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Mono S® PC 1.6/5 INSTRUCTIONS

1. Introduction

The Precision Column PC 1.6/5 (0.10 ml) pre-packed with Mono S is designed for fast, sensitive and high-resolving cation exchange separations of proteins, peptides, polynucleotides and other biomolecules in the micropreparative scale.

These instructions may be used as a guide for obtaining the best results from this column. File them under "Column Instructions" in the system handbook.

Additional information about column treatment, methods etc. can be found in the method manual.

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

2. Important user information

- Filter buffers and samples to ensure long column life. (Section 5.2)
- Equilibrate the column before use. (Section 6.1)
- Use a flow rate within the recommended interval. (Section 6.2)
- Keep the column clean and store it properly. (Sections 6.3, 7.1 and 7.2)

3. General description

3.1 Gel properties

Mono S (Sulphonate) is a strong cation exchanger based on a beaded hydrophilic polymer. The monodispersity was accomplished through a unique process developed by Prof. John Ugelstad of SINTEF, Trondheim, Norway.

Mono S beads have a particle size of 10 ± 0.5µm. The absence of fines gives the packed column a large void volume (40%) and therefore a low back-pressure.

The charged group on the gel is $-CH_2SO_3^-$. Ionic capacity is 0.14-0.18 mmol/ml gel.

The maximum protein/peptide capacity is approximately 0.5 - 3 mg depending on the type of sample and running conditions. The practical loading range is 0.5-500 µg.

Non-specific adsorption to MonoBeads® is negligible.

Recovery of protein activity is normally greater than 80%.

3.2 Chemical and physical stability

Mono S PC 1.6/5 can be used with aqueous solutions in the pH range 2-12. It is resistant to solutions of urea, guanidine-HCl, ethylene glycol and similar compounds. The column is also stable in alcohol/water solutions.

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

Dimethyl sulphoxide, dimethyl formamide, formic acid and similar solvents at concentrations ≤ 70% can be used with great care.

All oxidizing and other reactive substances should be avoided.

The maximum flow rate for Mono S PC 1.6/5 is 0.4 ml/min, and the operational pressure limit is 5 MPa (50 bar,750 psi). When running at a flow rate of 0.1 ml/min the column generally operates at a back pressure of 1.0-1.5 MPa.

All material in the column hardware 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 e.g. by metal ions.

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

Table 1. Properties of Mono S PC 1.6/5.

Column dimensions	1.6 mm x 50 mm
Gel volume	0.10 ml
Charged group	$-CH_2SO_3^-$
Ionic capacity (K^+)	0.14-0.18 mmol/ml gel
Particle size	10 ± 0.5 µm
Molecular weight range	$\leq 10^7$
Maximum protein/peptide capacity	0.5 - 3 mg
Practical loading range	0.5-500 µg
Detection limit, one peak.	10 ng/280 nm
Flow rate*	0.01-0.4 ml/min
Sample volume	≤ 50 ml
Recovery of protein activity	≥ 80%
pH-stability working range	2-12
pH stability cleaning	2-14
Operational pressure limit*	5 MPa
Temperature interval	4-40 °C

* Distilled water, 25 °C

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Edition AC

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4. Installation

4.1 Unpacking

Please check the contents against this list:

Description	Code No.	No. per pack
Mono S® PC 1.6/5	17-0672-01	1
Instructions		1

4.2 Connection of 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.

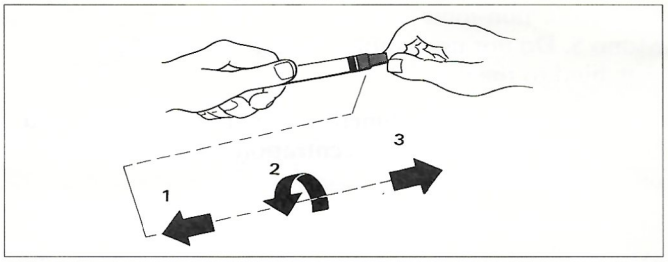


Fig. 1.

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

Follow the instructions in the software to install the column. Further information can be found in the system handbook. The procedure is illustrated in figures 2.1-2.4.

