00 - 20.42	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-260-L-10-SR
10	200 - 260	15.71 - 20.42	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-260-S-10-SR
10	380 - 500	29.85 - 39.27	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-500-L-10-SR
10	440 - 500	34.56 - 39.27	80	10	Porous Glass	Solvent Resistant (SR)	MF-10-500-S-10-SR
10	0 - 120	0.00 - 9.42	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-120-L-2-SR
10	60 - 120	4.71 - 9.42	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-120-S-2-SR
10	140 - 260	11.00 - 20.42	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-260-L-2-SR
10	200 - 260	15.71 - 20.42	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-260-S-2-SR
10	380 - 500	29.85 - 39.27	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-500-L-2-SR
10	440 - 500	34.56 - 39.27	80	2	Porous Glass	Solvent Resistant (SR)	MF-10-500-S-2-SR
15	0 - 120	0.00 - 21.21	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-120-L-10-SR
15	60 - 120	10.60 - 21.21	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-120-S-10-SR
15	140 - 260	24.74 - 45.95	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-260-L-10-SR
15	200 - 260	35.34 - 45.95	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-260-S-10-SR
15	380 - 500	67.15 - 88.36	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-500-L-10-SR
15	440 - 500	77.75 - 88.36	66.67	10	Porous Glass	Solvent Resistant (SR)	MF-15-500-S-10-SR
15	0 - 120	0.00 - 21.21	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-120-L-2-SR
15	60 - 120	10.60 - 21.21	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-120-S-2-SR
15	140 - 260	24.74 - 45.95	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-260-L-2-SR
15	200 - 260	35.34 - 45.95	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-260-S-2-SR
15	380 - 500	67.15 - 88.36	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-500-L-2-SR
15	440 - 500	77.75 - 88.36	66.67	2	Porous Glass	Solvent Resistant (SR)	MF-15-500-S-2-SR

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ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (um)	Frit Type	Column Type (application)	ELS Part No
20	0 - 120	0.00 - 37.70	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-120-L-10-SR
20	60 - 120	18.85 - 37.70	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-120-S-10-SR
20	140 - 260	43.98 - 81.86	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-260-L-10-SR
20	200 - 260	62.83 - 81.86	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-260-S-10-SR
20	380 - 500	119.38 - 157.08	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-500-L-10-SR
20	440 - 500	138.23 - 157.08	60	10	Stainless Steel	Solvent Resistant (SR)	MF-20-500-S-10-SR
20	0 - 120	0.00 - 37.70	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-120-L-2-SR
20	60 - 120	18.85 - 37.70	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-120-S-2-SR
20	140 - 260	43.98 - 81.86	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-260-L-2-SR
20	200 - 260	62.83 - 81.86	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-260-S-2-SR
20	380 - 500	119.38 - 157.08	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-500-L-2-SR
20	440 - 500	138.23 - 157.08	60	2	Stainless Steel	Solvent Resistant (SR)	MF-20-500-S-2-SR
25	0 - 120	0.00 - 58.90	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-120-L-10-SR
25	60 - 120	29.45 - 58.90	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-120-S-10-SR
25	140 - 260	68.72 - 127.63	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-260-L-10-SR
25	200 - 260	98.17 - 127.63	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-260-S-10-SR
25	380 - 500	186.53 - 245.44	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-500-L-10-SR
25	440 - 500	215.98 - 245.44	50	10	Stainless Steel	Solvent Resistant (SR)	MF-25-500-S-10-SR
25	0 - 120	0.00 - 58.90	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-120-L-2-SR
25	60 - 120	29.45 - 58.90	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-120-S-2-SR
25	140 - 260	68.72 - 127.63	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-260-L-2-SR
25	200 - 260	98.17 - 127.63	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-260-S-2-SR
25	380 - 500	186.53 - 245.44	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-500-L-2-SR
25	440 - 500	215.98 - 245.44	50	2	Stainless Steel	Solvent Resistant (SR)	MF-25-500-S-2-SR
50	0 - 120	0.00 - 235.62	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-120-L-10-SR
50	60 - 120	117.81 - 235.62	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-120-S-10-SR
50	140 - 260	274.89 - 510.51	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-260-L-10-SR
50	200 - 260	392.70 - 510.51	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-260-S-10-SR
50	380 - 500	746.13 - 981.75	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-500-L-10-SR
50	440 - 500	863.94 - 981.75	28	10	Stainless Steel	Solvent Resistant (SR)	MF-50-500-S-10-SR
50	0 - 120	0.00 - 235.62	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-120-L-2-SR
50	60 - 120	117.81 - 235.62	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-120-S-2-SR
50	140 - 260	274.89 - 510.51	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-260-L-2-SR
50	200 - 260	392.70 - 510.51	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-260-S-2-SR
50	380 - 500	746.13 - 981.75	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-500-L-2-SR
50	440 - 500	863.94 - 981.75	28	2	Stainless Steel	Solvent Resistant (SR)	MF-50-500-S-2-SR

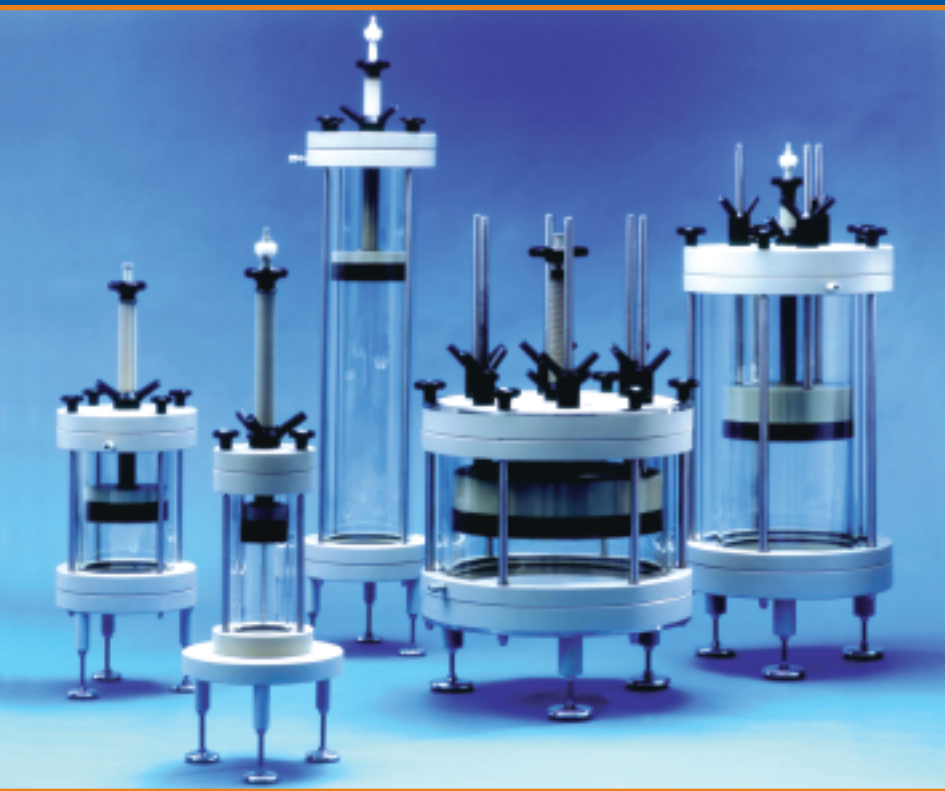
Media-Flex Lab[®] Columns

ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (μ m)	Frit Type	Column Type (application)	ELS Part No
5	0 - 120	0.00 - 2.36	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-120-L-10-AB
5	60 - 120	1.18 - 2.36	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-120-S-10-AB
5	140 - 260	2.75 - 5.11	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-260-L-10-AB
5	200 - 260	3.93 - 5.11	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-260-S-10-AB
5	380 - 500	7.46 - 9.82	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-500-L-10-AB
5	440 - 500	8.64 - 9.82	86.67	10	Polyethylene	Aqueous Buffer (AB)	MF-5-500-S-10-AB
5	0 - 120	0.00 - 2.36	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-120-L-5-AB
5	60 - 120	1.18 - 2.36	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-120-S-5-AB
5	140 - 260	2.75 - 5.11	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-260-L-5-AB
5	200 - 260	3.93 - 5.11	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-260-S-5-AB
5	380 - 500	7.46 - 9.82	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-500-L-5-AB
5	440 - 500	8.64 - 9.82	86.67	5	Polyethylene	Aqueous Buffer (AB)	MF-5-500-S-5-AB
10	0 - 120	0.00 - 9.42	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-120-L-10-AB
10	60 - 120	4.71 - 9.42	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-120-S-10-AB
10	140 - 260	11.00 - 20.42	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-260-L-10-AB
10	200 - 260	15.71 - 20.42	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-260-S-10-AB
10	380 - 500	29.85 - 39.27	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-500-L-10-AB
10	440 - 500	34.56 - 39.27	80	10	Polyethylene	Aqueous Buffer (AB)	MF-10-500-S-10-AB
10	0 - 120	0.00 - 9.42	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-120-L-5-AB
10	60 - 120	4.71 - 9.42	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-120-S-5-AB
10	140 - 260	11.00 - 20.42	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-260-L-5-AB
10	200 - 260	15.71 - 20.42	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-260-S-5-AB
10	380 - 500	29.85 - 39.27	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-500-L-5-AB
10	440 - 500	34.56 - 39.27	80	5	Polyethylene	Aqueous Buffer (AB)	MF-10-500-S-5-AB
15	0 - 120	0.00 - 21.21	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-120-L-10-AB
15	60 - 120	10.60 - 21.21	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-120-S-10-AB
15	140 - 260	24.74 - 45.95	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-260-L-10-AB
15	200 - 260	35.34 - 45.95	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-260-S-10-AB
15	380 - 500	67.15 - 88.36	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-500-L-10-AB
15	440 - 500	77.75 - 88.36	66.67	10	Polyethylene	Aqueous Buffer (AB)	MF-15-500-S-10-AB
15	0 - 120	0.00 - 21.21	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-120-L-5-AB
15	60 - 120	10.60 - 21.21	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-120-S-5-AB
15	140 - 260	24.74 - 45.95	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-260-L-5-AB
15	200 - 260	35.34 - 45.95	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-260-S-5-AB
15	380 - 500	67.15 - 88.36	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-500-L-5-AB
15	440 - 500	77.75 - 88.36	66.67	5	Polyethylene	Aqueous Buffer (AB)	MF-15-500-S-5-AB

ID (mm)	Bed Length min-max (mm)	Volume min-max (ml)	Pressure Limit (bar)	Frit Porosity (um)	Frit Type	Column Type (application)	ELS Part No
20	0 - 120	0.00 - 37.70	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-120-L-10-AB
20	60 - 120	18.85 - 37.70	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-120-S-10-AB
20	140 - 260	43.98 - 81.86	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-260-L-10-AB
20	200 - 260	62.83 - 81.86	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-260-S-10-AB
20	380 - 500	119.38 - 157.08	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-500-L-10-AB
20	440 - 500	138.23 - 157.08	60	10	Polyethylene	Aqueous Buffer (AB)	MF-20-500-S-10-AB
20	0 - 120	0.00 - 37.70	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-120-L-5-AB
20	60 - 120	18.85 - 37.70	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-120-S-5-AB
20	140 - 260	43.98 - 81.86	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-260-L-5-AB
20	200 - 260	62.83 - 81.86	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-260-S-5-AB
20	380 - 500	119.38 - 157.08	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-500-L-5-AB
20	440 - 500	138.23 - 157.08	60	5	Polyethylene	Aqueous Buffer (AB)	MF-20-500-S-5-AB
25	0 - 120	0.00 - 58.90	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-120-L-10-AB
25	60 - 120	29.45 - 58.90	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-120-S-10-AB
25	140 - 260	68.72 - 127.63	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-260-L-10-AB
25	200 - 260	98.17 - 127.63	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-260-S-10-AB
25	380 - 500	186.53 - 245.44	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-500-L-10-AB
25	440 - 500	215.98 - 245.44	50	10	Polyethylene	Aqueous Buffer (AB)	MF-25-500-S-10-AB
25	0 - 120	0.00 - 58.90	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-120-L-5-AB
25	60 - 120	29.45 - 58.90	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-120-S-5-AB
25	140 - 260	68.72 - 127.63	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-260-L-5-AB
25	200 - 260	98.17 - 127.63	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-260-S-5-AB
25	380 - 500	186.53 - 245.44	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-500-L-5-AB
25	440 - 500	215.98 - 245.44	50	5	Polyethylene	Aqueous Buffer (AB)	MF-25-500-S-5-AB
50	0 - 120	0.00 - 235.62	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-120-L-10-AB
50	60 - 120	117.81 - 235.62	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-120-S-10-AB
50	140 - 260	274.89 - 510.51	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-260-L-10-AB
50	200 - 260	392.70 - 510.51	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-260-S-10-AB
50	380 - 500	746.13 - 981.75	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-500-L-10-AB
50	440 - 500	863.94 - 981.75	28	10	Polyethylene	Aqueous Buffer (AB)	MF-50-500-S-10-AB
50	0 - 120	0.00 - 235.62	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-120-L-5-AB
50	60 - 120	117.81 - 235.62	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-120-S-5-AB
50	140 - 260	274.89 - 510.51	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-260-L-5-AB
50	200 - 260	392.70 - 510.51	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-260-S-5-AB
50	380 - 500	746.13 - 981.75	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-500-L-5-AB
50	440 - 500	863.94 - 981.75	28	5	Polyethylene	Aqueous Buffer (AB)	MF-50-500-S-5-AB

Upscale[®] Columns

From Pilot to Process . . .



HIGHLIGHTS:

- Simple Sanitary Design
- Low Dead Volume
- Reliable Mechanical Seal
- Low Maintenance
- Scalable

Advantages

◆ UNIQUE FLOW DISTRIBUTION SYSTEM

The base plate and piston are equipped with an efficient flow distribution system composed of an anti-jetting device and radial distribution channels utilizing a 20 µm frit. This system ensures a uniform flow of liquid onto the surface of the packing material and out of the column, resulting in improved separation performance. The fluid delivery (inlet tube) system connections are made at the exterior of the column, providing a more visible and reliable connection.

◆ PROVEN MECHANICAL SEAL TECHNOLOGY

The mechanical seal provides simple, accurate and repeatable seal adjustment. The sealing systems at each end of the column have been specifically designed to minimize dead volume, which may trap micro-organisms, retain contaminating substances and/or dilute the sample.

◆ EASE OF USE

The column is easily packed with a few simple adjustments of the coarse and fine seal adjustment controls. A large hub and handwheel make bed height adjustment virtually effortless.

◆ LIGHTWEIGHT DESIGN

The piston assembly is made from polypropylene for a lightweight feel. This facilitates assembly and disassembly for the user without unnecessary exertion or reliance on an external lifting apparatus.

◆ PROTECTIVE JACKET FOR TEMPERATURE CONTROL

The acrylic jacket acts as a temperature control unit and as protection for the interior glass from the production environment, thus ensuring the ruggedness of the column assembly, as well as maintaining operator safety.

UPSCALE® COLUMN MATERIALS OF CONSTRUCTION

Materials in Contact with the Solutions

Column Tube:

9 cm, 18 cm, 30 cm
* 45 cm

Borosilicate Glass
Acrylic

Tube Flanges, Base Plate, Piston, Sanitary Connections:

Piston Seal, O-Ring, Sanitary O-Ring:

Frit (20 µm):

Polypropylene
EPDM
Polyethylene

Materials Not in Contact with the Solutions

Jacket:

Tie Rods, Feet, Internal Central Screw:

Polymethacrylate
TP316L Stainless Steel

◆ HASSLE-FREE SCALE UP

The scale up of process from development through pilot scale through production scale is no problem.

◆ FULL RANGE OF SIZES

Upscale® columns are available in standard diameters of 9, 18, 30, and 45 cm and standard lengths of 30, 60, and 90 cm. Custom-made columns of up to 100 cm diameter are also available.

