µRPC C2/C18 SC 2.1/10

1. Introduction

The stainless steel column SC 2.1/10 (0.35 ml) prepacked with µRPC C2/C18 is designed for fast, sensitive, and high-resolving reversed phase separations of proteins, peptides, polynucleotides and other biomolecules in the micropreparative scale.

The SC column is designed for use with the SMART™ system. To be able to use the column with other high performance chromotography systems such as ÄKTA™ design, you need the Precision Column Holder (Code No 17-1455-01).

These instructions may be used as a guide for obtaining the best results from this column.

For further information about columns and equipment please contact your local representative for GE Healthcare.

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

µRPC C2/C18 is a porous microparticulate silica to which C2/C18 alkyl chains have been covalently bonded.

Particle size is 3 µm, and the average pore size is 120 Å.

The maximum protein/peptide capacity for µRPC C2/C18, SC 2.1/10 is 1–2 mg. However, to get an acceptable separation the practical loading range is usually 10 ng–500 µg depending on which peptides/proteins will be separated and which wavelength and running conditions are used. The detection limit for a separate peak at highest sensitivity is ≤ 1 ng. The amount of non-specific adsorption to µRPC C2/C18 is negligible.

3.2 Chemical and physical stability

µRPC C2/C18 SC 2.1/10 can be used with aqueous and organic solvents miscible in water in the pH-range 2–8. The degradation of the gel increases at pH values higher than 7–8 and lower than 2–3. Therefore, avoid long contact with eluents above pH 7.0 and below pH 2.0.

Additives like guanidine hydrochloride, urea, formic acid (≤ 60%), detergents and ion-pairing agents may be used.

The pressure limit over the column is equal to the system pressure limit for SMART System, 25 MPa (250 bar, 3625 psi). The recommended flow rate interval is 10–250 µl/min. It is possible to use higher flow rates but then the stability of the gel bed can not be guaranteed.

All material in the column hardware is made of stainless steel, fluoroplastics and titanium.

Table 1. Properties of µRPC C2/C18 SC 2.1/10

Column dimensions	2.1 mm × 100 mm
Gel volume	0.35 ml
Base matrix	Silica
Carbon chains	$-CH_2-CH_3$ $-(CH_2)_{17}-CH_3$
Particle size	3 µm
Efficiency, theoretical plates (N/m)*	≤ 100 000
Maximum protein/peptide capacity	1–2 mg
Practical loading range	10 ng–500 µg
Detection limit, one peak	≤ 1 ng
Flow rate	10–250 µl/min
pH-stability	2–8
Operational pressure limit	25 MPa (250 bar, 3625 psi)

* Measured in a special test system.

4. Installation

4.1 Unpacking

Please check the contents against this list:

Description	No. per pack	Code No.
µRPC C2/C18 SC 2.1/10	1	17-0704-01
Steerings for SC		17-0897-01
Instructions	1	

4.2 Connection of the column

To install the column, replace the sealing cap of the column inlet with the steering and remove the sealing cap of the column outlet. Use the commands in SMART Manager to install the column. Further information can be found in the system handbook. The procedure is illustrated in figures 1.1–1.4.

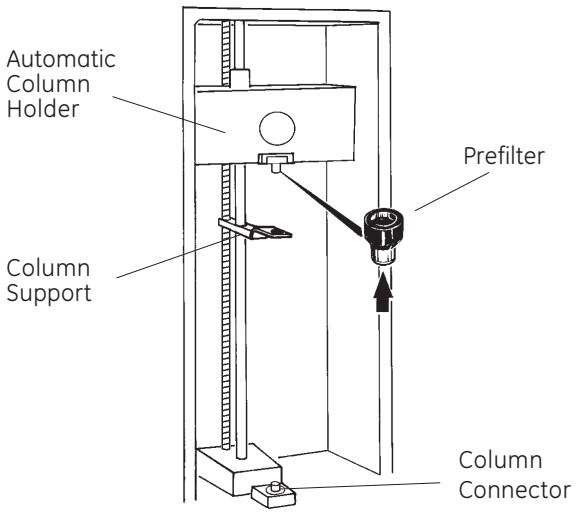

Fig 1.1. Check that the Automatic Column Holder is high enough to install the column. It is recommended to use Prefilter 0.8 mm or 3.2 mm (Code No. 18-1800-75 and 18-1800-76) to protect the column.

