Superdex

Superdex Peptide PE 7.5/300

Quick Information

Superdex Peptide PE 7.5/300 is a pre-packed PEEK column for high performance size exclusion chromatography of natural, recombinant or synthetic peptides and other small biomolecules.

Read the Instruction

The instructions on this page will help you get started quickly with your new column. The back of this instruction gives more in-depth information on optimisation and trouble shooting.

Column data

Matrix	Cross-linked agarose and dextran	
Bed volume	Approx. 13 ml	
Column efficiency, H^{-1}	>30 000	
Exclusion limit, M_w		
aqueous buffer, peptides	20 000	
pH stability	**Regular use**	**Cleaning**
	pH 1-14	pH 1-14
Temperature	**Regular use**	**Storage**
	4-40 °C	4-30 °C
Pressure over column	**Regular use**	**Do not exceed!**
	0.6-1.2 MPa	1.5 MPa
	6-12 bar	15 bar
	90-180 psi	220 psi
Flow rate, water at room temperature	**Regular use**	**Maximum**
	0.1-0.5 ml/min	0.7 ml/min

First time use

Equilibration of the column for first time use or after long time storage:

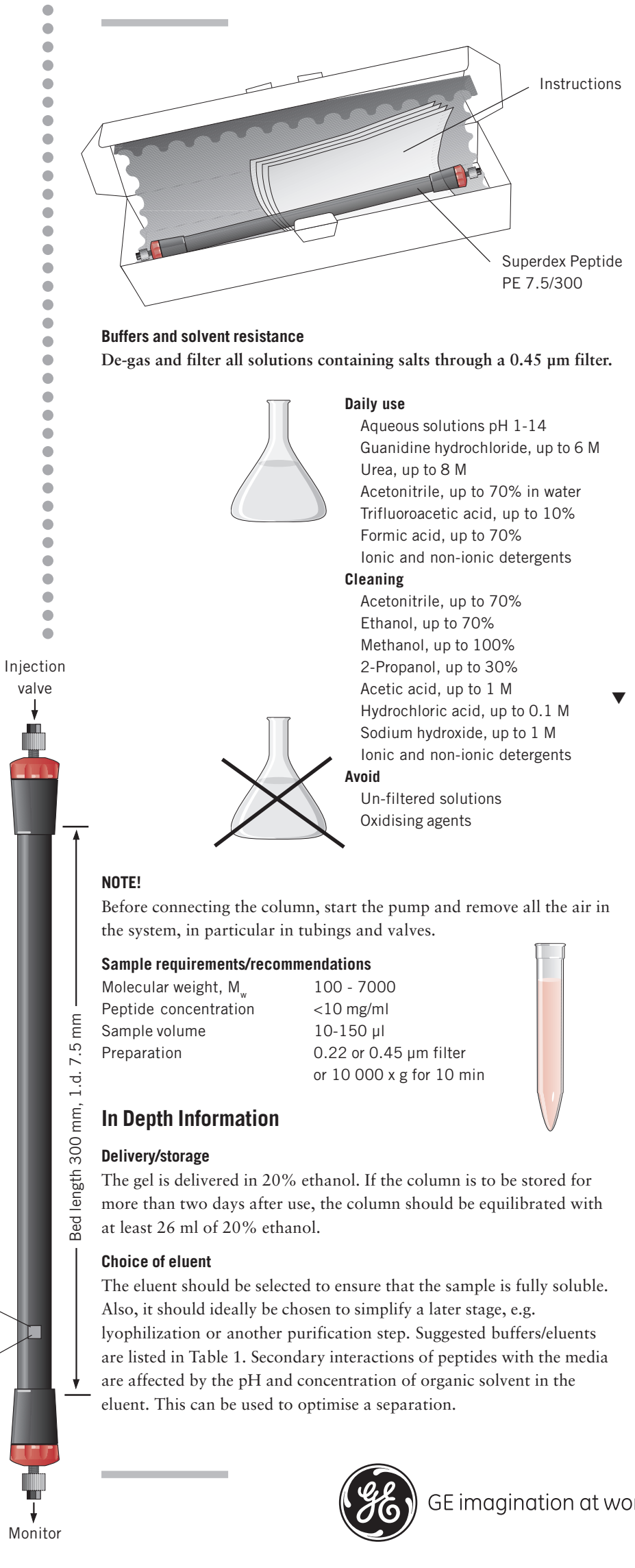
Equilibrate with at least 26 ml eluent buffer at 0.25 ml/min. Ensure that the back pressure over the column does not exceed 1.5 MPa (15 bar, 220 psi).