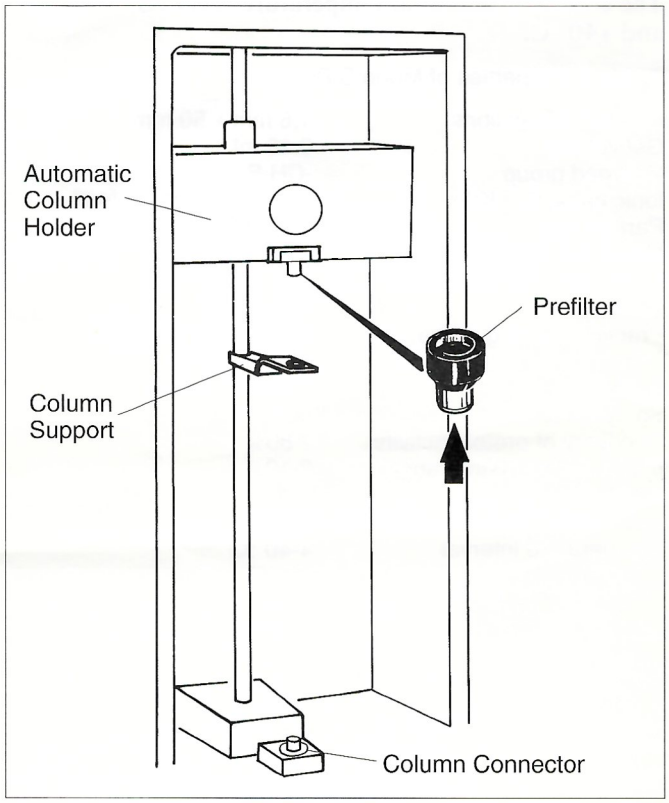


Fig. 2.1. Check that the Automatic Column Holder is high enough to install the column.

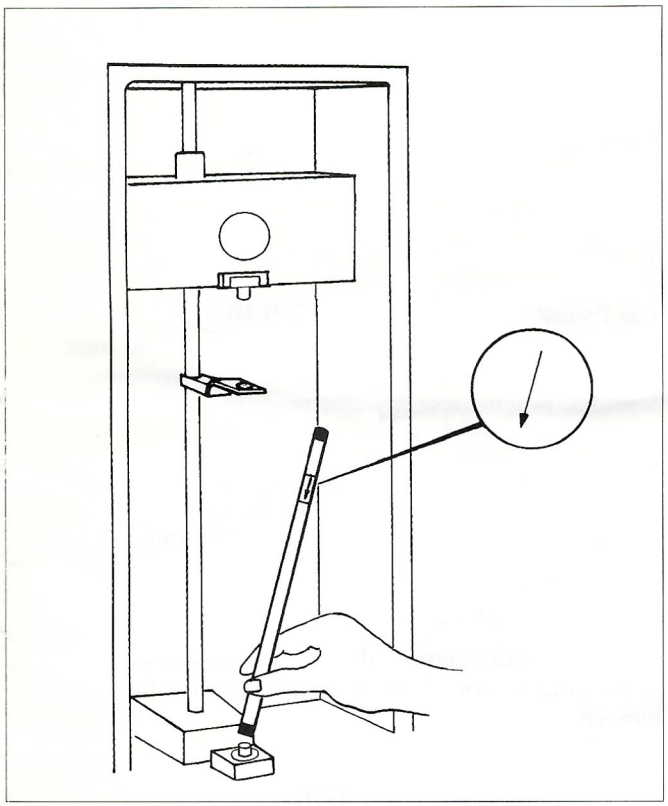


Fig. 2.2. Put the column on to the Column Connector. The arrow on the label, which should be pointing downwards, indicates the correct flow direction.