Upscale® Process Columns

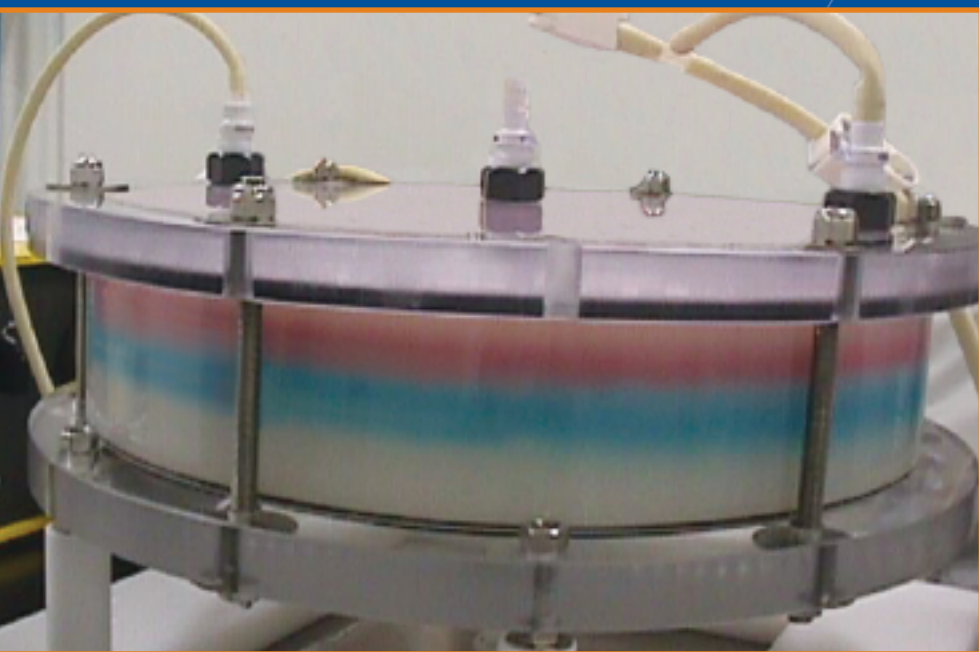


Dimensions (cm)	Pressure (bar)	Volume/ml Min/Max (L)	Bed Height Min/Max (cm)	Liter (cm)	ELS Part No
9 x 30	6	0.0 - 1.6	0 - 25	0.06	265111
9 x 60	6	0.9 - 3.5	15 - 55	0.06	265117
9 x 90	6	2.9 - 5.4	45 - 85	0.06	265118
18 x 30	4	0.0 - 6.4	0 - 25	0.25	265121
18 x 60	4	3.8 - 14.0	15 - 55	0.25	265127
18 x 90	4	11.5 - 21.6	45 - 85	0.25	265128
30 x 30	3	0.0 - 14.8	0 - 21	0.71	265131
30 x 60	3	7.8 - 36.0	11 - 51	0.71	265137
30 x 90	3	28.9 - 56.2	41 - 81	0.71	265138
*45 x 30	2	0.0 - 33.4	0 - 21	1.59	265141
*45 x 60	2	17.1 - 79.3	11 - 51	1.59	265147
*45 x 90	2	63.7 - 128.8	41 - 81	1.59	265148

*45 cm diameter available in acrylic only.

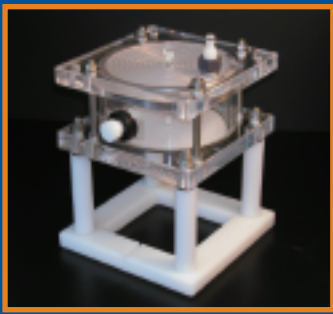
IsoKrom™ Columns

Economical/Sanitary
Process Scale Technology



HIGHLIGHTS:

- All wetted parts are either autoclavable, disposable and/or can be completely taken apart for thorough cleaning.
- Fixed volume, packing through the wall.
- Reduced dead-space by volume (V) and length (L).
- Simple & Robust - IsoKrom™ is designed around standard industrial parts, which lowers the price and assures quality.
- No metal parts contact the solution.



IsoKrom™ Columns

Acrylic columns for preparative low-medium pressure bio-chromatography available from ISO 9000 certified manufacturer

"In a multi-product environment, we install new mesh for each new product run to prevent carry-over contamination. In contrast to IsoKrom, frit replacement in other large columns is expensive, laborious and/or requires factory service".

J. Burton Lee, President,
Lee BioSolutions Inc., MO

"Processes have never been this robust with other systems...[IsoKrom] allows a much faster cycle time for a column run. Production personnel really appreciate when different operators, on different days and with different batches, can consistently reproduce the performance".

Fred Drewe, Ph.D, President,
Drewe Browning Strickler, TX

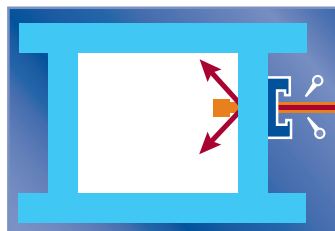
- Improved column sanitation with a packed bed in (CIP) as well as in a disassembled state.
- Inexpensive mesh replacement eliminates potential carry-over of sorbent and contaminants.
- No packing valves blocking the mesh, no stagnant pockets. Uniform unobstructed flow through the entire bed.
- Shorter dead-space translates into reduced clearance time ($=L^2$), and/or a more thorough CIP.
- Shortended time for column order delivery. Standard parts (Acrylic tube, Nylon mesh, etc.) are available on short notice.
- Due to lower column cost, each purification step can afford a separate dedicated column, with no need to clean and validate re-packing.
- No issues with rusting at low pH and/or at high Chloride, EDTA and Citrate concentration.

AVAILABLE STANDARD SIZES*

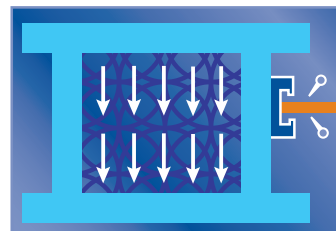
Tube ID (cm)	Volume (L) at 20-cm bed
9.5	1.4
13.3	2.8
22.9	8.2
33.0	17.1
47.0	34.7
66.0	68.5
79.0	98.0
102.9	166.2
177.8	496.6
238.8	895.5

*Custom sizes available upon request.

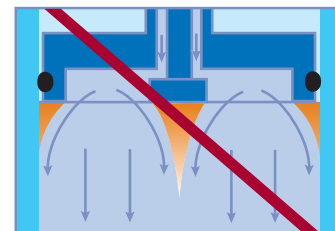
IsoKrom™ Columns



Pack sorbent slurry into precise fixed volume using sidepacking valve.



Packing valve is retracted flush with the wall eliminating dead volume in column.



Other columns employ center packing valve which creates dead volume.

- All wetted parts are either autoclavable, replaceable and/or can be completely taken apart for thorough cleaning.
- No metal parts contact the solution: no issues with rusting at low pH and/or at high NaCl concentration.



essentialLife Solutions

Upscale® Column Manual

Revision 2.0

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and initial inspection

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II.3 - Base sub-assembly
II.4 - Piston sub-assembly
II.5 - Flow distributor
II.6 - Connectors and tubing

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III.2 - Packing
III.3 - Cleaning in place

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I - INTRODUCTION

Essential Life Solutions Upscale® glass columns are designed to meet the requirements for large scale purification of biological molecules using low pressure liquid chromatography techniques.

Upscale® columns consist of a borosilicate glass tube protected by an acrylic jacket, a base plate and an adjustable piston. The piston range allows the user to select a variety of column heights and volumes.

The base plate and the piston are equipped with a flow distributor system, composed of an anti-jetting device and radial distribution channels equipped with a 20 µm (standard) frit.

Upscale® columns feature an efficient flow distribution system which ensures uniform flow of liquid onto the surface of the packing material and out of the column.

The sealing systems at the lower and upper portion of the column have been designed to minimize dead volume which may trap micro-organisms, retain contaminating substances and/or dilute the sample. All connections have a sanitary design.

The column is easily packed with few simple adjustments.

This piston range is set and pressure is applied on the upper seal by simply operating two handles, which reduces the time required for set-up of the column.

Upscale® columns are composed of biocompatible materials and withstand solutions commonly used in low pressure liquid chromatography (acidic and basic solutions, detergents, polar organic solvents).

I.1 – Removing the column from packaging – Initial inspection

Upon receipt of the column, check that all parts are included (see detail of packaging content in appendix 9) and that there has not been damage to the column during shipment.

Columns are shipped with piston seal compressed and on 300 and 450 mm dia. Columns, pressure rod handles are clamped. Unclamp pressure rod handles and decompress piston seal by turning handles fully counter clockwise, then adjust piston height to remove from package (see figure 1).

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II - GENERAL DESCRIPTION

II.1 - THE COLUMN (figure 1)

The design and the materials of construction are similar for all column diameters and heights.

The geometric features of Upscale® glass columns are summarized in table I.

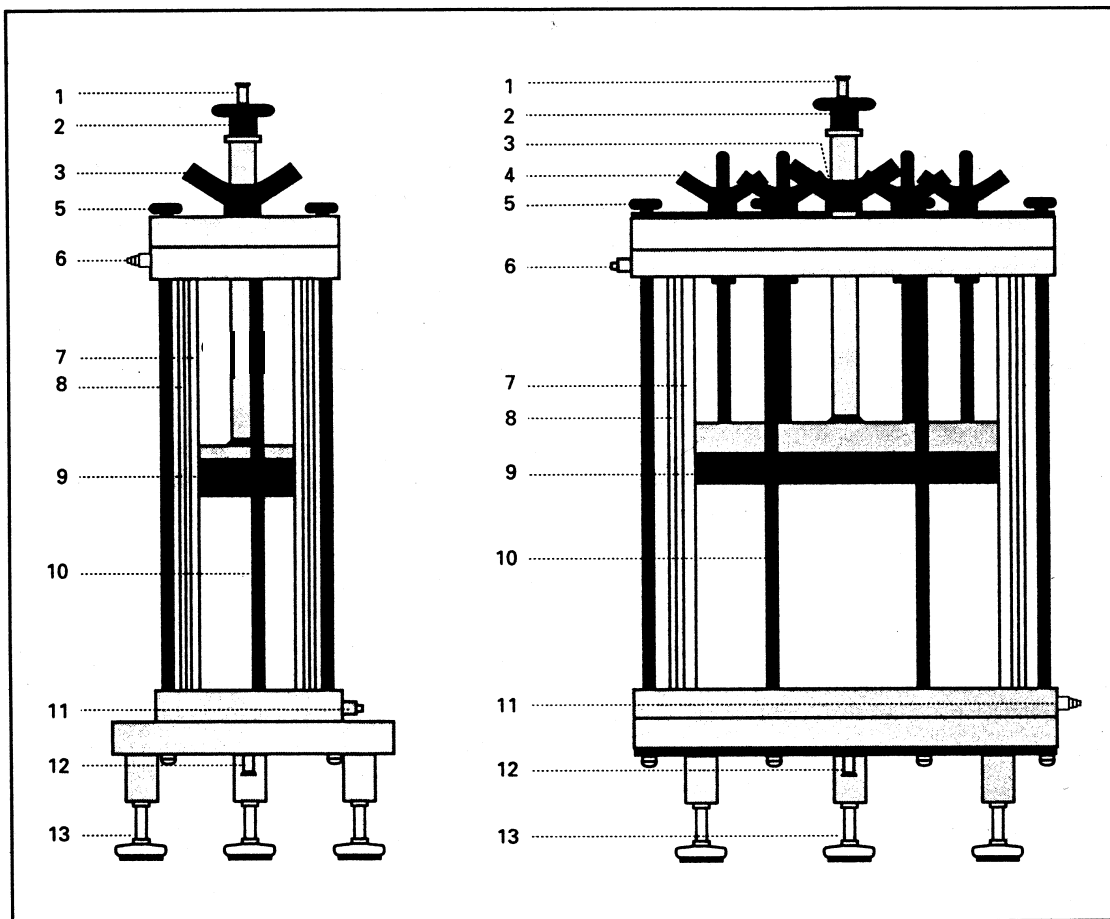


Figure 1: General Description of Upscale® Columns

- | | |
|---|---|
| 1 - Sanitary connection (column inlet) | 7 - Column tube (borosilicate glass) |
| 2 - Seal actuation hand wheel | 8 - Acrylic protective jacket |
| 3 - Bed height adjustment handle | 9 - Adjustable piston-to-tube seal (EPDM) |
| 4 - Pressure rod clamp handle
(only for 300 and 450 mm dia. columns) | 10 - Tie rod column tube unit |
| 5 - Piston / tie rod nuts | 11 - Heat exchange port |
| 6 - Heat exchange port | 12 - Sanitary connection (column outlet) |
| | 13 - Adjustable foot |

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Table I: Geometric features (1) of Upscale® Columns

Part No.	Column	I.D. (mm)	Max. Useful Length (mm)	Piston Stroke (mm)	Volume (L)
265111	90 x 300	90	250	250	0 - 1.6
265117	90 x 600	90	550	300	.9 - 3.5
265118	90 x 900	90	850	300	2.9 - 5.4
265121	180 x 300	180	250	250	0 - 6.4
265127	180 x 600	180	550	300	3.8 - 14
265128	180 x 900	180	850	300	11.5 - 21.6
265131	300 x 300	300	210	210	0 - 14.8
265137	300 x 600	300	510	300	7.8 - 36
265138	300 x 900	300	810	300	28.9 - 56.2
265141	450 x 300	450	210	210	0 - 33.4
265147	450 x 600	450	510	300	17.1 - 79.3
265148	450 x 900	450	810	300	63.7 - 128.8

(1) Essential Life Solutions may at times change the features of the columns described in this manual without notice.

(2) 1 inch = 25.4mm

The available diameters and tube heights and the adjustable piston range allows selection of the optimal volume and geometry for the chromatographic process.

The running conditions for Upscale® glass columns are detailed in appendix 1: *Operating specifications*.

Scaling up is simplified because flow distribution efficiency is maintained for all column sizes. Chemical compatibility and manipulation operations are identical across the range of sizes (see appendix 1: *Operating specifications*, and appendix 3: *Chemical resistance*).

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Upscale® columns consist of three sub-assemblies (fig. 2):

- the column tube
- the piston with adjustable stroke
- the base assembly

Assembly and disassembly of the column into these three sub-assemblies for routine maintenance is easy. The protocol is detailed in section III.1.

NOTE: It is not recommended to disassemble the sub-assemblies (e.g. to remove the jacket or the central screw of the piston). The sub-assemblies are set by the manufacturer and do not require maintenance. Essential Life Solutions should be consulted for any operation which is not detailed in this manual.

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II.2 - THE COLUMN TUBE SUB-ASSEMBLY

The column tube sub-assembly (glass tube + jacket) consists of two polypropylene flanges connected with stainless steel tie rods which must not be disassembled.

Upscale® columns feature an acrylic jacket which protects the glass tube. The jacket also allows the user to circulate heat exchange fluid between the jacket and glass barrel.

Each flange of the tube sub-assembly is equipped with a threaded hose barb connector for 6 mm internal diameter flexible tube. The thread is ¼" gas Whitworth straight pipe thread. These connectors allow the column to be connected to a cooling/heating circuit. **Pressure in the jacket should not exceed 0.5 bar (7.25 psi).**

The bottom flange carries two o-rings which with o-rings in place provide the seal with the base plate and frit.

