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Mini S® PC 3.2/3 INSTRUCTIONS

1. Introduction

The Precision Column PC 3.2/3 (gel volume 0.24 ml) pre-packed with Mini S® is for fast, sensitive and high-resolving cation exchange separations of proteins, peptides, polynucleotides and other biomolecules in the micropreparative scale.

Mini S is based on MiniBeads™, which are monodisperse 3 µm polymer beads. Also available within the MiniBeads family is Mini Q™ pre-packed in PC 3.2/3, which is for anion exchange separations.

To ensure that Mini S PC 3.2/3 is a high quality product each column is thoroughly tested during production. To get optimal results and keep the column in good condition, follow the recommendations given below.

These instructions should be filed under "Column Instructions" in SMART® System handbook.

For further information about columns and equipment, please contact your local representative for Pharmacia Biotech.

2. General description

2.1 Gel properties

Mini S (Sulphonate) is a strong cation exchanger based on a beaded hydrophilic polymer. The chemistry of the beads was developed at Pharmacia Biotech and the monodispersity was accomplished through a unique process developed by Prof. John Ugelstad of SINTEF, Trondheim, Norway.

Mini S beads are non-porous and have a particle size of 3 µm. The charged group on the gel is $-CH_2-SO_3^-$. Ionic capacity is 0.016–0.030 mmol/ml gel.

The maximum protein capacity for Mini S PC 3.2/3 is approximately 1–1.5 mg. When applying a solution of ribonuclease A or lysozyme the capacity was determined to be 1.3 mg. However, to minimize the risk of losing non-bound material in the flow-through fractions, decreased resolution or detection problems, a practical loading range is ≤ 200 µg.

Mass recovery on a column in use is normally ≥ 90% of applied sample. However, the recovery is lower on a new column due to non-specific adsorption. To minimize the non-specific adsorption, saturate the column with a suitable protein solution before applying the sample.

2.2 Chemical and physical stability

For separations on Mini S PC 3.2/3 aqueous solutions in the pH range 3–11 can be used. For short term use, for example in a cleaning procedure the pH stability is 1–14.

Mini S can be used with solutions such as 100% acetonitrile, 75% acetic acid, 2 M NaOH and 1 M HCl. However, to minimize the risk of destroying the column, do not keep it in these solutions for longer periods of time.

Anionic or non-ionic detergents can be used with Mini S. Do not use cationic buffers or detergents as they bind to the gel matrix.

All oxidizing and other reactive substances should be avoided.

The column should be stored in 20% ethanol.

The maximum flow rate for Mini S PC 3.2/3 is 1.0 ml/min, and the maximum pressure limit is 10 MPa (100 bar, 1500 psi). When running at a flow rate of 0.4 ml/min, the column generally operates at a back pressure of 4 MPa.

All material in the column body is made of glass and fluoroplastics, whereas the filter is made of titanium. This composition makes the column totally biocompatible, and hence there is no risk of corrosion or sample contamination by e.g. metal ions.

The column operates at temperatures between +4 °C and +40 °C.

71-7132-00
Edition AC

Pharmacia Biotech

Table 1. Properties of Mini S PC 3.2/3.

Column dimensions	3.2 mm x 30 mm
Gel volume	0.24 ml
Charged group	$-CH_2-SO_3^-$
Ionic capacity (K^+)	0.016–0.030 mmol/ml gel
Particle size	3 µm
Max. protein capacity	1–1.5 mg
Practical loading range	≤ 200 µg of protein
Max. flow rate*	1.0 ml/min
Protein mass recovery**	≥ 90%
pH-stability	
working range	3–11
cleaning range	1–14
Max. pressure limit	10 MPa (100 bar, 1500 psi)
Temperature interval	+4 ° to +40 °C

* In water, at room temperature.
** Mass recovery on a column in use is normally ≥90% of the applied sample. However, the recovery is lower on a new column due to non-specific adsorption. To minimize the non-specific adsorption, saturate the column with a suitable protein solution before applying the sample.