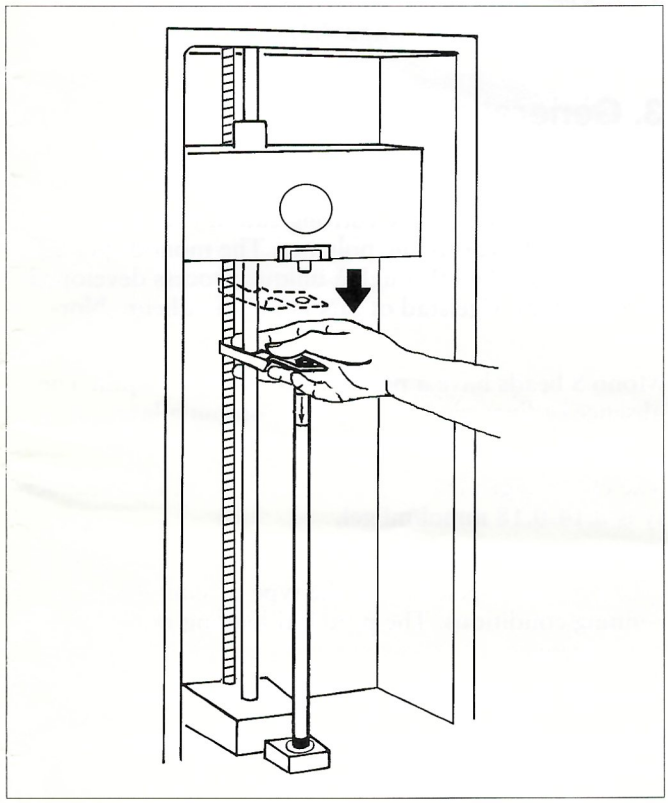


Fig. 2.3. Align the column vertically with the Column Support.

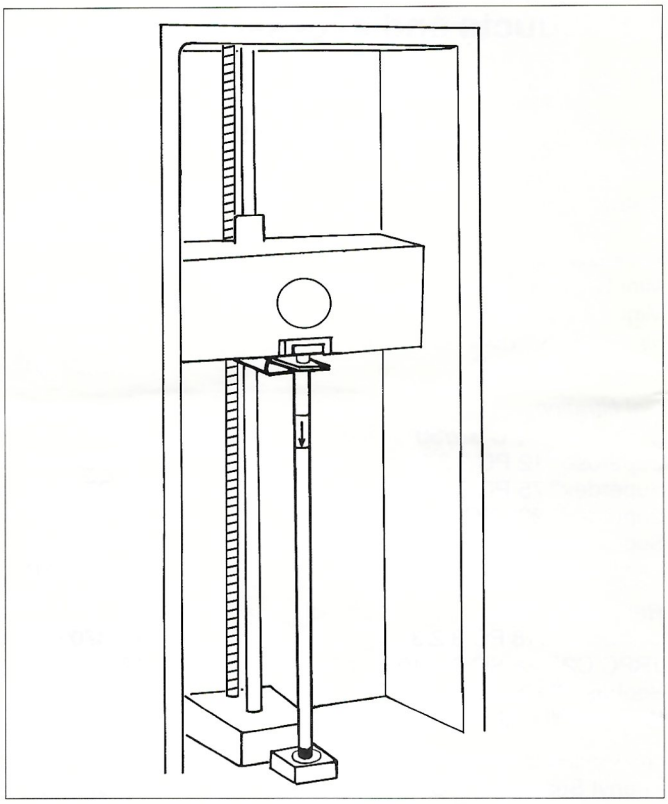


Fig. 2.4. The column is now connected.

5. Getting started

5.1 Choice of running conditions

Cationic 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 Mono S.

The pH of the buffer should be about 1 pH unit below the pI of the substances of interest. 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.

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 are two examples of buffer systems for cation exchange chromatography (provided the pI of the substance of interest is not too acid) :

1. Start buffer: 20 mM formic acid, pH 4.0
   Elution buffer: 20 mM formic acid, pH 4.0 + 0.5 M LiCl
2. Start buffer: 20 mM MES, pH 6.0
   Elution buffer: 20 mM MES, pH 6.0 + 0.5 M NaCl

Table 2 gives suggested buffers for various pH intervals. Operational pH intervals are approximately ± 0.5 pH units from pKa of the buffer.

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

Table 3. Cations and suggested concentrations for gradient elution.

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

Comment on table 3: Two millilitres is a reasonable gradient volume. Increase the cation concentration if the substance of interest is not eluted in the gradient.

Further information about chemical and physical stability is found in section 3.2.

Table 2. Suggested buffers for various pH intervals. Operational pH intervals are approximately ± 0.5 pH units from pKa of the buffer

Based on experiments performed in our laboratories and determined at 25°C.