Try these conditions first

Equilibrate with at least 26 ml eluent buffer at 0.5 ml/min. Longer equilibration may be needed with eluents containing detergents. Equilibration is not necessary between runs with the same eluent buffer.

Eluent:	0.1% TFA in 30% acetonitrile
Flow rate:	0.5 ml/min, room temperature
Sample volume:	10 µl

Please read the back of this instruction for information on optimising a separation.



Buffers and solvent resistance

De-gas and filter all solutions containing salts through a 0.45 µm filter.

Daily use

- Aqueous solutions pH 1-14
- Guanidine hydrochloride, up to 6 M
- Urea, up to 8 M
- Acetonitrile, up to 70% in water
- Trifluoroacetic acid, up to 10%
- Formic acid, up to 70%
- Ionic and non-ionic detergents

Cleaning

- Acetonitrile, up to 70%
- Ethanol, up to 70%
- Methanol, up to 100%
- 2-Propanol, up to 30%
- Acetic acid, up to 1 M
- Hydrochloric acid, up to 0.1 M
- Sodium hydroxide, up to 1 M
- Ionic and non-ionic detergents

Avoid

- Un-filtered solutions
- Oxidising agents

NOTE!

Before connecting the column, start the pump and remove all the air in the system, in particular in tubings and valves.

Sample requirements/recommendations

Molecular weight, M_w	100 - 7000
Peptide concentration	<10 mg/ml
Sample volume	10-150 µl
Preparation	0.22 or 0.45 µm filter
	or 10 000 x g for 10 min

In Depth Information

Delivery/storage

The gel is delivered in 20% ethanol. If the column is to be stored for more than two days after use, the column should be equilibrated with at least 26 ml of 20% ethanol.

Choice of eluent

The eluent should be selected to ensure that the sample is fully soluble. Also, it should ideally be chosen to simplify a later stage, e.g. lyophilization or another purification step. Suggested buffers/eluents are listed in Table 1. Secondary interactions of peptides with the media are affected by the pH and concentration of organic solvent in the eluent. This can be used to optimise a separation.

Table 1. Suggested buffers/eluents.

pH	Buffer/Eluent	Properties/Application example
1.0	70% formic acid	For strongly hydrophobic peptides. Poor UV transparency at 214 nm. Volatile.
1.3	0.05 M HCl	Good solubility for peptides. Good UV transparency.
5.0	0.1 M ammonium acetate	Volatile
7.0	0.05 M phosphate+ 0.15 M NaCl	Physiological conditions
7.8	0.15 M ammonium hydrogen carbonate	Volatile. Should be used fresh.
	Acetonitrile 30%+ TFA 0.1%	For separation of hydrophobic compounds

Optimisation

Standard protocol

Start with a high flow rate, e.g. 0.5 ml/min and a sample volume of 10 µl.

If the results are unsatisfactory, consider the following:

Action	Effect
Change flow rate	Improved resolution with lower flow rate for high molecular weight components. Decreased resolution with lower flow rate may be seen for small components.
Smaller sample volume	Improved resolution.
Change pH	Selectivity change.
Change concentration of organic solvent	Selectivity change.

For more information, please refer to the two poster reprints listed below.

Column cleaning

After 10-20 separation cycles, a regular cleaning cycle should be performed.