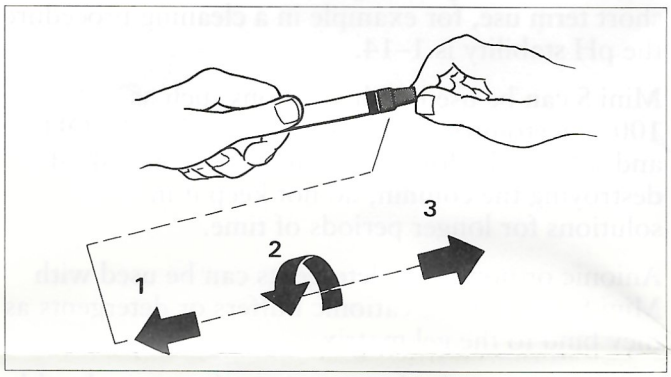


Fig. 1. 1. Hold the protective jacket tightly and press the cap inwards.
2. Turn the cap counter-clockwise.
3. Pull the cap outwards.

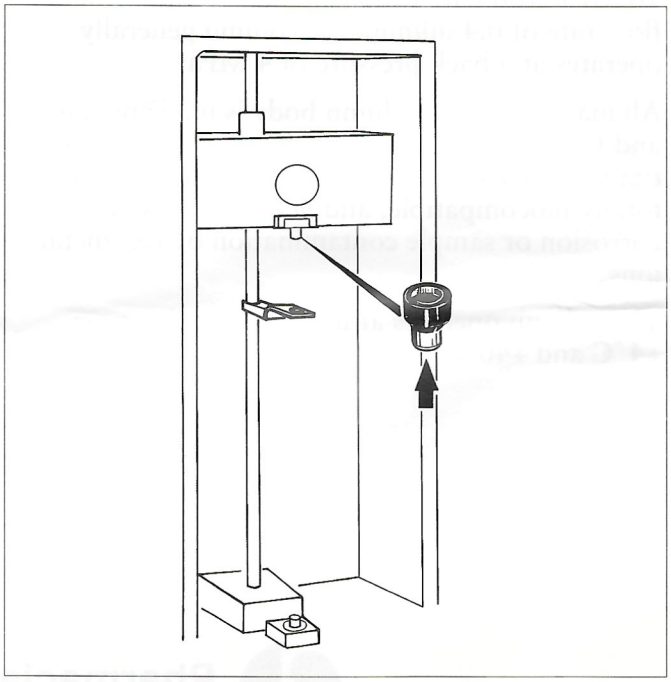


Fig. 2.1. Check that the Automatic Column Holder is high enough to install the column.

3. Installation

3.1 Unpacking

The package contains one column, Mini S PC 3.2/3, Code No. 17-0687-01, and instructions.

3.2 Connecting the column

The two protective plastic caps at either end of the column are held in place by bayonet fittings. To remove them, follow the steps in Figure 1.

Follow the instructions in SMART Manager to install the column. The procedure is illustrated in Figures 2.1-2.4. Further information can be found in SMART System Handbook.

4. Getting started

4.1 Choice of running conditions

Cation exchangers should be used with anionic or zwitterionic buffers. Anionic (e.g. SDS) or non-ionic detergents (e.g. octylglucoside) may be used. Avoid cationic buffers and detergents since they bind to Mini S.

The pH of the buffer should be at least 1 pH unit below the pI of the substances of interest so that they are positively charged and bind to the gel matrix. The pI of a molecule can be determined by running an electrophoretic titration curve.

Buffer concentration should be around 20-50 mM. For highly sensitive separations, it is sometimes advisable to have a lower concentration, about 5-10 mM, to avoid buffer disturbances.