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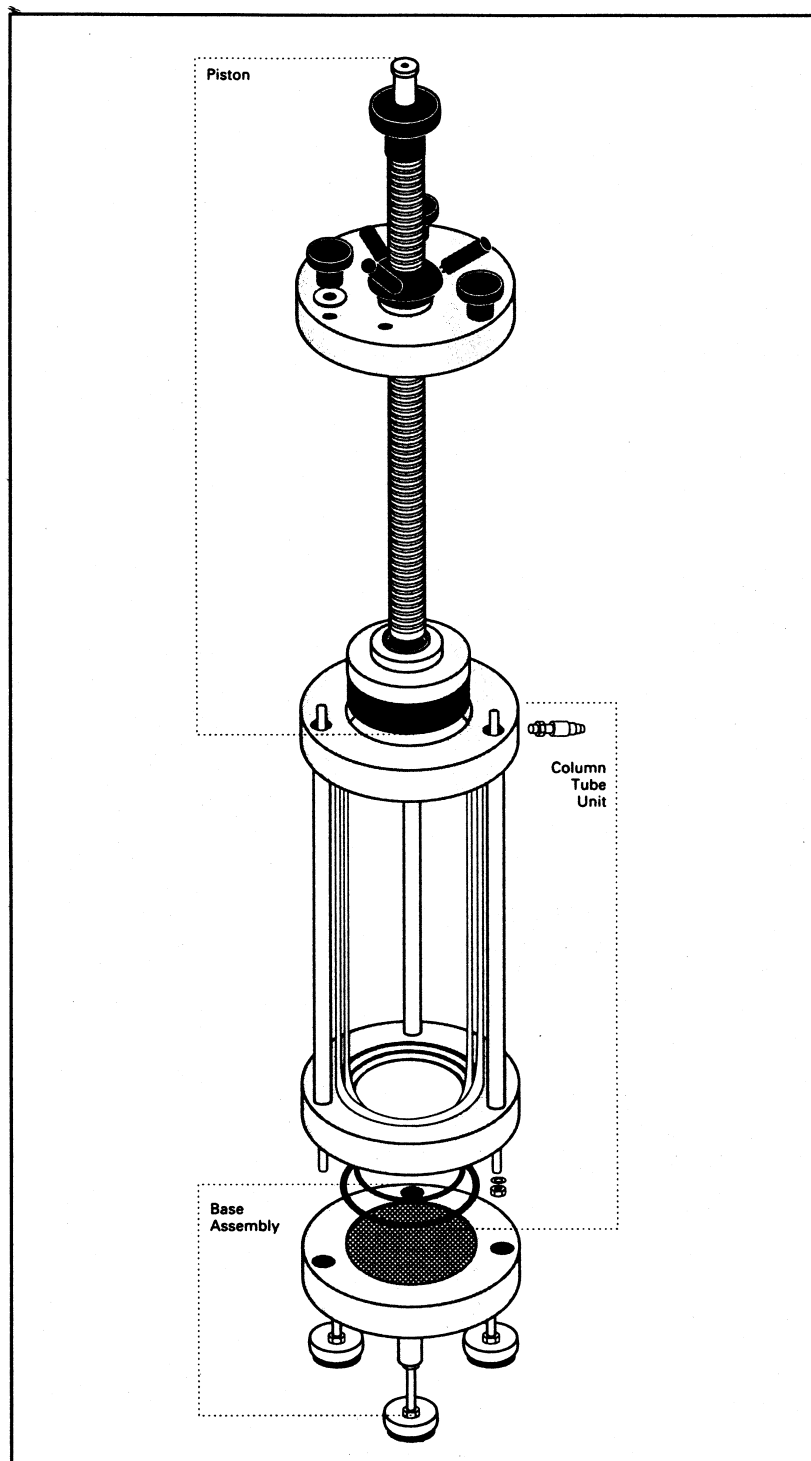


Figure 2: Main sub-assemblies of the Upscale® columns

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II.3 - BASE ASSEMBLY

The base plate (fig. 3) of Upscale® glass columns contains a flow distributor topped by a polyethylene frit. The frit diameter is slightly larger than that of the glass tube and both are sealed with o-rings. Therefore there is minimum dead volume in the lower part of the column. The base plate is fixed to the column tube sub-assembly via the extending stainless steel tie rods of the tube sub-assembly.

The adjustable column feet are fixed on the base plate. A bubble level is supplied so that the column can be easily adjusted to the vertical position. The heights of the individual feet can be adjusted by first loosening the 19 mm jam nut adjacent to the polypropylene stand off and then turning the 19 mm head of the leveling bolt adjacent to the foot pad. Retighten the jam nut when adjustments are complete. Adjustable locking column stabilization assemblies can be provided for the 300 and 450 mm diameter as optional accessories.

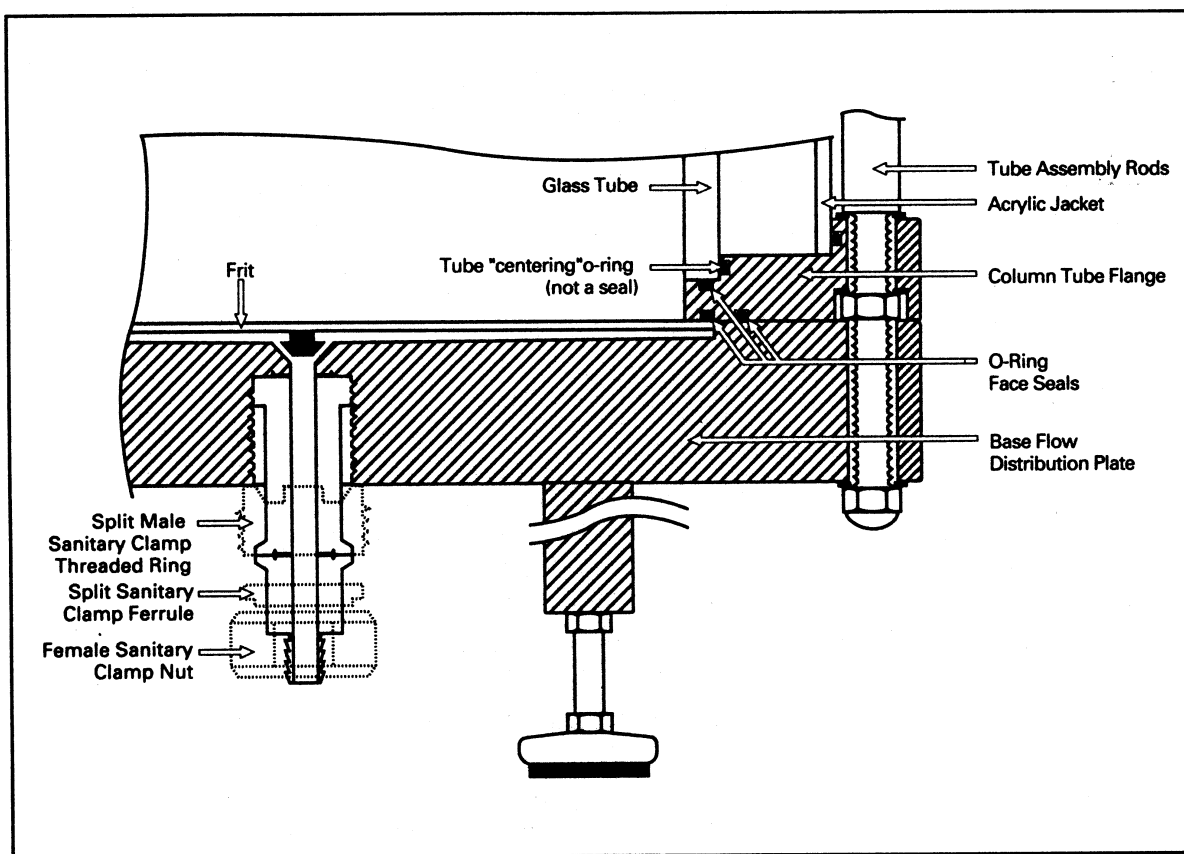


Fig. 3 : Base assembly

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II.4 - PISTON SUB-ASSEMBLY

CAUTION: Do not attempt to adjust piston height before pressure rod clamps (300 and 450 mm columns only) have been disengaged and the adjustable piston-to-tube seal has been loosened.

The piston (fig. 4) allows the user to adjust the column bed height and volume (table I).

It is easily introduced into the column tube and is then fixed in place with hand tightened nuts on the stainless steel tie rods. The piston is raised or lowered in the column with the bed height adjuster handle.

The piston to glass barrel seal is obtained by compressing the piston seal actuated by clockwise rotation of the handle located on the end of the central screw. The descending motion during actuation of the seal is designed to eliminate dead volume. This reduces sample dilution, band broadening, accumulation of contaminating products and bacterial proliferation.

On large diameter columns (300 and 450 mm), additional stainless steel pressure rods link the flow distributor of the upper plate of the column. The rods are disengaged from the upper plate during movement of the piston and should be engaged when the final position of the piston has been reached. The pressure rods are engaged and disengaged by hand-tightening the pressure rod handles.

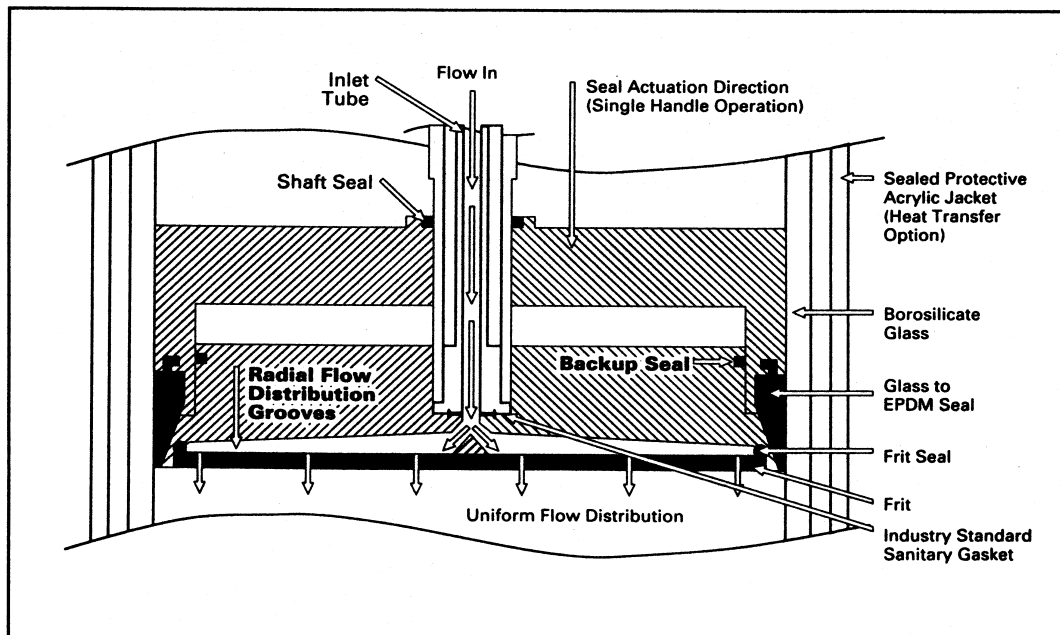


Fig. 4 : Cross section of piston flow adapter assembly

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II.5 - FLOW DISTRIBUTOR (figure 5)

The two (top and bottom) flow distributors are identical except for a slightly larger diameter on the bottom. The column may be used in ascending as well as in descending flow direction.

The flow distribution system consists of :

- an anti-jetting device
- radial distribution channels
- 20 um (standard) polyethylene frit

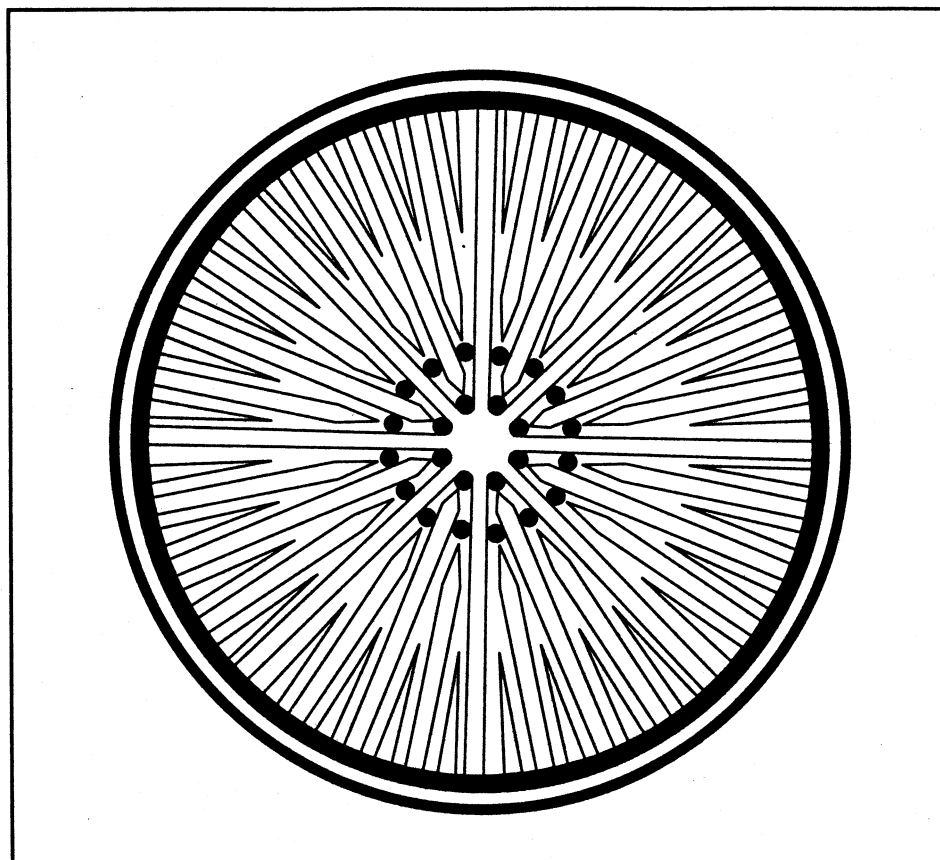


Figure 5: Upscale® flow distribution system

The design of the grooved radial distribution channels minimizes dead volume under the frit and optimizes flow distribution.

The polyethylene frit provides the support for the column packing material and increases efficiency of flow distribution by inducing a slight pressure drop between the anti-jetting device and

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the packing material. An o-ring has been placed around the frit in order to prevent channeling of flow around the frit.

The polyethylene frits should be handles with care to maintain cleanliness.

Wetting the frits with 20% EtOH is strongly recommended before operation!

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II.6 - CONNECTORS AND TUBING

The column inlet and outlet are equipped with sanitary connections (fig. 6), which are free of points of retention and are in accordance with international sanitary standards.

The following tubing is recommended to minimize back pressure and reduce contamination:

- ~ for 90, 180 mm dia. columns : 6mm (=1/4") I.D. tubing with sanitary clamp connection
- ~ for 300, 450 mm dia. columns : 9.5 mm I.D. (3/8" I.D.) tubing with sanitary clamp connection

Each column is supplied with **2 clamps and 4 sanitary gaskets**, as well as **2 sanitary flanges with barbed fitting connectors** for tubing as above.

The sanitary flanges with barb fitting provides connection of the column to other accessories, which may not have sanitary flanges, via flexible tubing.

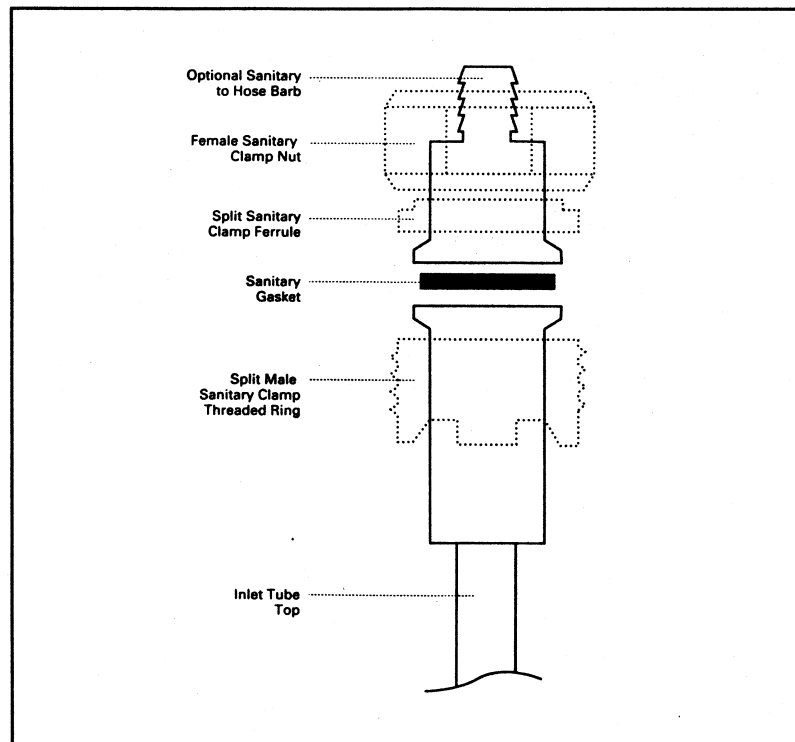


Fig. 6 : Sanitary connection

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III – METHOD OF USE

III.1 - PISTON AND BASE ASSEMBLY / FRIT REPLACEMENT

III.1.1 – Column disassembly

Follow the instructions below to inspect and wash the three column sub-assemblies:

1) Piston removal:

- Decompress the piston seal:
 - * Check that no pressure is applied in the column.
 - * Unscrew the seal handwheel.