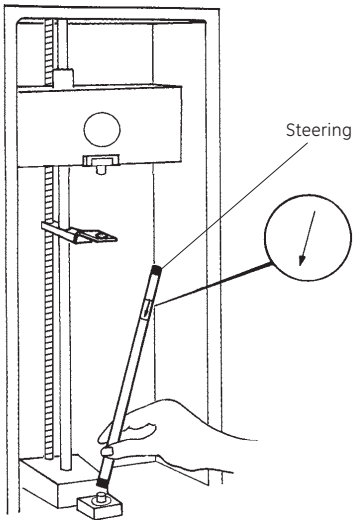

Fig 1.2. Put the column onto the Column Connector. The arrow on the label, which indicates the correct flow direction, should point downwards.

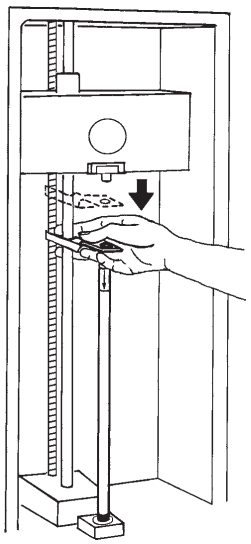

Fig 1.3. Align the column vertically with the Column Support.

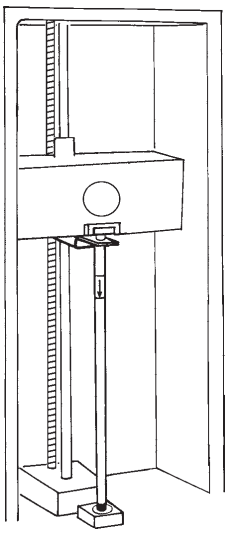

Fig 1.4. The column is now connected.

5. Getting started

5.1 Choice of running conditions

Reversed phase chromatography for biomolecules is normally performed by mixing an aqueous eluent and an organic solvent according to a programmed gradient elution.

The hydrophobicity of biomolecules depends on the ionization state of amino and carboxyl groups, and whether the molecules are denatured or not. To help achieve ion suppression a pH below 4 is recommended. Trifluoroacetic acid (TFA) at concentrations of ≤ 0.3% is often used since it also has an ion-pairing effect, increasing the hydrophobicity of the molecules.

A list of some suitable eluents for RPC is found in table 2.

The eluting power of an organic solvent depends on its concentration and polarity. The elution strength increases in the order water < methanol < ethanol < acetonitrile < 2- propanol < 1-propanol.

Most separations on µRPC C2/C18 can be performed using an acetonitrile gradient, but for very hydrophobic samples a propanols gradient could be preferable.

Note: *Propanols are much more viscous than acetonitrile and therefore give higher back pressures.*

Table 2. Some suitable eluents for RPC.

Eluent	Conc.	Comments
Volatile, low pH:		
Trifluoroacetic acid (TFA)	≤ 0.3%	Form ion-pairs Low UV-absorbance
Pentafluoroproprionic acid (PFPA)	≤ 0.3%	More hydrophobic than TFA
Heptafluorobutyric acid	≤ 0.3%	More hydrophobic than TFA
Perchloric acid	≤ 0.3%	
Ammonium acetate	10–50 mM	
Non-volatile, low or neutral pH:		
Phosphoric acid pKa_1= 2.12 pKa_2= 7.21	10–50 mM	Less hydrophobic than TFA. Adjust chosen pH with NaOH.

Comment to table 2: It is recommended that the pH be increased to more than 2.0 to prevent from degradation of the gel.

A list of suitable organic components for the mobile phase is found in table 3.

Table 3. Suitable organic components for the mobile phase.

Solvent	Comments
Acetonitrile	Good UV-transparency,low viscosity
Ethanol	Viscous, rather less effective eluent than acetonitrile.
Methanol	Quite viscous, half to two-thirds eluting power of acetonitrile
2-propanol	Very viscous, adequate UV-transparency. (iso-propanol) More powerful eluent than acetonitrile.
1-propanol (n-propanol)	Very viscous, adequate UV transparency. More powerful eluent than acetonitrile.