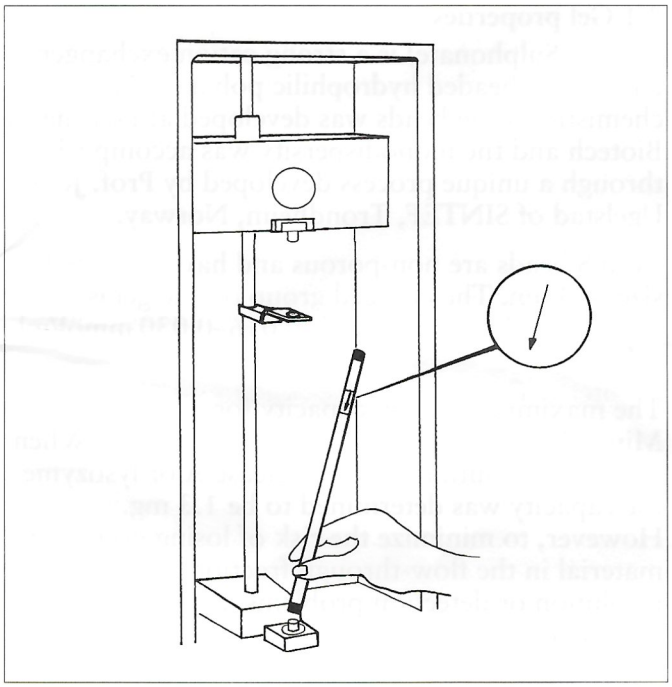


Fig. 2.2. Put the column on to the Column Connector. The arrow on the label, which should be pointing downwards, indicates the correct flow direction.

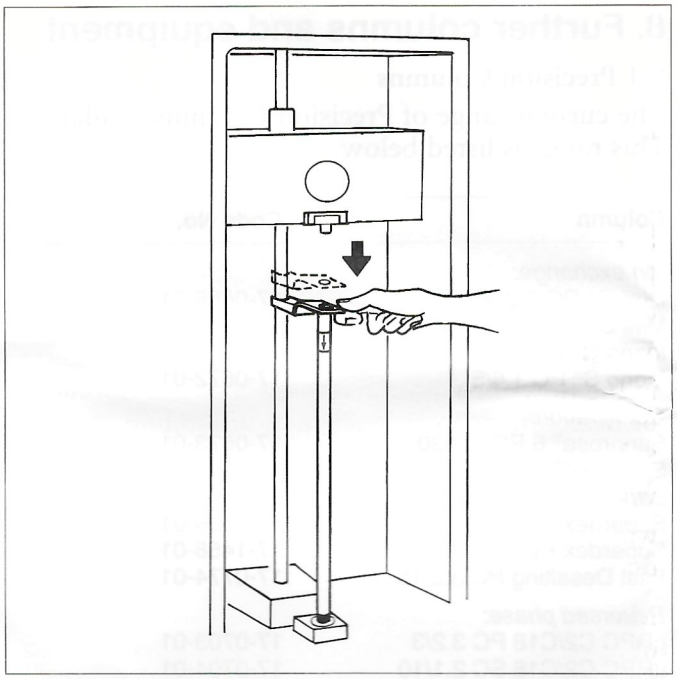


Fig. 2.3. Align the column vertically with the Column Support.

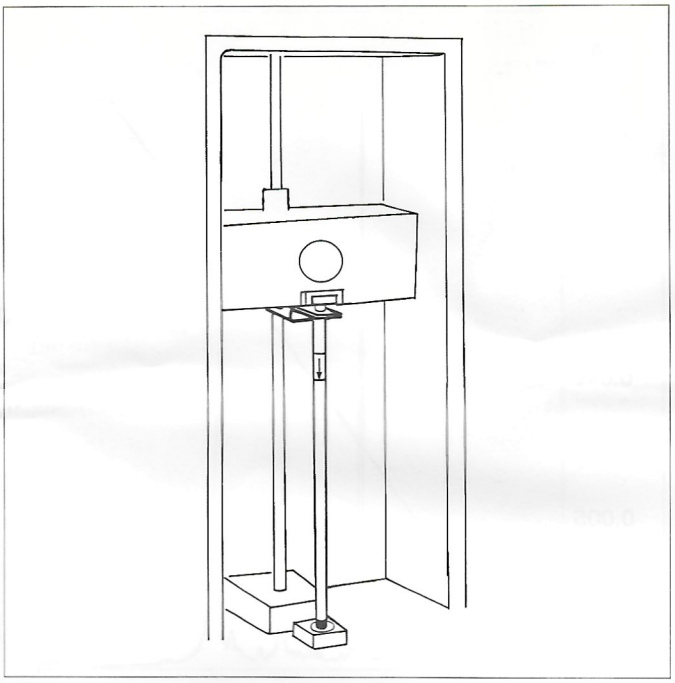


Fig. 2.4. The column is now connected.

The UV-absorption profile of buffers can vary from batch to batch owing to contaminants. Perform a blank gradient run before running a sample at a high sensitivity.

The following is an example of a buffer system for cation exchange chromatography (provided the pI of the substance of interest is not too acid):

Start buffer: 20 mM MES, pH 6.0
Elution buffer: 20 mM MES with 0.5 M NaCl, pH 6.0

Table 2 lists suggested buffers for various pH intervals.