Substance	pKa(25 °C)	dpKa/°C[1]	Conc.(mM)	Counter-ion[2]
Maleic acid	2.00		20	Na^+
Malonic acid	2.88		20	Na^+ / Li^+
Citric acid	3.13	-0.0024	20	Na^+
Formic acid	3.75	+0.0002	50	Na^+ / Li^+
Butanedioic acid	4.21	-0.0018	50	Na^+
Acetic acid	4.75	+0.0002	50	Na^+ / Li^+
MES	6.10	-0.0110	50	Na^+ / Li^+
Phosphate	7.20	-0.0028	50	Na^+
HEPES	7.48	-0.0140	50	Na^+ / Li^+
BICINE	8.26	-0.0180	50	Na^+

1. The pKa value changes if the temperature is lowered.
2. Sodium is the most commonly used counter-ion, alternatives are listed in table 3.

5.2 Preparation of buffers and samples

The water 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 for samples ≤ 200 µl (Code No. 17-0774-01). It is also possible to use the disposable columns, details of which can be found in section 9.2.

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 passed through 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 column life considerably.

6. Running procedure

6.1 Column equilibration

The gel is delivered in 20% ethanol with potassium (K^+) 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 4.2 and fill the system with the chosen buffers. Set the operational pressure limit to 5 MPa (50 bar, 750 psi).
2. Flush out the storage solution with 0.1 ml of start buffer at a flow rate of 0.1 ml/min.
3. Change to the desired counter-ion by washing with the eluent buffer. Continue until the system is completely free of air bubbles and the UV-signal is stable at the selected sensitivity. The minimum wash volume required is 0.5 ml.
4. Equilibrate until stable baseline or a minimum of 0.5 ml of start buffer.

6.2 Sample application and elution

Ensure that the sample has been prepared as described in section 5.2.

The maximum protein/peptide capacity for Mono S PC 1.6/5 is approximately 0.5 - 3 mg, as it is dependent on which proteins/peptides will be separated and which running conditions are used. Therefore a practical loading range is around 0.5-500 µg. This range minimizes the risk of losing non-bound material in the flow-through fractions, decreased resolution or detection problems.

The detection limit at 280 nm for a separate peak is about 10 ng. At 214 nm the detection limit is roughly 10 times lower.

The maximum sample volume is 50 ml. For volumes of 2 ml or less, use the sample loops listed in section 9.3. For volumes larger than 2 ml, use Superloop 10 ml or 50 ml (Code No.19-7585-01 or 19-7850-01).

The recommended flow rate is 0.1 ml/min, but it can be varied between 0.01-0.4 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 2 ml (0.1 ml/min, 20 min) is usually sufficient. If the protein/peptide of interest is not eluted in the gradient, then increase gradient-time and/or cation concentration.

6.3 Column regeneration

To start a new run, clean the column with 0.5 ml of elution buffer, before equilibrating with 0.5 ml of start buffer. This procedure can be included in the method.

If it is suspected that not everything has been eluted, inject 0.5 ml of a more concentrated salt solution (e.g. 1-2 M NaCl).

If detergents are not being used in the run, but have been included in previous ones, rinse the column with 0.5 ml of water before equilibrating with start buffer.

Should the column be dirty, then follow the cleaning procedures described in section 7.2 before equilibration is carried out.

7. Maintenance

7.1 Storage and prevention of microbial growth

Before storing for longer periods, wash the column sequentially with 0.5 ml of 1-2 M NaCl, 0.5 ml of water and 0.5 ml of 20 % ethanol. Check that the UV-signal is stable before proceeding to the next wash.

An alternative to ethanol is an anti-microbial agent in a storing buffer. This agent may be present in the buffers during chromatography, if so make sure it does not interact with the sample substances.

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

To prevent draining 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.

7.2 Column cleaning

The following observations may indicate a dirty column, which will require a more rigorous washing:

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

Use own knowledge of possible contamination when choosing a cleaning method. The following is a list of recommended washing solutions. Inject them through a 2 ml loop (Code No. 18-5897-01) on to the column (flow rate ≤ 0.1 ml/min depending on the back-pressure) and then rinse with 0.2-0.5 ml of water. Always ensure the baseline is stable before proceeding to the next wash.