1. Wash the column with 13 ml 0.5 M sodium hydroxide or 30-70% acetonitrile at a flow rate of 0.25 ml/min.
2. Rinse the column with 26 ml of water at a flow rate of 0.5 ml/min.
3. Before the next run, equilibrate the column with at least 26 ml eluent buffer until the UV base line and pH are stable.

If you suspect that the column is still contaminated, refer to the "Trouble shooting" section below.

Trouble shooting

Symptom	Remedy
Increased back pressure over column and/or loss of resolution	Fill the column with a cleaning agent and allow it to stand for 2 hours in room temperature. 70% Acetonitrile, 1 M sodium hydroxide, 1 M acetic acid and/or 0.1 M hydrochloric acid may be used. Fill the column with pepsin 1 mg/ml in 0.1 M acetic acid including 0.5 M NaCl and leave it at room temperature overnight or 1 hour at 37 °C. After enzymatic treatment, repeat steps 1-3 in the cleaning procedure described in the section "Column cleaning" above.
Air in the column	Reverse the flow direction and pump 40-50 ml of well de-gassed eluent buffer at a flow rate of 0.25 ml/min. The quality of the packed bed will not normally be affected.

DO NOT OPEN THE COLUMN!

Column performance control

We recommend checking the column performance at regular intervals. The efficiency of the column can be checked with the following procedure.

Sample: 25 µl acetone, 5 mg/ml
Eluent: Distilled water
Flow rate: 0.5 ml/min, room temperature
Detection: 0.5 AUFS, 254 nm

Column efficiency, expressed as plater per metre H^{-1}, is calculated using following equation

$$H^{-1}=5.54 \times (v_r/w_h)^2 \times (1000/L)$$

where

H^{-1}= number of theoretical plates/metre
L= bed height (mm)
v_r= peak elution distance (mm)
w_h= peak width at half peak height (mm)

An alternative to the efficiency test to check column performance is to run a function test. We recommend the test described in figure 1.

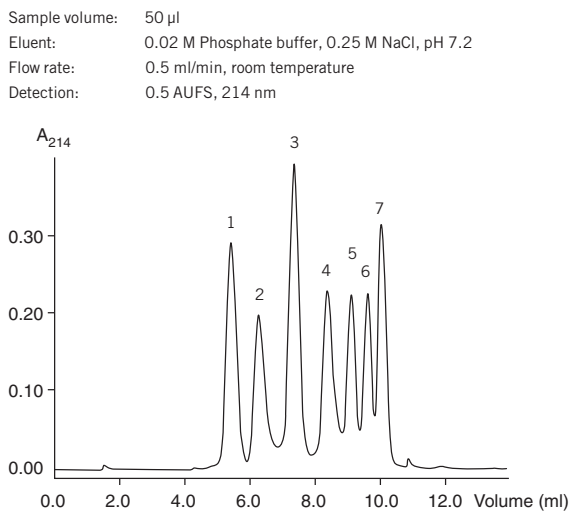


Fig 1. Typical chromatogram from function test of Superdex Peptide PE 7.5/300.

Sample:

1. Cytochrome C	0.2 mg/ml	M_w 12 500	Boehringer Mannheim 103870
2. Aprotinin	0.2 mg/ml	M_w 6 500	SIGMA A-1153
3. Gastrin I	0.2 mg/ml	M_w 2 126	SIGMA G-1276
4. Substance P	0.2 mg/ml	M_w 1 348	SIGMA S-6883
5. $(Glycine)_6$	0.2 mg/ml	M_w 360	SIGMA G-5630
6. $(Glycine)_3$	0.2 mg/ml	M_w 189	SIGMA G-1377
7. Glycine	8 mg/ml	M_w 75	SIGMA G-7126

Accessories

Designation	No. per pack	Code No.
Tubing connectors:		
Union 1/16" female/ M6 male (HPLC to FPLC)	6	18-1112-57
Union M6 female/ 1/16" male (FPLC to HPLC)	8	18-1112-58
Poster reprints:		
Superdex Peptide I	1	18-1060-84
Superdex Peptide II	1	18-1060-85

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