Operational pH intervals are approximately ± 0.5 pH units from the pKa of the buffer. For separations on Mini S PC 3.2/3, aqueous solutions in the pH range 3–11 can be used. For short term use, for example in a cleaning procedure, the pH stability is 1–14.

Specific cations have different elution strengths. Table 3 gives various cations and suggested concentrations.

Table 3. Cations and suggested concentrations for gradient elution.

K^+	0.35 M
NH_4^+	0.35 M
Na^+	0.50 M
$HN^+(CH_3)_3$	0.50 M
Li^+	0.50 M

Comment to table 3: 4.8 ml is a reasonable gradient volume. Increase the cation concentration if the substance of interest is not eluted.

Further information about chemical and physical stability is found in section 6.2.

Table 2. Suggested buffers for various pH intervals.
Suggestions are based on experiments performed in our laboratories at 25 °C.

Substance	pKa(25 °C)	dpKa/ °C[1]	Concentration	Counter-ion[2]
Citric acid	3.13	-0.0024	20 mM	Na^+ / Li^+
Formic acid	3.75	+0.0002	20 mM	Na^+ / Li^+
Acetic acid	4.75	+0.0002	50 mM	Na^+ / Li^+
Malonic acid	5.69		50 mM	Na^+ / Li^+
MES	6.10	-0.0110	20 mM	Na^+ / Li^+
Phosphate	7.20	-0.0028	50 mM	Na^+
HEPES	7.48	-0.0140	50 mM	Na^+ / Li^+
BICINE	8.26	-0.0180	50 mM	Na^+

[1] The pKa value changes if the temperature is changed.

[2] Sodium is the most commonly used counter-ion, alternatives are also given when appropriate.

4.2 Preparing buffers and samples

The water used for the buffers should be Milli-Q* or of a corresponding quality. Use HPLC grade solvents, salts and buffers. Degas and filter all solutions through a 0.22-0.45 µm filter. Be sure to select a solvent resistant filter if the buffer contains an organic solvent.

When possible, dissolve or dilute the sample in start buffer. Buffer exchange is easily accomplished by gel filtration e.g. using Sephadex® G-25 Superfine with Fast Desalting PC 3.2/10 (Code No. 17-0774-01) for samples ≤ 100 µl. It is also possible to use disposable columns such as PD-10 columns with Sephadex G-25 (Code No. 17-0851-01).

Pass the sample through a 0.22–0.45 µm filter. If necessary, centrifuge the sample first (10 000 x *g* for 10 min). Be sure to select a solvent resistant filter if the sample is dissolved in an organic solvent.

The sample may be applied directly to the column only if:

- the volume is very small and there is a risk of sample loss due to the dead-volume of the filter.
- the sample has previously undergone one or more chromatography steps, and is therefore estimated to be pure enough without filtration.

However, if a sample is applied directly there is a greater risk that the column will become blocked.

Never apply a turbid solution (indicates sample insolubility).

Note: Careful handling of solutions and samples increases the life of the column considerably.

5. Running procedure

5.1 Column equilibration

The gel is delivered in 20% ethanol with sodium (Na^+) as the counter-ion. To equilibrate the column for first time use or after long term storage, proceed according to steps 1-4 below.

1. Install the column by following the instructions in section 3.2 and fill the system with the chosen buffers. Set the operational pressure limit to 10 MPa (100 bar, 1500 psi).
2. Flush out the storage solution with 0.24 ml of start buffer (e.g. 20 mM MES, pH 6.0) at a flow rate of 0.1 ml/min.
3. If the counter-ions are to be changed, wash with minimum five column volumes (1.2 ml) of the eluent buffer. Continue until the system is completely free of air bubbles and the UV-signal is stable at the selected sensitivity.
4. Equilibrate until stabel baseline or with at least five column volumes of start buffer.

5.2 Sample application and elution

Ensure that the sample has been prepared as described in section 4.2.

To minimize the risk of losing non-bound material in the flow-through fractions, decreased resolution or detection problems, a practical loading range is ≤ 200 µg.

When separating compounds on SMART System with µPeak Monitor, single peaks can be detected on the pg level.

Mass recovery on a used column is normally ≥90% of applied sample. However, the recovery is lower on a new column due to non-specific adsorption. To minimize the non-specific adsorption, saturate the column with a suitable protein solution before applying the sample.