- 2 ml 1 M NaCl
- 2 ml 2 M NaOH
- 2 ml 1 M HCl
- 2 ml 75% acetic acid
- 2 ml 1% TFA.
- 2 ml 0.5% SDS in 0.5 M NaOH
- 2 ml 30% acetonitrile
- 2 ml 0.5% non-ionic detergent (e.g. octylglucoside) in 3 M HAc

A common cleaning method used in our laboratories is:

1) 2 ml 2 M NaOH
2) 0.2 ml of water
3) 2 ml 1 M NaCl
4) 0.2 ml of water
5) 2 ml 75% HAc
6) 0.2 ml of water
7) 2 ml 1 M NaCl
8) 0.2 ml of water

If the column 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 the enzymatic treatment, repeat the cleaning process.

8. Quality control and trouble-shooting

8.1 Quality control

To guarantee that Mono S PC 1.6/5 is a product of the highest quality each column passes through an efficiency test, which shows that is has an approved theoretical plate number and that the correlation between flow rate and back-pressure is good.

8.2 Increased back-pressure

There are many reasons for increased back-pressure. One of the first checks should be 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 5.2.

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 0.2 ml of elution buffer through it at 0.05 ml/min.
2. Return to normal flow direction and run for 5 min at 0.1 ml/min.

If the problem persists, clean the column according to the description in section 7.2.

8.3 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.

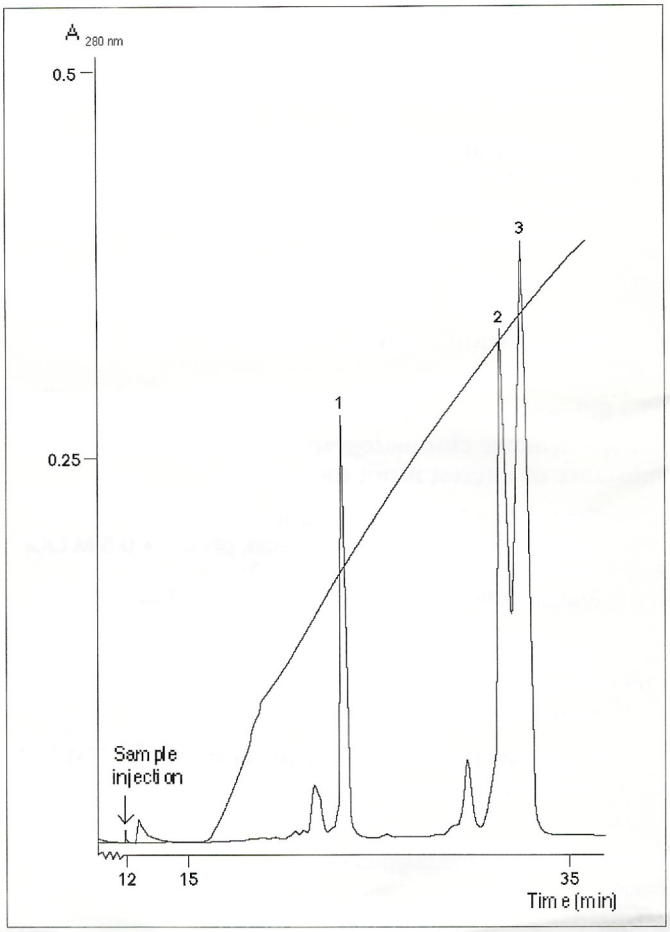


Fig. 3. Function test for Mono S PC 1.6/5

Sample: 25 µl of 1. Ribonuclease A, 1 mg/ml
2. Cytochrome C, horse heart, 0.5 mg/ml
3. Lysozyme, chicken egg white, 0.5 mg/ml
Buffer A: 0.01 M Hepes, pH 8.0
Buffer B: 0.01 M Hepes, 0.40 M LiCl, pH 8.0
Flow rate: 0.1 ml/min
Gradient: 2 ml
Detector: UV-M, 280 nm, 0.5 AUFS

9. Products and accessories

9.1 Precision Columns

Precision columns available. The column range is being expanded.

Column	Bed volume	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

Products	Code No.
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	18-3858-01
Superloop 10 ml	19-7585-01
Superloop 50 ml	19-7850-01
Superloop 150 ml	18-1023-85
Prefilter 0.8 mm	18-1800-75
Prefilter 3.2 mm	18-1800-76
Precision Column Holder	17-1455-01

9.3 Spare parts for the column

Products	Code No.
Protective caps (2/pk)	18-1015-28

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