- Remove the piston from the column tube after unscrewing all nuts on the tie rods. For 300 and 450 mm diameter columns, to simplify handling of the frit, it is recommended to turn the column piston over and rest it upside down on the pressure rods.

- CAUTION: Be sure pressure rod handles are clamped. A tool is provided to remove frit retaining ring. Remove frit.

2) Base plate removal :

- Remove the base plate by unscrewing the blind nuts at the bottom of the tie rods. Lift column tube assembly off base and gently set it on tie rods. Remove frit.

- Wash all the parts with a non-abrasive detergent or a diluted acid or basic solution (0.5 N) then rinse with demineralized water.

III.1.2-Column assembly

1) Installing the polyethylene frits :

The frits should be kept in their individual packaging until use to maintain cleanliness. After removing this packaging, the frits should be handled with care in order to prevent damage or contamination. The largest diameter frit is inserted into the flow distributor of the base plate. The frits should be wetted before running the column. Essential Life Solutions recommends 20% EtOh

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(Ethanol) wetting agent. Frits should be immersed, if possible. The smaller diameter frit of the piston distributor is inserted after ensuring that the o-ring is correctly installed in its groove around the flow distribution cell. A retainer clip is supplied to maintain the piston distributor frit in position.

- 2) Place the base plate equipped with its frit on the spot where the column will be filled or will be running, since its is not recommended to move large columns once packed. Inspect the two o-rings on the bottom of the column tube. Put the column tube (o-ring side down) on the base plate and fix the whole unit by screwing the acorn nuts symmetrically on the tie rods. Be sure to include the washers.
- 3) Introduce the piston into the column tube. The unit (piston and column tube) is fastened by hand tightening the nuts on the tie rods. Connect the column to the fluid pumping system (see Appendix 4). Make the axis of the column tube plumb (in the vertical position) by adjusting the individual foot heights and using the level bubble.

Practical column packing recommendations are given in III.2.2.

III.2 - PACKING THE COLUMN

III.2.1. - Introduction

Proper column packing is critical factor in successful chromatography. The use of a good packing procedure will avoid the creation of heterogeneous areas in the packed bed and gradients of bead size from the top to the bottom of the column.

The protocols described below are valid for semi-rigid and rigid supports such as TRISACRYL R and SPHERODEX R supports, and for non-rigid supports such as ULTROGEL R.

III.2.2 - Packing protocol for rigid or semi-rigid supports (e.g. TRISACRYL R and SPHERODEX R)

- 1) Transfer the chromatographic support into a large container. After the gel has settled, remove the excess liquid above the gel.

CAUTION : In case sodium azide is present in the supernatant of the chromatographic support, the pH of the 1 M NaCl solution should not be lower than pH 5.5!

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- 2) Add a 1 M NaCl, pH 7 solution until the total volume reaches 125 – 140 % of the settled gel volume (e.g. 10 liters of settled support mixed with 2.5 – 4 liters of solutions). Additional liquid can be added if needed for ease in transferring.
- 3) Stir the suspension manually until it is homogeneous, making sure that no air bubbles appear, and that the suspension remains fluid (do not allow the support to settle).
- 4) Remove air trapped in the bottom flange of the column by one of the methods 4a or 4b below :

4a – While the outlet valve is closed, introduce about 0.3 column volume of a 1 M NaCl, pH 7 solution into the column (fig. 7A). Open the outlet valve and allow the liquid to flow until only 0.1 column volume remains (fig. 7B and 7C). Close the outlet valve.

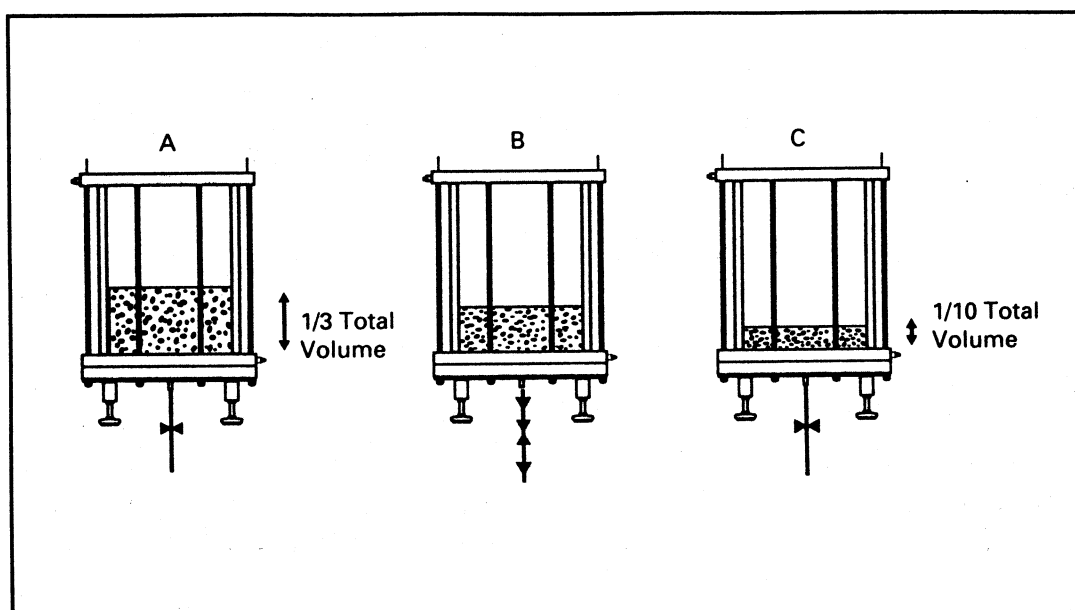
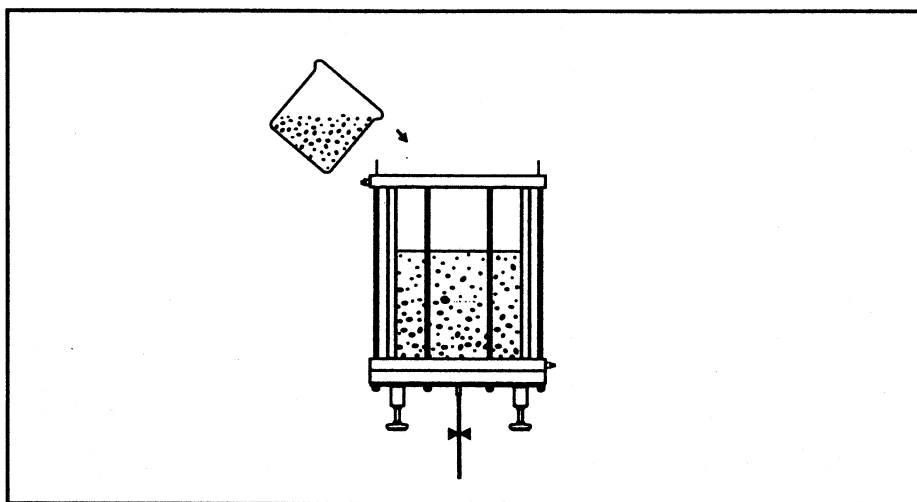


Fig. 7 : Preliminary operations before introducing the suspension :
7A : Introduce 1/3 of total column volume (1 M NaCl, pH 7).
7B : Remove the excess of solution and air bubbles.
7C : Maintain about 0.1 column volume and close the outlet.

4b – Connect the outlet valve to a pump and fill the column with the 1 M NaCl, pH 7 solution until it reaches 1/10 of the column volume. Close the outlet valve and disconnect the pump.

5) While the outlet valve of the column is closed, gently pour the homogeneous suspension of the chromatographic support down the side wall of the column to avoid trapping air (fig. 8A).



**Fig. 8 : Packing the column :
Gently pour along side of glass tube**

- 6) Once the suspension has been totally transferred, stir again manually until the suspension becomes homogeneous, being careful to avoid contact with the column wall and air entrapment.
- 7) Introduce the piston while the inlet valve of the column is opened (fig. 9A). During this operation, allow the excess buffer to discharge from the column and remove air bubbles from the piston (by tilting piston) as the piston is lowered into the column (fig. 9B). Fix the piston on the column tube using the nuts provided. Lower the piston gradually to the appropriate height.

NOTE : pressure rod handles must be turned fully counter clockwise on 300 and 450 m diameter columns before adjusting piston height.

- 8) When the piston contacts the suspension upper surface, tighten the piston seal. Lock pressure rod handles fully clockwise on 300 and 450 mm diameter columns.
- 9) At this point, close the inlet valve, connect a bubble trap to the column inlet (optional) downstream of the feed pump (fig. 9C). Make sure to keep air bubbles out of the column.

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- 10) Open the inlet and outlet valve of the column and start the pump at a moderate flow rate with 1 M NaCl pH 7.0. The pump flow rate should be continuously adjusted to generate a pressure of 0.2 – 1 bar (3-14.5 psi), at the inlet of the column (fig. 9D). Alternatively a flow rate 1.3 times the highest process flow is recommended. When the height of the bed is stabilized, stop the pump and close the outlet valve.

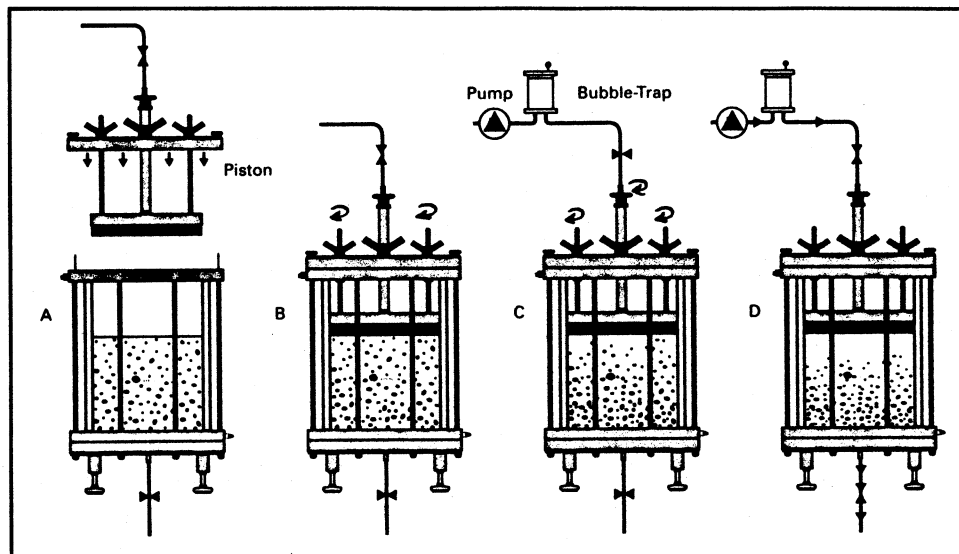


Fig. 9 : Piston installation :

- 9A :** Introduce the piston. Tilt piston to remove air bubbles, if necessary.
9B : Lower the piston on the gel suspension (inlet valve opened).
9C : Compress the piston seal, connect bubble-trap (optional) and pump.
9D : Pack with pump (inlet and outlet opened). Use process flow rate.

- 11) Back-off compression of the piston seal (turn piston seal actuation handwheel counter clockwise) (and pressure rods on 300 and 450 mm diameter columns) leaving the seal lightly in contact with the glass column wall, and lower the piston gradually. When the frit contacts the surface of the packed chromatographic support, tighten the piston seal (fig. 10) (and pressure rods).

NOTE : To fully pack the column, allow for a large quantity of 1 M NaCl, pH 7 solution (10 column volumes minimum).

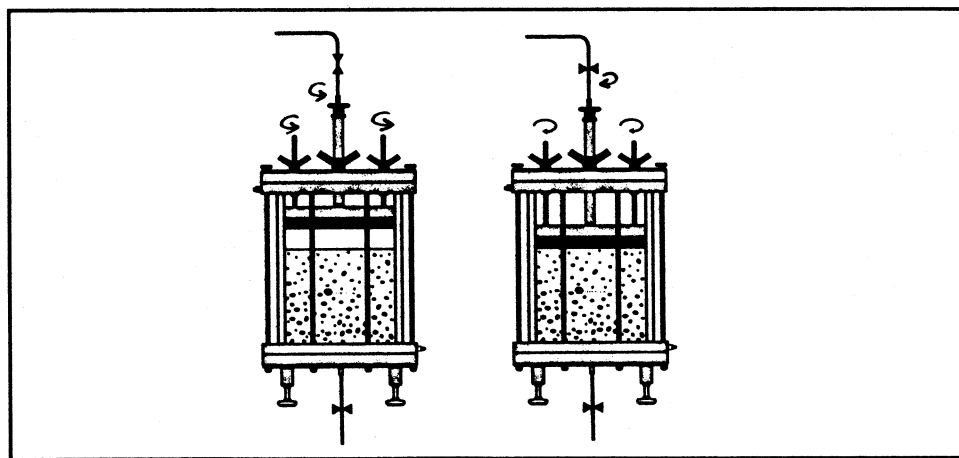


Fig. 10 : Final adjustment of the piston on the surface of packed gel

- 1) Open the inlet valve of the column and start the pump. The packed column is now ready for operation.

CAUTION : pH should never be lower than 5 – 5.5 as long as sodium azide is present in the storage buffer.

III.2.3 - Packing procedure for non-rigid supports (e.g. BioSeptra ULTROGEL R AcA and A, HA-ULTROGEL R)

Follow instructions detailed in section III.2.2 from point 1 to point 6, then allow the support to settle. It is recommended to open the outlet valve to allow flow, which speeds up the bead sedimentation, and prevents them from classifying according to particle size. Then follow the instructions of section III.2.2 from point 7 to point 12, referring to the maximum packing pressure for the support used.

III.2.4 – Packing evaluation

Chromatographic efficiency is dependent upon the packing procedure and particle size. The column packing method can change efficiency, resolution, peak tailing and / or fraction dilution.

A simple method for the evaluation of packed bed quality (appendix 5) to determine the

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height equivalent to a theoretical plate (HETP) of the support.

III.3 – CLEANING

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 example in appendix 6).

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 III.2) 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 um filters in-line at column inlet and outlet.

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TABLE II: Cleaning procedures according to the nature of the absorbed material to be eliminated.

TREATMENT	REGENERATION (elution of strongly absorbed molecules)	STERILIZATION	DEPYROGENATION
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	Hydrolysis of adsorbed proteins	Ineffective	Ineffective
Pepsin treatments pH 1.5-2	Hydrolysis 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	"	Ineffective	Possible elimination of pyrogens
Non ionic detergents in acidic solution pH~3 (acetic acid)	"	Ineffective	Possible elimination of pyrogens
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 hydroxide + 0.1-1 M hydrochloric acid	Removal of various small charged molecules and pigments	Ineffective	Elimination of pyrogens
0.1-1 M sodium hydroxide	Removal of bound hydrophobic proteins, lipopolysaccharides and other unknown contaminants	Effective	Elimination of pyrogens
60% ethanol, 0.5-1 M acetic acid	Elimination of lipids, pigments, lipopolysaccharides and other lipophilic substances	Very Effective	Elimination of pyrogens
50-80% acetic acid	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
60% ethanol, 0.1 M and 1 M HCl	Elimination of various charged molecules and lipids	Unknown	Elimination of pyrogens

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IV - MAINTENANCE

Columns have a simple design utilizing few parts, for simple cleaning and maintenance.