The following is an example of a suitable eluent system for reversed phase chromatography:

Eluent A: 0.1% TFA in water
Eluent B: 0.1% TFA in acetonitrile

Detergents should be used with great care as they may adsorb to the gel matrix because of hydrophobic interactions. If running a detergent-containing sample, first inject a blank to see what peaks the detergent will give.

5.2 Preparation of eluents and samples

The water used for the eluents should be of an ultra pure quality. Use HPLC grade solvents, salts and buffers. Degas and filter all solutions through a 0.22 µm filter. Be sure to select a solvent resistant filter when the eluent contains an organic solvent.

Samples containing high salt- or high urea/guanidine hydrochloride concentrations may be applied directly on to the column. It may have a negative effect on the application if the sample is dissolved in a large volume with high concentrations of organic solvents or detergents. In these cases, a concentration by evaporation or an eluent exchange is recommended.

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 contains 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 is extremely dilute and there is a risk of sample loss due to adsorption to 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 of the column becoming blocked.

Never apply a turbid solution (indicates sample insolubility).

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

6 Running procedure

6.1 Column equilibration

The column contains in 20% ethanol. To equilibrate the column for first time use, after long term storage or when changing solvents, 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 eluents.
2. Flush out the storage solution with a minimum of 0.6 ml of eluent B (e.g. 0.1% TFA in acetonitrile) at a flow rate of 200 µl/min. Continue until the system is completely free of air bubbles and the UV-signal is stable at the selected sensitivity.
3. Run a 5 minute gradient from 100% down to 0% eluent B.
4. Equilibrate with at least 1.5 ml of eluent A (e.g. 0.1% TFA in water).

6.2 Sample application and elution

Ensure that the sample has been prepared according to the description in section 5.2.

The practical loading range for µRPC C2/C18 SC 2.1/10 is approximately 10 ng–500 µg, while the detection limit for a separate peak is ≤ 1 ng. It is dependent on which peptides/proteins will be separated and which wavelength and running conditions are used. Generally it is possible to load larger amounts of proteins than peptides. Within this loading range the risk of losing non-bound material in the flow-through fractions, decreased resolution or detection problems is minimized.

The sample volume is of minor importance when gradient elution is used, but can affect the resolution of early eluting components. For volumes of 2 ml or less, use the sample loops listed in section 9.2. For volumes > 2 ml, use Superloop™ 10 ml or 50 ml (Code No.19-7585-01 or 19-7850-01)

A recommended flow rate is in the interval 10–250 µl/min. It is possible to use a higher flow rate, but then the stability of the gel bed cannot be guaranteed.

Note: *Depending on the sample composition it may be necessary to lower the flow rate during sample application to avoid a high backpressure.*

Use either linear or stepwise gradients for elution.
A suggested start gradient for separations of peptides/proteins is 0–100 % eluent B in a volume of 5 ml (200 µl/min, 25 min). Lower flow rates and more shallow gradients usually improve the resolution. If the peptide/protein of interest is not eluted in the gradient, then change to a more hydrophobic organic component.

6.3 Column regeneration

To start a new run, follow the procedure in 6.1 (points 3–4). This procedure can be included in the method.

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

7. Maintenance

7.1 Storage and prevention of microbial growth

After daily use of the column, wash it with a minimum of 3.5 ml of 20 % ethanol or a pure organic solvent.

Check that the UV-signal is stable before finishing the wash.

To ensure maximum column life, do not store the column in solvents containing TFA or other additives.

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 that requires washing:

- increased backpressure (20–50% increase from the first time)
- loss of resolution
- decreased sample recoveries

Proceed as follows:
Wash the column by running a 30 minute gradient 0% B – 100% B – 0% B at a flow rate of 50 µl/min. If the column is not fully cleaned, the procedure can be repeated.