For sample application there are sample loops between 5 µl and 50 ml. For volumes of 2 ml or less, use the sample loops listed in section 8.3. For volumes larger than 2 ml, use Superloop 10 ml or 50 ml (Code No.19-7585-01 or 19-7850-01).

When performing a separation, a typical flow rate is 0.4 ml/min. The maximum flow rate is 1.0 ml/min.

Note: Depending on sample composition, it may be necessary to reduce the flow rate during sample application to avoid a high back-pressure.

Use either linear or stepwise gradients for elution. A gradient volume of 4.8 ml (0.4 ml/min, 12 min) is usually sufficient. If the protein/peptide of interest is not eluted in the gradient, then increase cation concentration.

5.3 Column regeneration

To start a new run, clean the column with 1.2 ml (5 column volumes) of elution buffer, before equilibrating with start buffer. This procedure can be included in the method. If it is suspected that material still remains on the column, inject 0.5 ml of a more concentrated salt solution (e.g. 1-2 M NaCl).

If detergents have not been used in the run, but have been included in previous ones, rinse the column with 1.2 ml (5 column volumes) of water before equilibrating with start buffer.

* Milli-Q is a registered trademark of Millipore Corp.

Should the column be contaminated, follow the cleaning procedures described in section 6.2 before equilibrating the column.

6. Maintenance

6.1 Storage and prevention of microbial growth

Before storing for longer periods, wash the column sequentially with 1.2 ml (5 column volumes) of 1-2 M NaCl, 1.2 ml of water and 1.2 ml of 20% ethanol. Check that the UV-signal is stable before proceeding to the next wash.

To prevent drainage during storage, always fill the empty space above the filters with liquid before replacing the protective caps. It is also advisable to keep the column wrapped in plastic or in a beaker filled with 20% ethanol.

Note: There is a risk that ethanol will leak into the space between the protective jacket and the column, but this will not affect the column.

The column should be stored between +4 °C and +30 °C. If stored for more than two weeks, place the column in a refrigerator.

6.2 Column cleaning

The following observations may indicate a dirty column which needs more rigorous washing:

- increased back-pressure
- colour change in the gel bed
- loss of resolution
- decreased sample recoveries

Bear in mind the possible sources of contamination when choosing a cleaning method. As a standard method for cleaning the following procedure is recommended:

1. 1 ml 1 M NaCl
2. 1 ml 1 M NaOH
3. 1 ml 1 M HCl
4. 1 ml 1 M NaCl

It is sometimes possible to decrease the volume of each washing solution. Rinse with 0.5 ml water between each step.

Note: Do not store the column in 1 M HCl or 1 M NaOH.

If the peaks are broader or the retention times are longer after cleaning, saturate the column with a surplus of a suitable protein before applying the sample.

Depending on the character of the contaminant, the following cleaning solutions may also be appropriate:

- 1% TFA
- 30% CH_3CN
- 2 M NaOH, 1 M NaCl, 75% HAc, 1 M NaCl

If the column performance is still not restored, try injecting a solution of 1 mg/ml pepsin in 0.1 M acetic acid and 0.5 M NaCl and then leave it overnight at room temperature or 1 hour at 37 °C. Depending on the contamination, other enzymes can be used, e.g. DNAse. After this enzymatic treatment, repeat the cleaning process.

7. Trouble-shooting

7.1 Increased back-pressure

There are many causes of increased back-pressure. First check the tubings and, if used, Prefilter 0.8 mm or 3.2 mm (Code No. 18-1800-75 and 18-1800-76).

Note: Prefilters are not meant to substitute the sample treatment described in section 4.2.

If increased back-pressure is caused by the column, try to restore it by performing the following steps:

1. Reverse the flow direction through the column by installing it upside-down and pumping 0.5 ml of elution buffer through it at 0.05 ml/min.
2. Return to normal flow direction and run for 5 min at 0.24 ml/min.

Treatment with sonication for 5 minutes may in some cases reduce high back pressures.

If the problem persists, clean the column according to the procedure described in section 6.2.

7.2 Checking the column packing

Though not as critical in gradient as in isocratic techniques, a well-packed column is important for a good separation. For best performance, ensure that the column is clean and has been correctly installed.

If the column packing is the suspected cause of reduced resolution, repeat the function test described in Figure 3 and compare the chromatograms.