It is recommended to inspect the frits regularly and to wash them with detergent at each packing operation.

The flow distributors and the internal surface of the glass tube should be washed with detergent, then rinsed with demineralized water. No abrasive should be used on glass.

To maximize frit lifetime, it is recommended to filter all solutions pumped into the column (e.g. 0.2 μ m for buffers and 0.45 μ m for protein solutions). A clogged frit may give loss of resolution, capacity and efficiency. If this occurs, it is necessary to wash the frit as soon as possible, or to replace it. If the pressure increase caused by the frit clogging is not too high (lower than the mechanical resistance of the column or support), it is possible to continue the run by decreasing the flow rate.

The seals of the column (two o-rings on the bottom flange of the glass tube, one o-ring in the piston flow distributor) should be regularly inspected and should be replaced if damaged.

V – WARRANTY

Essential Life Solutions offers a one- year limited guarantee on these columns covering defects in material and workmanship if used under conditions detailed in the instruction manual provided with the column. This guarantee does include frits, o-rings and glass tube.

Appendices

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APPENDIX 1- SPECIFICATIONS

* **Temperature Range:** +4 Deg C to + 40 Deg C (+39 Deg F to + 104 Deg F)

* **Pressure Range:**

Column Diameter:	mm	9	180	300	450
	inches	3.5	7	11.8	17.7
Working Pressure:	bar	6	4	3	2
	psi	87	58	43	29
Test Pressure:	bar	9	4.5	3.5	2.25
	psi	130	65	51	32

* 1 inch = 25.4 mm

** 1 bar = 14.5 psi

*** Working pressure = max. pressure continuously applied

**** Test pressure = max. pressure applied for quality control

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APPENDIX 2 - MATERIALS OF CONSTRUCTION

Materials in contact with the solutions:

*Borosilicate Glass.....Column Tube

Note: Acrylic column tubes available on request.

*Polypropylene.....Tube Flanges
Base Plate
Piston
Sanitary Connections

*EPDM.....Piston Seal
O-Ring
Sanitary O-Ring

*POLYETHYLENE.....Frits

Materials not in contact with the solutions:

*POLYMETHACRYLATE.....Jacket
*TP316L STAINLESS STEEL.....Tie Rods
Feet
Internal Central Screw

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APPENDIX 3 - CHEMICAL RESISTANCE

The Materials in contact with the solution are suitable for use with the following substances:

- *Acetic acid 50%
- *Hydrochloric acid 1N
- *Sodium hydroxide 1 N
- *Pure ethanol

NOTE:

Due to the wide range of solutions available please contact Essential Life Solutions prior to the purchase of your column.

APPENDIX 4 - SYSTEM CONFIGURATION EXAMPLES

*MAIN COMPONENTS OF A CHROMATOGRAPHIC SYSTEM

The hydraulic environment of a column includes:

- The feed pump, the outlet of which may be equipped with a purge valve and a pressure probe if required. The pressure measurement monitors the packing and purification operations, and in some cases is used to stop feed the pump as soon as the maximum level of pressure has been exceeded.
- The filtration system which prevents the introduction of impurities into the pump. The buffer solutions are generally filtered in line, whereas the samples must be previously clarified before their injection onto the column. The filtration system is connected to a set of valves (1 four-way valve and 2 three-way valves) either in line when the buffers are introduced into the column, or by-passed when the sample is injected.
- The bubble-trap system which eliminates introduction of air bubbles into the column, thus prevents the support from drying. The bubble-trap is by-passed when the sample is injected, in order to prevent diluting the sample.
- A four-way valve selects the direction of the fluids into the column (ascending or descending flow).
- A four-way or three-way valve connecting the column outlet to waste or fraction collection.

Several detectors at the column outlet allow the user to follow the purification (UV absorbance detector, conductivity and pH-meter).

Before the pump, a valve manifold allows user to select the solution to be injected into the column.

The valves and the pump are either manually or automatically operated. The system may be equipped with many probes providing greater safety (shutdown based on pressure limits, fluid levels or air trapped in the system).

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***EXAMPLE**

Fig. 11 shows an installation using a desalting column. This type of support allows the separation of macromolecules from low molecular weight substances, or to equilibrate a sample in the buffer of a determined composition.

The first step (fig. 11A) consists of equilibrating the support in a buffer with appropriate ionic strength and pH. The bubble-trap and the filters are in line and the outlet is connected to waste.

The sample is loaded in the second step (fig. 11B). The bubble-trap and the filter are by-passed. Collection starts as soon as the UV peak appears at the column outlet (the detectors are not shown in this diagram).

The column is then washed with the equilibration buffer through the bubble-trap and the filter (fig. 11C). Collection continues until the UV signal has reached the baseline.

In a final step (fig. 11D), the column is regenerated and / or sterilized and / or depyrogenated (see section III.3). This operation is not necessarily performed after all cycles.

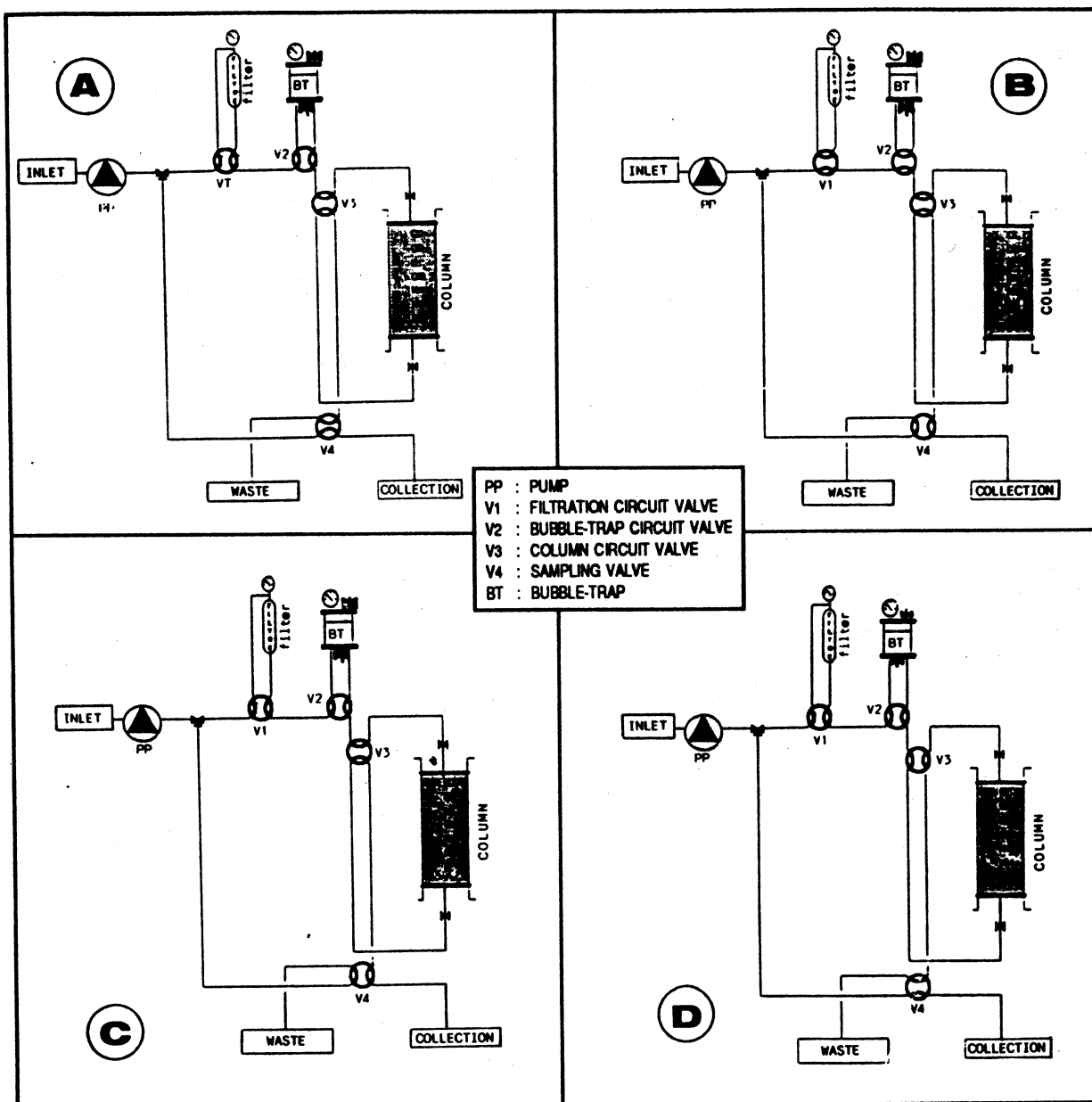


Fig. 11 : Examples of use : desalting

- 11A : Equilibration with buffer No. 1 (column protected by bubble-trap and filter)
- 11B : Sample injection (bubble-trap and filter by-passed) and beginning of collection
- 11C : Column washing and end of collection (column protected by bubble-trap and filter)
- 11D : Regeneration (option of ascending flow)

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APPENDIX 5 - EVALUATION OF THE PACKED COLUMN EFFICIENCY

This evaluation is made by injecting a sample into the column and by following its elution, generally by conductivity or UV absorbance.

*PROTOCOL:

- Equilibrate the chromatographic support in an appropriate buffer (e.g. 0.05 M Tris-HCl, pH 7.4 buffer for gel filtration or ion exchange supports such as DEAE, QA supports, or 0.05 M, pH 5.5 acetate buffer for ion exchange supports such as COOH or SP supports).
- Inject, as close as possible to the support (avoiding bubble-trap) a sample which will not interact with the support (e.g. 2.5% column volume of a sample constituted of 2% acetone in the buffer).
- Record the elution peak at the column outlet (UV absorbance at 280nm).
- Calculate the HETP using the following formulae:

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

$$N = 16(\text{Ve}/W)^2 \quad N = 5.54(\text{Ve}/\text{WO5})^2$$

L = Column height (cm)

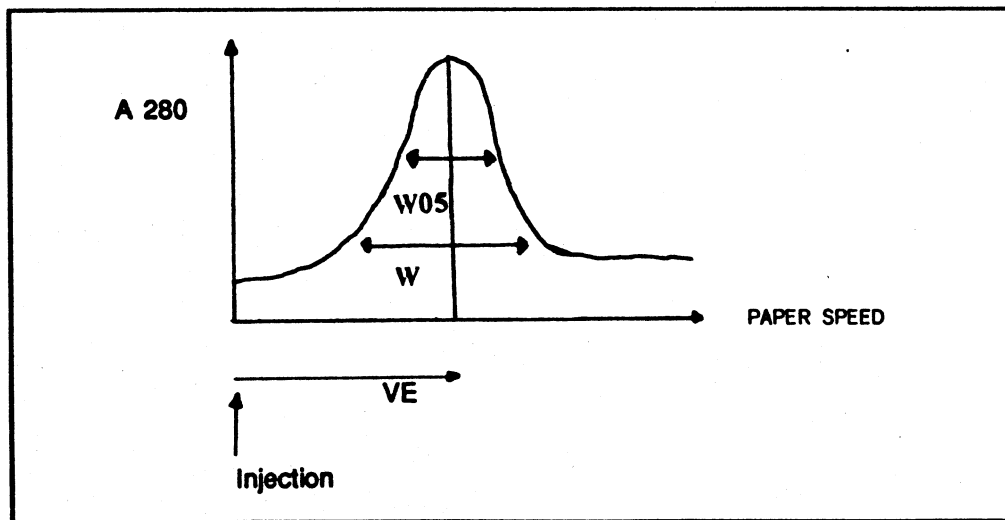
N = Number of theoretical plates

Ve = Elution volume (l)

W = Peak width at the bottom (l)

WO5 = Half of the peak width (l)

Ve, W, WO5 must be expressed in the same unit (i.e. volume, time, length).



The lower the HETP value is, the better the packing quality is.
HETP standard values are supplied for each type of chromatographic support.

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APPENDIX 6 - EXAMPLE OF A STERILIZATION / DEPYROGENATION METHOD

In the following example, DEAE TRISACRYL PLUS chromatographic support is depyrogenated and sterilized in a 40% ethanol – 0.2 N sodium hydroxide solution.

The passage of the solution is performed at a flow rate of 1 column volume per hour, during three hours. The resin is then rinsed with a concentrated (0.5 M Tris-HCl) buffer before being reequilibrated in the appropriate buffer.

The control of depyrogenation is followed by sampling at the column outlet (analysis by LAL test). The sensitivity of the test is 0.0125 EU/ml.

CAUTION : The 40% ethanol – 0.2 % sodium hydroxide solution is not compatible with phosphate buffers. It is necessary to insert a wash step between these two solutions, in order to avoid a saline precipitation on the column.

~ Experimental conditions:

- ~ Column volume.....3.6 liters
- ~ Height570 mm
- ~ Diameter.....90 mm

~ Injected solutions:

- ~40% ethanol – 0.2 N NaOH solution, for 3 hours at 57 cm.hour
- ~0.5 M Tris-HCl, pH 7 buffer, for 15 min. at 114 cm/hour
- ~0.05 M Tris-HCl, pH 7 buffer, for 1 hour at 114 cm/hour

~ Results:

Results are detailed in Table III and in Fig. 12.

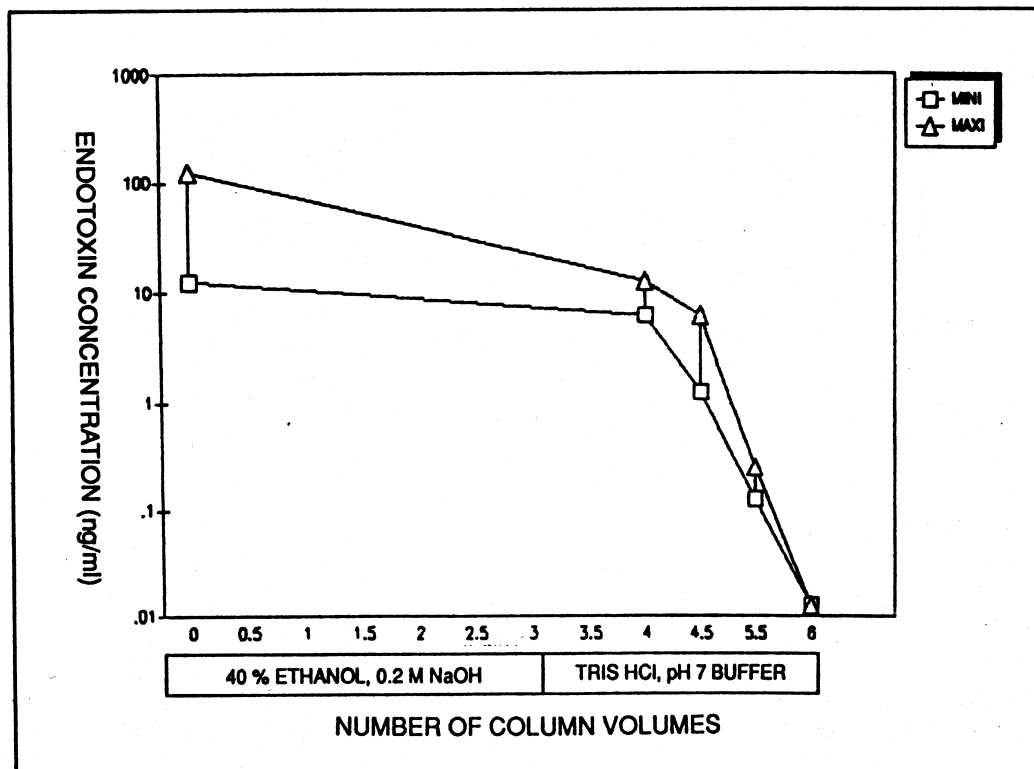
In the described experiment, the final level of endotoxin which may be present in the column after depyrogenation is lower than the LAL test detection level (0.0125 EU/ml).