Eluent A: 0.1% TFA in water.
Eluent B: 0.1% TFA in acetonitrile or iso-propanol

Note: *If iso-propanol is used the backpressure is considerably higher.*

8. Trouble-shooting

8.1 Quality control test on µRPC C2/C18 SC 2.1/10

To guarantee that µRPC C2/C18 SC 2.1/10 is a high quality product that gives reproducible results, the following quality control tests are carried out:

- Each column passes an efficiency test, which shows that it has an approved theoretical plate number and good correlation between flow rate and backpressure.
- Each lot undergoes a function test which is described in Figure 2.

8.2 Increased back-pressure

There are many reasons for increased backpressure. The first check 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 backpressure 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 run 0.6 ml of eluent B through it at 50 µl/min.
2. Return to normal flow direction and run for 6 min at 100 µl/min.

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

8.3 Checking the column function

To check the column function, repeat the function test described in figure 2 and compare the chromatograms. For best performance ensure that the column is clean and has been correctly installed.

Sample:	1. Angiotensin III Ile^{7}
	2. Angiotensin III Val^{4}
	3. Angiotensin III
	4. Angiotensin I
	500 ng of each peptide in a total volume of 5 µl.
Eluent A:	0.1 % TFA in 10 % acetonitrile
Eluent B:	0.1 % TFA in 90 % acetonitrile
Flow rate:	180 µl/min
Gradient:	15–25 % B in 10 min
Detector:	214 nm

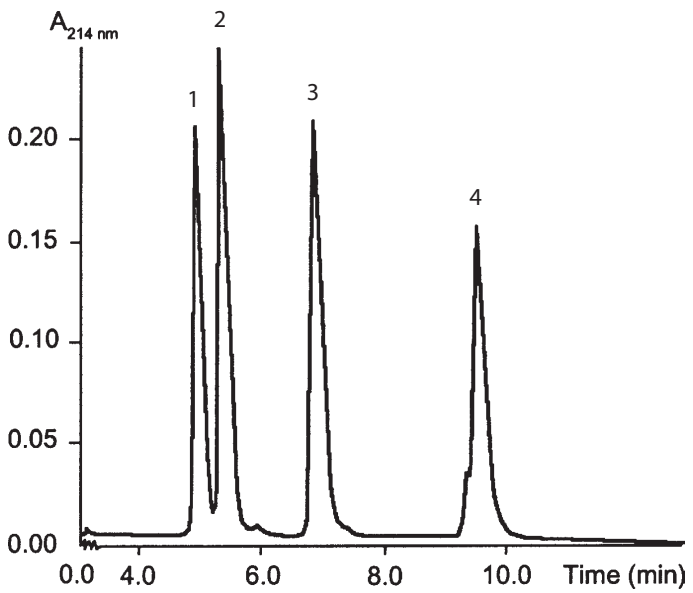

Fig 2. Function test for µRPC C2/C18 SC 2.1/10.

9. Products and accessories

9.1 Precision Columns

Column	Bed volume	Code No.
Mono Q™ PC 1.6/5	0.10 ml	17-0671-01
Mono S™ PC 1.6/5	0.10 ml	17-0672-01
Mini Q™ PC 3.2/3	0.24 ml	17-0686-01
Mini S™ PC 3.2/3	0.24 ml	17-0687-01
Superose™ 6 PC 3.2/30	2.4 ml	17-0673-01
Superose™ 12 PC 3.2/30	2.4 ml	17-0674-01
Superdex™ 75 PC 3.2/30	2.4 ml	17-0771-01
Superdex™ 200 PC 3.2/30	2.4 ml	17-1089-01
Superdex Peptide PC 3.2/30	2.4 ml	17-1458-01
µRPC C2/C18 SC 2.1/10	0.35 ml	17-0704-01

9.2 Other products

Products	Code No.
Sample loops, teflon (5, 50, 100, 200, 500 µl)	18-0404-01
Sample loops, 1 ml and 2 ml	18-5897-01
Union, M6 Female/1/16" Male	18-3858-01
Superloop 10 ml	19-7585-01
Superloop 50 ml	19-7850-01
Precision Column Holder	17-1455-01
Prefilter 0.8 mm	18-1800-75
Prefilter 3.2 mm	18-1800-76

9.3 Spare parts

Product	No. per pack	Code No.
Steerings for SC	2	17-0897-01
Sealing cap	2	17-0879-01

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