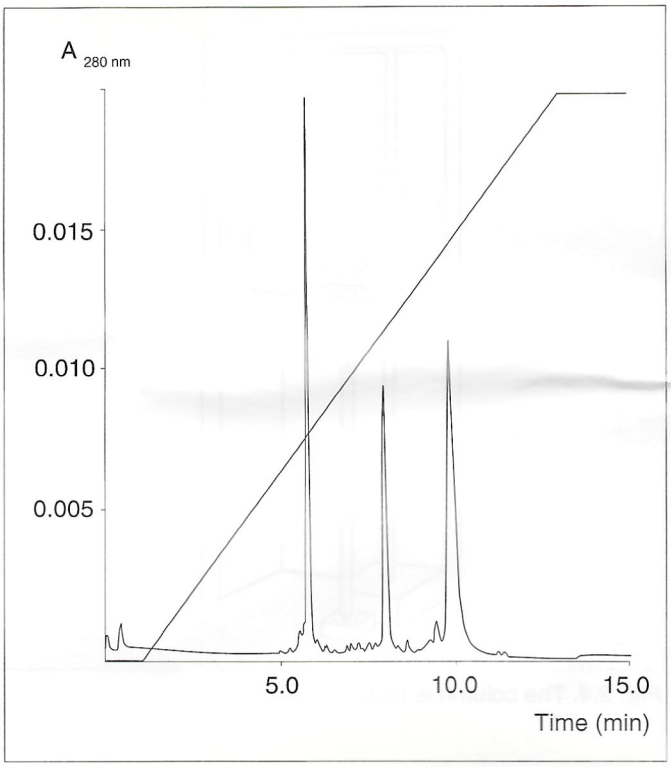


Fig. 3. Typical chromatogram from function test of Mini S PC 3.2/3.

Column:	Mini S PC 3.2/3
Instrument:	SMART® System
Sample:	Chymotrypsinogen A, Ribonuclease A, Lysozyme (1:3:1), 6 µg in 50 µl
Buffer A:	20 mM acetate-NaOH pH 5.0
Buffer B:	Buffer A with 0.4 M NaCl
Flow rate:	400 µl/min
Gradient:	0 - 100% B in 12 min

8. Further columns and equipment

8.1 Precision Columns

The current range of Precision Columns available. This range is listed below.

Column	Code No.
Ion exchange:	
Mini Q® PC 3.2/3	17-0686-01
Mini S® PC 3.2/3	17-0687-01
Mono Q® PC 1.6/5	17-0671-01
Mono S® PC 1.6/5	17-0672-01
Gel filtration:	
Superose® 6 PC 3.2/30	17-0673-01
Superose® 12 PC 3.2/30	17-0674-01
Superdex® 75 PC 3.2/30	17-0771-01
Superdex® 200 PC 3.2/30	17-1089-01
Superdex Peptide® PC 3.2/30	17-1458-01
Fast Desalting PC 3.2/10	17-0774-01
Reversed phase:	
µRPC C2/C18 PC 3.2/3	17-0703-01
µRPC C2/C18 SC 2.1/10	17-0704-01
Sephasil™ C8 SC 2.1/10	17-0769-01
Sephasil™ C18 SC 2.1/10	17-0904-01
Hydrophobic interaction:	
Phenyl Superose® PC 1.6/5	17-0772-01
Affinity chromatography:	
NHS-activated Superose® PC 3.2/2	17-0773-01

9.2 Other products

Sample loop 5 µl	18-1800-70
Sample loop 10 µl	18-1800-71
Sample loop 20 µl	18-1800-72
Sample loop 50 µl	18-1800-73
Sample loop 100 µl	18-1800-74
Sample loop 200 µl	18-1800-90
Sample loop 500 µl	18-1800-91
Sample loop 1000 µl + 2000 µl	18-5897-01
Union M6 female/1/16" male, plastic	18-3858-01
Superloop 10 ml	19-7585-01
Superloop 50 ml	19-7850-01
Superloop 150 ml	18-1023-83
Prefilter 0.8 mm	18-1800-75
Prefilter 3.2 mm	18-1800-76
Precision Column Holder	17-1455-01
Column Holder 10 cm	17-1550-01

9.3 Spare parts for glass columns

Protective caps (2/pk)	18-1015-28

Pharmacia Biotech

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