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Table III : Example of a depyrogenation procedure in column

INJECTED SOLUTION	ELUATE	ENDOTOXIN CONC. (ng/ml)	ENDOTOXIN CONC. (ng/ml)
40 % ETHANOL 0,2 N NaOH	Nber OF VOLUMES	MINI	MAXI
	0	12.5	125
	0.5		
	1		
	1.5		
	2		
	2.5		
3			
TRIS HCl 0.5 M, pH 7 BUFFER	3.5		
TRIS HCl 0.05 M, NaCl 0.15 M pH 7 BUFFER	4	6.25	12.5
	4.5	1.25	6.25
	5.5	0.125	0.250
	6	0.0125	0.0125

Fig. 12 : Elimination of endotoxins during a depyrogenation procedure in column



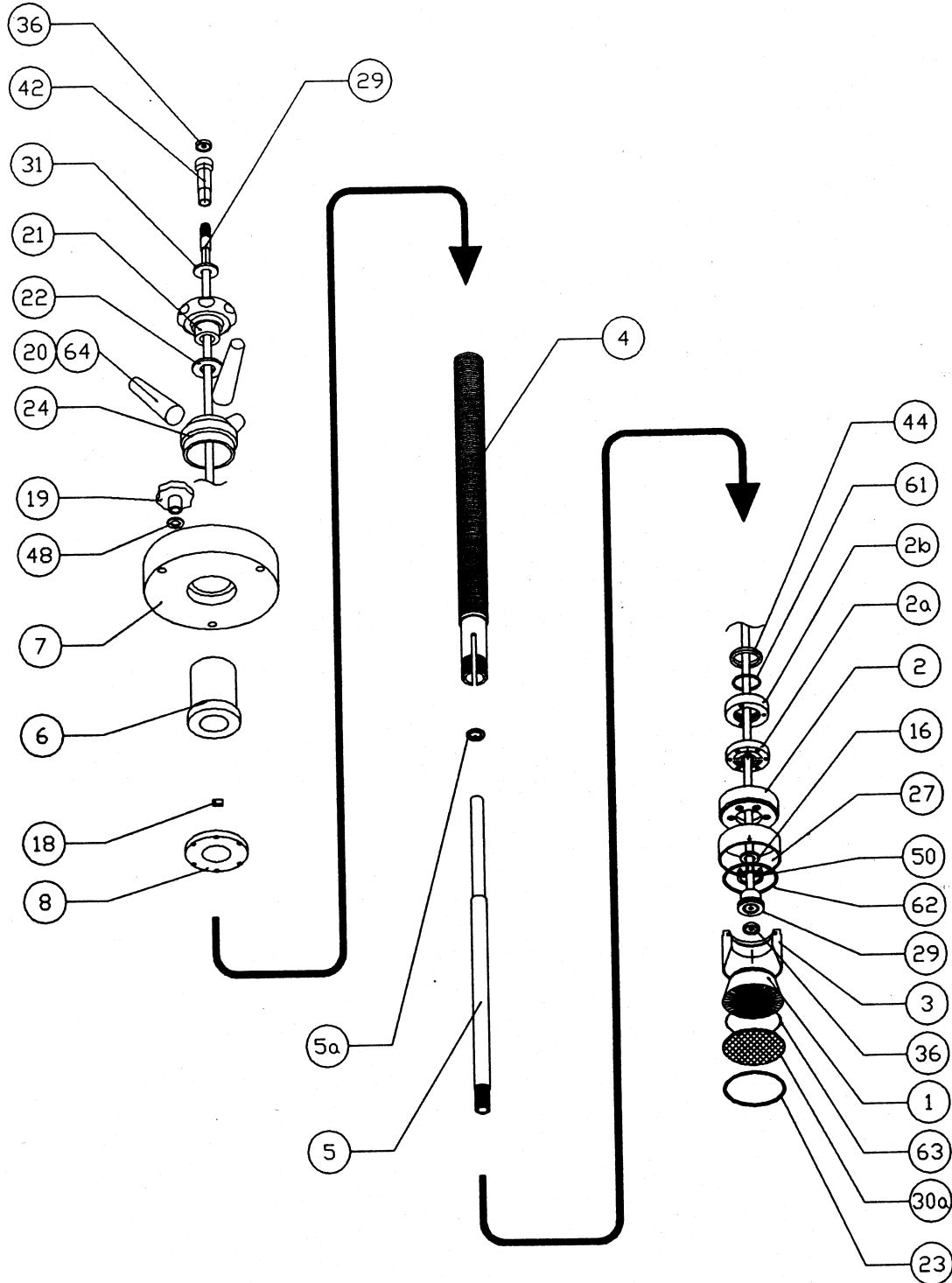
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APPENDIX 7 - PARTS LIST

- * **90 mm Diameter Columns**
- * **180 mm Diameter Columns**
- * **300 mm Diameter Columns**
- * **450 mm Diameter Columns**

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90 mm Column Piston



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90 mm Column - Parts List

Part No.	Ref .No.	Description	Qua- n.	Material
266011	1	Flow Distribution Cell	1	Grey PP
200001	2	Seal Screwing Plate	1	Grey PP
200002	2a	Screwing Nut	1	316L SS
200003	2b	Wiper Support ID 90/180	1	Grey PP
266012	3	Piston Seal	1	EPDM
266013	4	Central Screw ID 90/180	1	Grey PP
266304	5	Internal Central Screw ID 90/180	1	316L SS
266035	5a	Internal Central Screw Washer	1	316L SS
266306	6	Drive Bolt	1	316L SS
266032	7	Piston Support	1	Grey PP
262863	8	Holding Washer	1	Grey PP
266053	9	Top Tube Flange	1	Grey PP
200004	10	Jacket - H300mm	1	PMMA
200005	10	Jacket - H900mm	1	PMMA
266014	10	Jacket - H600mm	1	PMMA
263781	11	Glass Tube - H300mm	1	Borosilica- te Glass
263782	11	Glass Tube - H600mm	1	Borosilica- te Glass
263783	11	Glass Tube - H900mm	1	Borosilica- te Glass
263734	12	Bottom Tube Flange	1	Grey PP
263735	13	Base Plate	1	Grey PP
266036	14	Stand Foot ID 90/180	3	Grey PP
266046	15	Foot ID 90/180	3	316L SS
263791	16	M12 Washer	1	316L SS
200032	17	Tie Rod - H900 ID 90/180	3	316L SS
266305	17	Tie Rod - H300 ID 90/180	3	316L SS
266353	17	Tie Rod - H600 ID 90/180	3	316L SS
200016	18	Key ID 90/180	1	316L SS
266015	19	Piston / Tie Nut ID 90/180	3	Bakelite
266320	20	Plastic Handle	3	Bakelite
266321	21	Seal Actuation Handle ID 90/180/300	1	Bakelite & SS

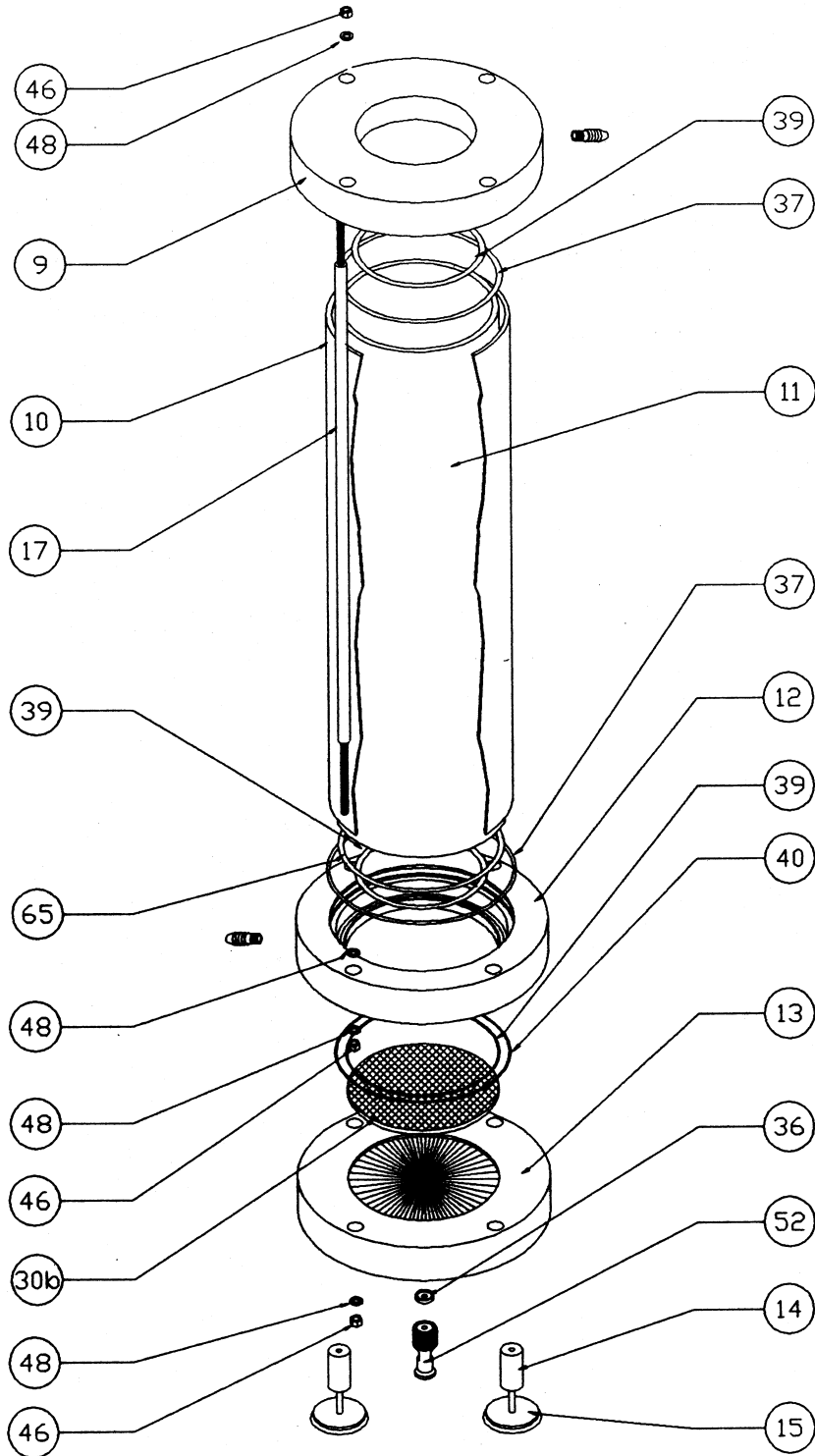
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90 mm Column - Parts List

Part No.	Ref .No.	Description	Qua- n.	Material
266322	22	Stop Washer	1	316L SS
266016	23	Frit Retaining Ring	1	Grey PP
266323	24	Bed Height Adjustment Handle Support	1	Black Delrin
200006	27	Seal Clip	1	Grey PP
266325	28	Clip Key	1	Black PP
266017	29	Inlet Tube	1	Grey PP
266018	30a	Top Frit	1	Porous PE
266019	30b	Bottom Frit	1	Porous PE
286101	31	Control Wheel Washer	1	Black Delrin
200007	36	ID 6 Sanitary Gasket ID 90/180	2	EPDM
200008	37	Jacket O-Ring	2	EPDM
200009	39	Glass Tube & Bottom Frit O-Rings	3	EPDM
266020	40	Base Plate O-Ring	1	EPDM
266031	42	Inlet Sanitary Connection	1	Grey PP
266022	44	ID 40 Wiper	1	EPDM
266024	46	M8 Nut ID 90/180	6	SS
266045	47	M8 Blind Nut ID 90/180	3	SS
266342	50	Gage	1	Grey PP
266344	52	ID 6 Outlet Sanitary Conn. ID 90/180	1	Grey PP
200011	61	Internal Central Screw O-Ring	1	EPDM
200012	62	Flow Distribution Plate O-Ring	1	EPDM
200013	63	Top Frit O-Ring	1	EPDM
200039	64	Handle Axis	3	316L SS
263742	65	Glass Tube Centering O-Ring ID 90/180	1	EPDM

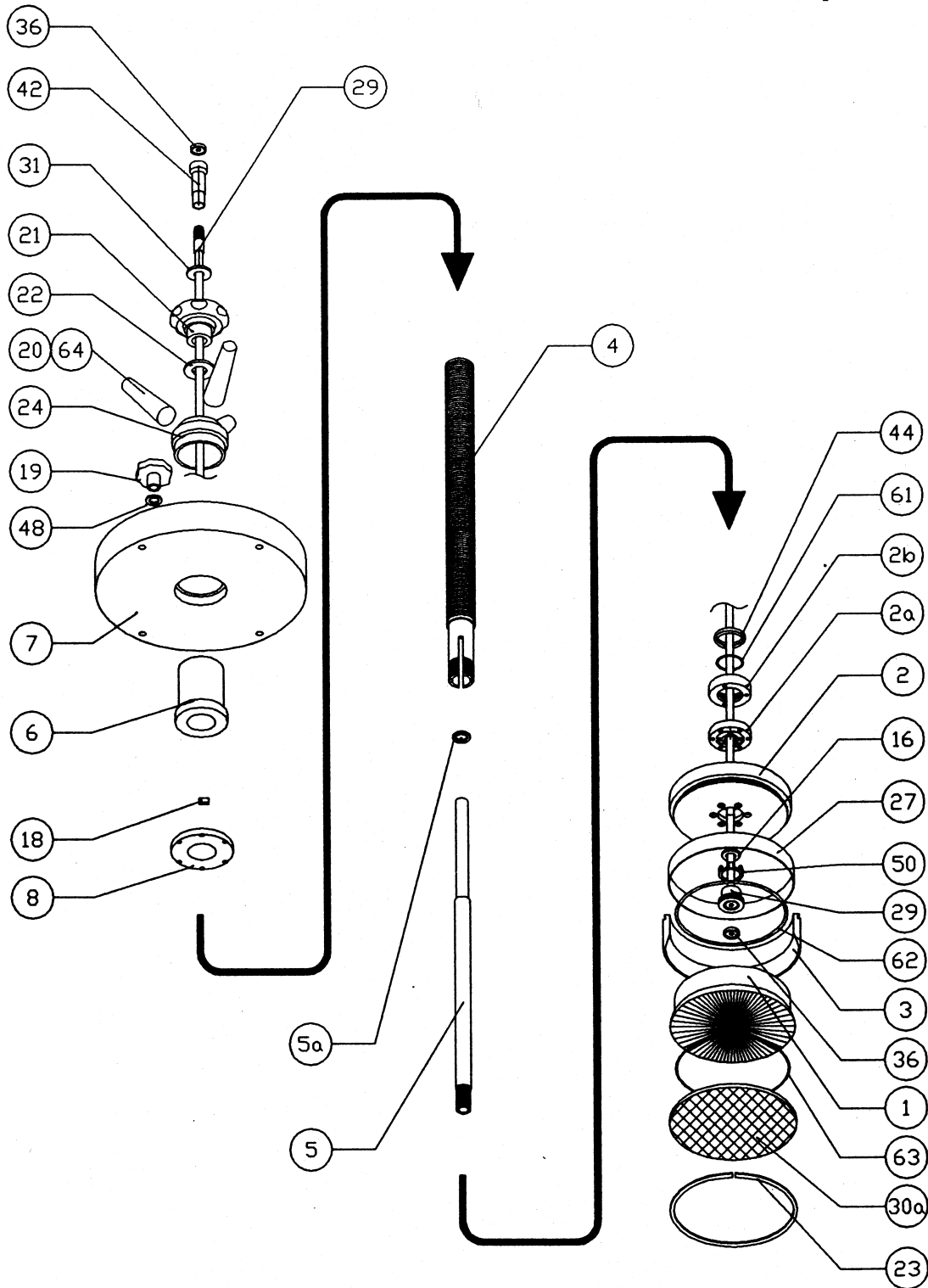
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180 mm Column Body



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180 mm Column Piston



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180 mm Column - Parts List

Part No.	Ref .No.	Description	Qua- n.	Material
266033	1	Flow Distribution Cell	1	Grey PP
200014	2	Seal Screwing Plate	1	Grey PP
200002	2a	Screwing Nut	1	316L SS
200003	2b	Wiper Support ID 90/180	1	Grey PP
266034	3	Piston Seal	1	EPDM
266013	4	Central Screw ID 90/180	1	Grey PP
266304	5	Internal Central Screw ID 90/180	1	316L SS
266035	5a	Internal Central Screw Washer	1	316L SS
266306	6	Drive Bolt	1	316L SS
263737	7	Piston Support	1	Grey PP
262863	8	Holding Washer	1	Grey PP
263738	9	Top Tube Flange	1	Grey PP
200017	10	Jacket - H300mm	1	PMMA
200018	10	Jacket - H600mm	1	PMMA
200019	10	Jacket - H900mm	1	PMMA
263794	11	Glass Tube - H300mm	1	Borosilica- te Glass
263795	11	Glass Tube - H600mm	1	Borosilica- te Glass
263796	11	Glass Tube - H900mm	1	Borosilica- te Glass
263739	12	Bottom Tube Flange	1	Grey PP
263740	13	Base Plate	1	Grey PP
266036	14	Stand Foot ID 90/180	3	Grey PP
266046	15	Foot ID 90/180	3	316L SS
263791	16	M12 Washer	1	316L SS
200032	17	Tie Rod - H900 ID 90/180	3	316L SS
266305	17	Tie Rod - H300 ID 90/180	3	316L SS
266353	17	Tie Rod - H600 ID 90/180	3	316L SS
200016	18	Key ID 90/180	1	316L SS
266015	19	Piston / Tie Nut ID 90/180	3	Bakelite
266320	20	Plastic Handle	3	Bakelite
266321	21	Seal Actuation Handle ID 90/180/300	1	Bakelite & SS

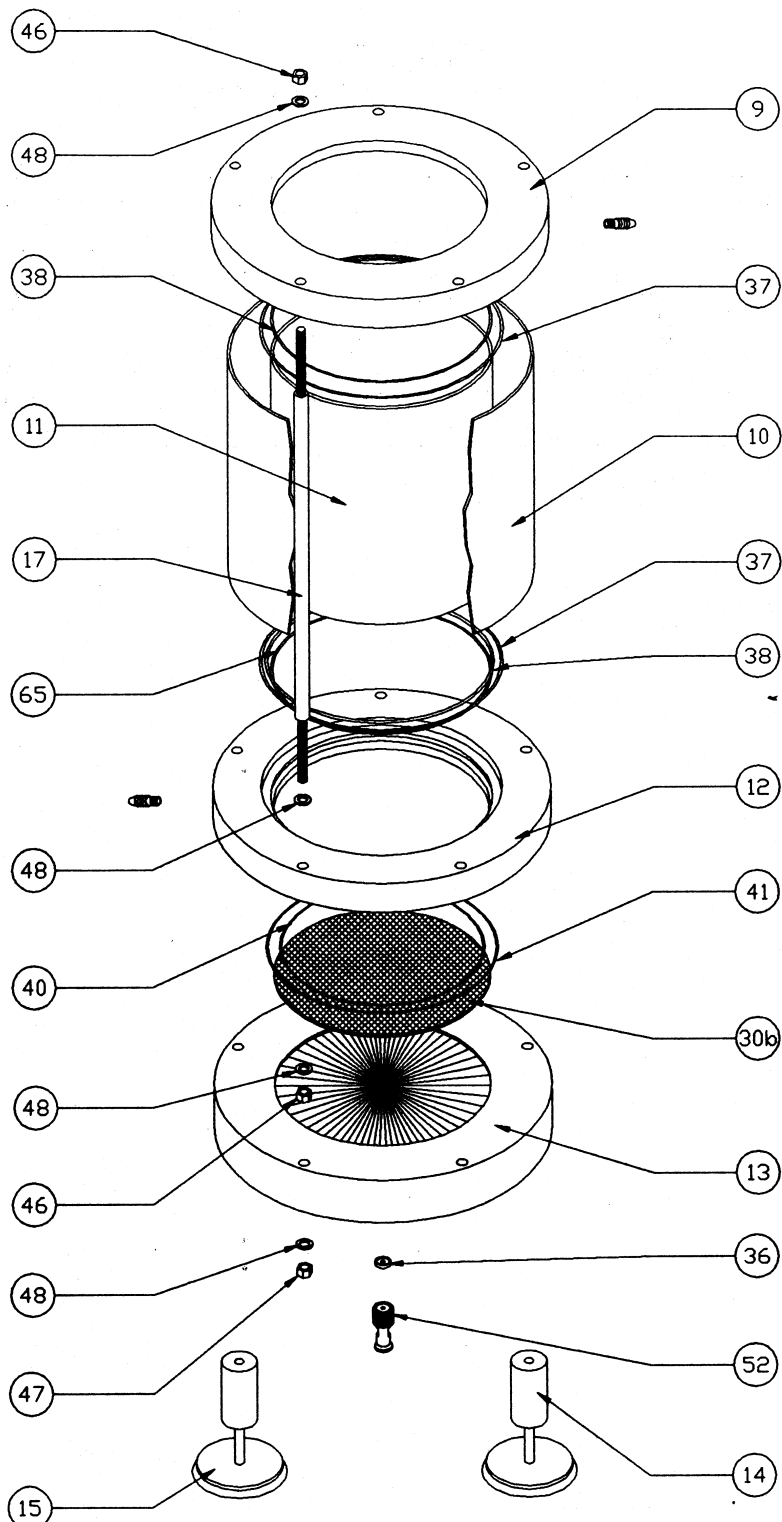
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180 mm Column - Parts List (con't)

Part No.	Ref .No.	Description	Qua- n.	Material
266322	22	Stop Washer	1	316L SS
266037	23	Frit Retaining Ring	1	Grey PP
266323	24	Bed Height Adjustment Handle Support	1	Black Delrin
200021	27	Seal Clip	1	Grey PP
266325	28	Clip Key	1	Black PP
266038	29	Inlet Tube	1	Grey PP
266039	30a	Top Frit	1	Porous PE
266040	30b	Bottom Frit	1	Porous PE
286101	31	Control Wheel Washer	1	Black Delrin
200007	36	ID 6 Sanitary Gasket ID 90/180	2	EPDM
200023	37	Jacket O-Ring	2	EPDM
266041	39	Glass Tube & Bottom Frit O-Rings	3	EPDM
200024	40	Base Plate O-Ring	1	EPDM
266031	42	Inlet Sanitary Connection	1	Grey PP
266022	44	ID 40 Wiper	1	EPDM
266024	46	M8 Nut ID 90/180	6	SS
266045	47	M8 Blind Nut ID 90/180	3	SS
266342	50	Gage	1	Grey PP
266344	52	ID 6 Outlet Sanitary Conn. ID 90/180	1	Grey PP
200011	61	Internal Central Screw O-Ring	1	EPDM
200026	62	Flow Distribution Plate O-Ring	1	EPDM
200027	63	Top Frit O-Ring	1	EPDM
200039	64	Handle Axis	3	316L SS
263742	65	Glass Tube Centering O-Ring ID 90/180	1	EPDM

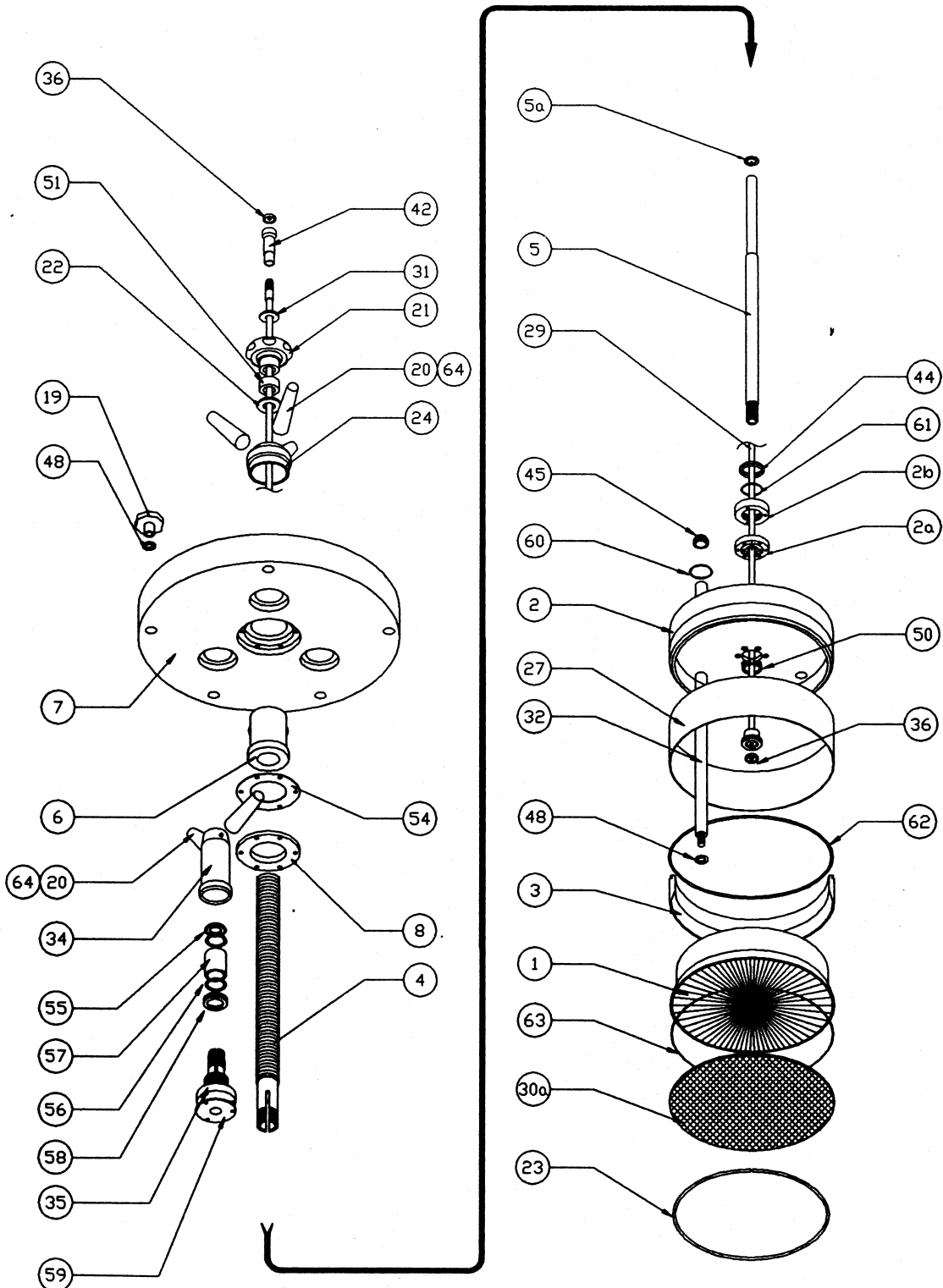
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300mm Column Body



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300mm Column Piston



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300mm Column Parts

Part No.	Ref .No.	Description	Qua- n.	Material
266300	1	Flow Distribution Cell	1	Grey PP
266301	2	Seal Screwing Plate	1	Grey PP
200002	2a	Screwing Nut	1	316L SS
263801	2b	Wiper Support ID 300/450	1	Grey PP
266023	3	Piston Seal	1	EPDM
263812	4	Central Screw ID 300/450	1	Grey PP
263802	5	Internal Central Screw ID 300/450	1	316L SS
266035	5a	Internal Central Screw Washer	1	316L SS
266306	6	Drive Bolt	1	316L SS
266307	7	Piston Support	1	Grey PP
262863	8	Holding Washer	1	Grey PP
266309	9	Top Tube Flange	1	Grey PP
266310	10	Jacket - H300mm	1	PMMA
266311	10	Jacket - H600mm	1	PMMA
266312	10	Jacket - H900mm	1	PMMA
263784	11	Glass Tube - H900mm	1	Borosilica- te Glass
263788	11	Glass Tube - H300mm	1	Borosilica- te Glass
263789	11	Glass Tube - H600mm	1	Borosilica- te Glass
266313	12	Bottom Tube Flange	1	Grey PP
266314	13	Base Plate	1	Grey PP
266315	14	Stand Foot ID 300/450	3	Grey PP
266316	15	Foot ID 300/450	3	316L SS
266317	17	Tie Rod - H300 ID 300/450	3	316L SS
266318	17	Tie Rod - H600 ID 300/450	3	316L SS
266319	17	Tie Rod - H900 ID 300/450	3	316L SS
267252	19	Piston / Tie Nut ID 300/450	3	Bakelite
266320	20	Plastic Handle	3	Bakelite
266321	21	Seal Actuation Handle ID 90/180/300	1	Bakelite & SS
266322	22	Stop Washer	1	316L SS
266026	23	Frit Retaining Ring	1	Grey PP
266323	24	Bed Height Adjustment Handle Support	1	Black Delrin
266324	27	Seal Clip	1	Grey PP
266325	28	Clip Key	1	Black PP
266027	29	Inlet Tube	1	Grey PP
266029	30a	Top Frit	1	Porous PE

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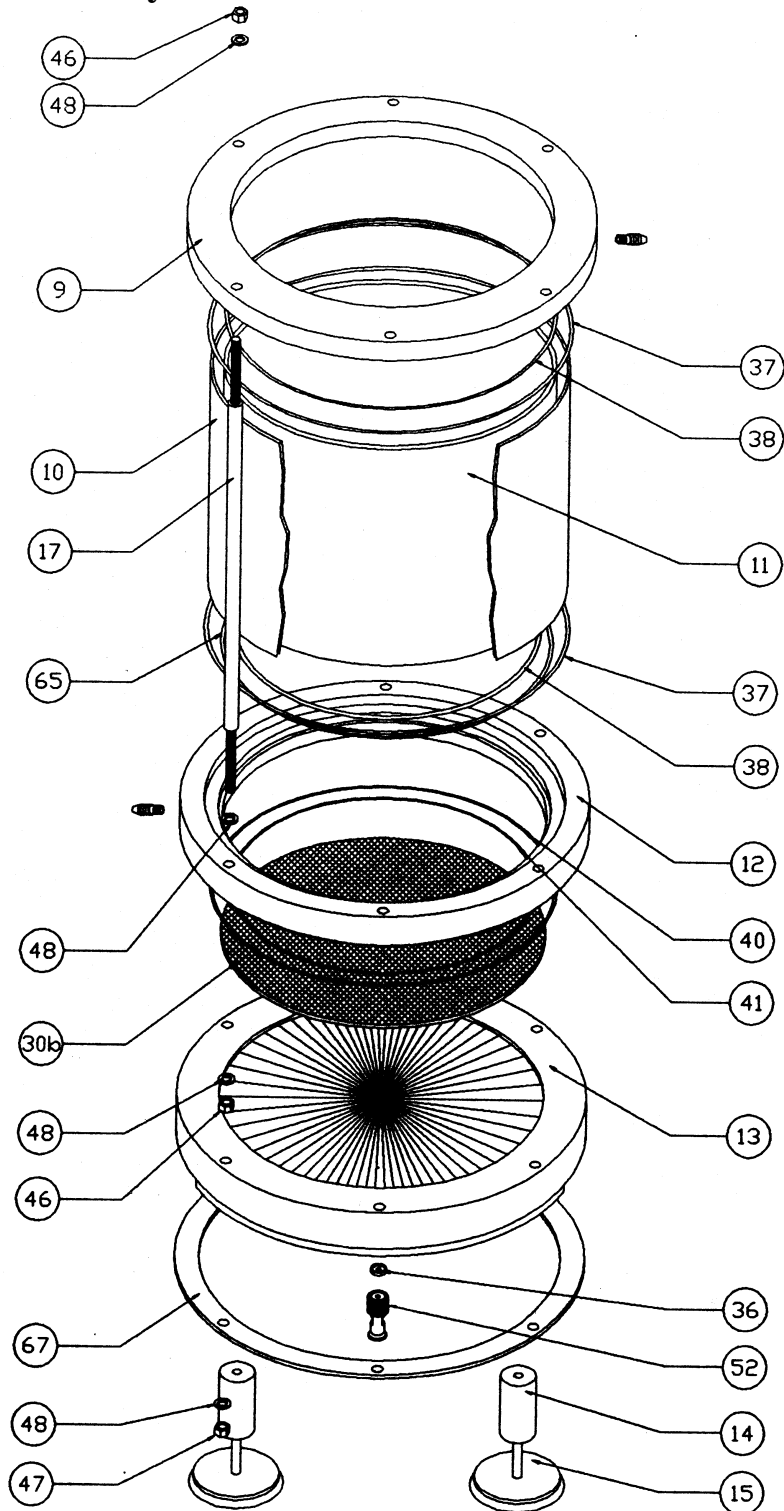
300mm Column Parts (con't)

Part No.	Ref .No.	Description	Qua- n.	Material
266028	30b	Bottom Frit	1	Porous PE
286101	31	Control Wheel Washer	1	Black Delrin
262629	32	Pressure Rod ID 300/450	3	Black Delrin
266327	34	Coupling Key	3	Nylon
266328	35	Coupling Support	3	Nylon
266329	36	ID 9.525 Sanitary Gasket ID 300/450	2	EPDM
266330	37	Jacket O-Ring	2	EPDM
266030	38	Glass Tube O-Rings	2	EPDM
266331	40	Bottom Frit O-Ring	1	EPDM
266332	41	Base Plate O-Ring	1	EPDM
266031	42	Inlet Sanitary Connection	1	Grey PP
266022	44	ID 40 Wiper	1	EPDM
266339	45	ID 20 Wiper ID 300/450	3	EPDM
266340	46	M12 Nut ID 300/450	10	SS
266341	47	M12 Blind Nut ID 300/450	5	SS
263791	48	M12 Washer	28	316L SS
266342	50	Gage	1	Grey PP
266343	51	Brace	1	Black Delrin
267251	52	ID 9.525 Outlet San. Conn. ID 300/450	1	Grey PP
266345	54	Slipping Washer ID 300/450	1	PTFE
266348	55	Bead Washer ID 300/450	3	SS
266349	56	ID 4mm Beads ID 300/450	132	304 SS
266303	57	Schuttle	3	SS
266350	58	Threaded Bead Washer ID 300/450	3	SS
266351	59	Coupling Washer ID 300/450	3	SS
266352	60	Pressure Tie Rod O-Ring ID 300/450	3	EPDM
200011	61	Internal Central Screw O-Ring	1	EPDM
266333	62	Flow Distribution Plate O-Ring	1	EPDM
266334	63	Top Frit O-Ring	1	EPDM
200039	64	Handle Axis	9	316L SS
263822	65	Glass Tube Cent. O-Ring	1	EPDM
270202	66	Leveling Eye	3	SS
270203	67	SS Ring	2	SS

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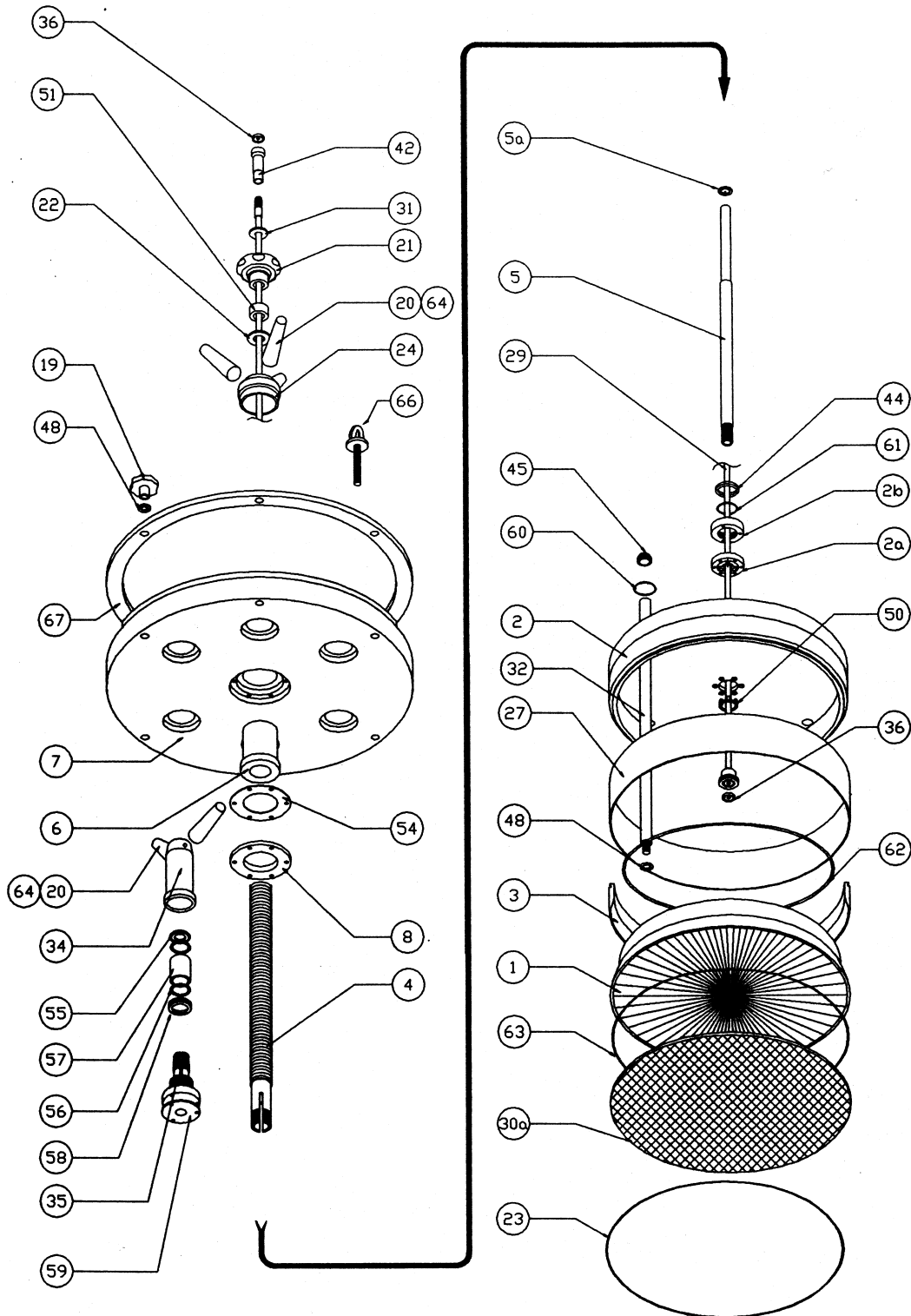
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450mm Column Body



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450mm Column Piston



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450mm Column Parts List

Part No.	Ref .No.	Description	Qua- n.	Material
200028	1	Flow Distribution Cell	1	Grey PP
200029	2	Seal Screwing Plate	1	Grey PP
200002	2a	Screwing Nut	1	316L SS
263801	2b	Wiper Support ID 300/450	1	Grey PP
266044	3	Piston Seal	1	EPDM
263812	4	Central Screw ID 300/450	1	Grey PP
263802	5	Internal Central Screw ID 300/450	1	316L SS
266035	5a	Internal Central Screw Washer	1	316L SS
266306	6	Drive Bolt	1	316L SS
2263743	7	Piston Support	1	Grey PP
262863	8	Holding Washer	1	Grey PP
263744	9	Top Tube Flange	1	Grey PP
200033	10	Jacket - H300mm	1	PMMA
200034	10	Jacket - H600mm	1	PMMA
200035	10	Jacket - H900mm	1	PMMA
263785	11	Glass Tube - H600mm	1	Borosilica- te Glass
263786	11	Glass Tube - H900mm	1	Borosilica- te Glass
263787	11	Glass Tube - H300mm	1	Borosilica- te Glass
263745	12	Bottom Tube Flange	1	Grey PP
263746	13	Base Plate	1	Grey PP
266315	14	Stand Foot ID 300/450	3	Grey PP
266316	15	Foot ID 300/450	3	316L SS
266317	17	Tie Rod - H300 ID 300/450	6	316L SS
266318	17	Tie Rod - H600 ID 300/450	6	316L SS
266319	17	Tie Rod - H900 ID 300/450	6	316L SS
267252	19	Piston / Tie Nut ID 300/450	6	Bakelite
266320	20	Plastic Handle	15	Bakelite
263811	21	Seal Actuation Hand Wheel	1	Bakelite & SS
266322	22	Stop Washer	1	316L SS
266047	23	Frit Retaining Ring	1	Grey PP
266323	24	Bed Height Adjustment Handle Support	1	Black Delrin
200036	27	Seal Clip	1	Grey PP
266325	28	Clip Key	1	Black PP

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450mm Column Parts List (con't)

Part No.	Ref .No.	Description	Qua- n.	Material
266048	29	Inlet Tube	1	Grey PP
266049	30a	Top Frit	1	Porous PE
266050	30b	Bottom Frit	1	Porous PE
286101	31	Control Wheel Washer	1	Black Delrin
262629	32	Pressure Rod ID 300/450	6	Black Delrin
266327	34	Coupling Key	6	Nylon
266328	35	Coupling Support	6	Nylon
266329	36	ID 9.525 Sanitary Gasket ID 300/450	2	EPDM
200037	37	Jacket O-Ring	2	EPDM
266051	38	Glass Tube O-Rings	2	EPDM
266335	40	Bottom Frit O-Ring	1	EPDM
266336	41	Base Plate O-Ring	1	EPDM
266031	42	Inlet Sanitary Connection	1	Grey PP
266022	44	ID 40 Wiper	1	EPDM
266339	45	ID 20 Wiper ID 300/450	6	EPDM
266340	46	M12 Nut ID 300/450	12	SS
266341	47	M12 Blind Nut ID 300/450	6	SS
263791	48	M12 Washer	36	316L SS
266342	50	Gage	1	Grey PP
266343	51	Brace	1	Black Delrin
267251	52	ID 9.525 Outlet San. Conn. ID 300/450	1	Grey PP
266345	54	Slipping Washer ID 300/450	1	PTFE
266348	55	Bead Washer ID 300/450	6	SS
266349	56	ID 4mm Beads ID 300/450	132	304 SS
266303	57	Schuttle	6	SS
266350	58	Threaded Bead Washer ID 300/450	6	SS
266351	59	Coupling Washer ID 300/450	6	SS
266352	60	Pressure Tie Rod O-Ring ID 300/450	6	EPDM
200011	61	Internal Central Screw O-Ring	1	EPDM
200041	62	Flow Distribution Plate O-Ring	1	EPDM
266338	63	Top Frit O-Ring	1	EPDM
200039	64	Handle Axis	15	316L SS
263823	65	Glass Tube Cent. O-Ring	1	EPDM

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Spare Parts & Accessories

267255	90mm Frit Kit consisting of:		
Part No.	Description	Qua-n.	Material
266018	Top Frit	5	Porous PE
266019	Bottom Frit	5	Porous PE
266020	Base Plate O-Ring	1	EPDM
200013	Top Frit O-Ring	1	EPDM
266325	Clip Key	1	Grey PP
266016	Frit Retaining Ring	1	Grey PP
200009	Glass Tube & Bottom O-Ring	1	EPDM

267256	180mm Frit Kit consisting of:		
Part No.	Description	Qua-n.	Material
266039	Top Frit	5	Porous PE
266040	Bottom Frit	5	Porous PE
200024	Base Plate O-Ring	1	EPDM
200027	Top Frit O-Ring	1	EPDM
266325	Clip Key	1	Grey PP
266037	Frit Retaining Ring	1	Grey PP
266041	Glass Tube & Bottom O-Ring	1	EPDM

267257	300mm Frit Kit consisting of:		
Part No.	Description	Qua-n.	Material
266029	Top Frit	5	Porous PE
266028	Bottom Frit	5	Porous PE
266332	Base Plate O-Ring	1	EPDM
266334	Top Frit O-Ring	1	EPDM
266325	Clip Key	1	Grey PP
266026	Frit Retaining Ring	1	Grey PP
266331	Glass Tube & Bottom O-Ring	1	EPDM

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Spare Parts & Accessories (con't)

267258	450mm Frit Kit consisting of:		
Part No.	Description	Quan.	Material
266049	Top Frit	5	Porous PE
266050	Bottom Frit	5	Porous PE
266336	Base Plate O-Ring	1	EPDM
266338	Top Frit O-Ring	1	EPDM
266325	Clip Key	1	Grey PP
266047	Frit Retaining Ring	1	Grey PP
266335	Glass Tube & Bottom O-Ring	1	EPDM

267253	90/180mm Fitting Kit consisting of:		
Part No.	Description	Quan.	Material
263733	Jacket ID 6 Hose Barb Connection	2	Grey PP
200007	ID 6 Sanitary Gasket	4	EPDM
273420	25 Sanitary Collar	2	Plastic
274002	Adaptor Sanitary Flange / ID 6 Hose Barb Fitting	2	EPDM

267254	300/450mm Fitting Kit consisting of:		
Part No.	Description	Quan.	Material
263733	Jacket ID 6 Hose Barb Connection	2	Grey PP
266329	ID 9.525 Sanitary Gasket	4	EPDM
273420	25 Sanitary Collar	2	Plastic
263790	Adaptor Sanitary Flange / ID 9.525 Hose Barb Fitting	2	EPDM

OTHER ACCESSORIES			
PART NUMBER	COLUMN SIZE	DESCRIPTION	NOTE
263731	ID 300	Adjustable Column Stabilization Assembly	None
263732	ID 450	Adjustable Column Stabilization Assembly	None
263767	ID 90	Accessory Kit	See Packing Content for List of Parts
263768	ID 180	Accessory Kit	See Packing Content for List of Parts
263623	ID 300	Accessory Kit	See Packing Content for List of Parts
266043	ID 450	Accessory Kit	See Packing Content for List of Parts

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Packing Content:

90 and 180 Diameter Columns:

*** 1 Complete Column**

*** 1 Accessory Kit (P/N 263767 for ID 90 and P/N 263768 for ID 180) including the following:**

- 1 set of 5 top porous frits
- 1 set of 5 bottom porous frits
- 1 frit clip
- 1 set of two o-rings for tube flange
- 2 top frit o-rings
- 4 sanitary gaskets ID 6 mm
- 2 sanitary collars DN 25
- 1 set of four washers
- 1 set of four nuts
- 2 hose barb connections ID 6 mm
- 2 adapters sanitary flange / hose barb fitting
- 1 clip dismounting device
- 1 bubble level
- 1 column manual
- 1 wrench of 13 mm

300 and 450 Diameter Columns:

*** 1 Complete Column**

*** 1 Accessory Kit (P/N 263623 for ID 300 and P/N 266043 for ID 450) including the following:**

- 1 top porous frit
- 1 bottom porous frit
- 1 frit clip
- 1 set of two o-rings for tube flange
- 2 top frit o-rings
- 4 sanitary gaskets ID 9.525 mm
- 2 sanitary collars DN 25
- 1 set of four washers
- 1 set of four nuts
- 2 hose barb connections ID 6 mm
- 2 adapters sanitary flange / hose barb fitting
- 1 clip dismounting device
- 1 bubble level
- 1 column manual
- 1 wrench of 13 mm

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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 Essentail Life Solutions appreciate your business!

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