SOURCE

SOURCE 15Q PE 4.6/100

Quick Information

SOURCE™ 15Q PE 4.6/100 is a prepacked PEEK column for anion exchange chromatography. It gives fast and high capacity preparative separations of proteins, peptides, polynucleotides and other biomolecules.

Read these instructions

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

Column data

Type of exchanger:	Strong anion	
Matrix:	Polystyrene/divinyl benzene	
Bead structure:	Rigid, spherical, porous monodisperse	
Particle size:	15 µm	
Bed volume:	Approx. 1.7 ml	
Recommended loading (proteins):	≤ 35 mg/column	
pH stability range:	**Long term**	**Short term**
	2-12	1-14
Temperature range:	**Regular use**	**Storage**
	+4 to +40 °C	+4 to +30 °C
Pressure over column:	**Regular use**	**Maximum**
	0.5–2.5 MPa	4 MPa
	5–25 bar	40 bar
	70–360 psi	580 psi
Flow rate:	**Recommended**	**Maximum**
(water at room temperature)	0.5-2.5 ml/min	5.0 ml/min

First time use

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

1. 5 ml starting buffer at 1.0 ml/min.
2. 5 ml elution buffer at 2.0 ml/min.
3. 5 ml starting buffer at 2.0 ml/min.

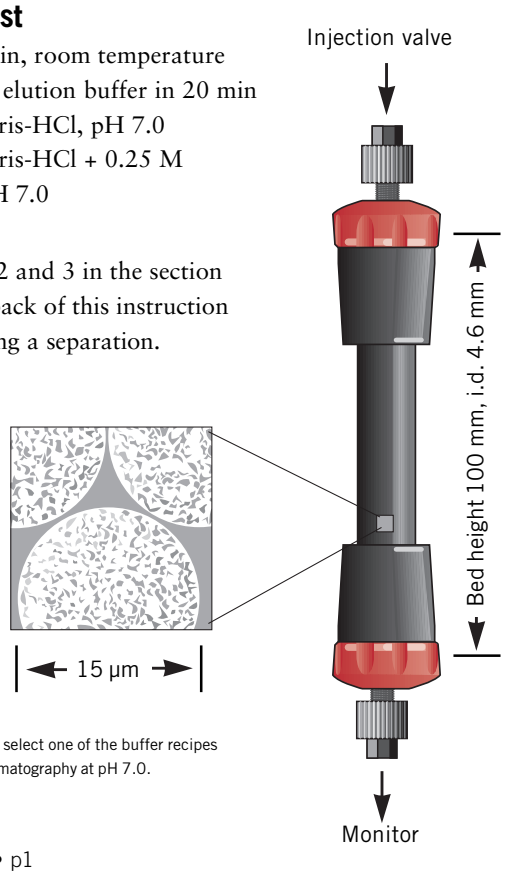
Note: Before connecting the column to a chromatography system, start the pump and remove all air in the system, in particular tubings and valves.

Try these conditions first

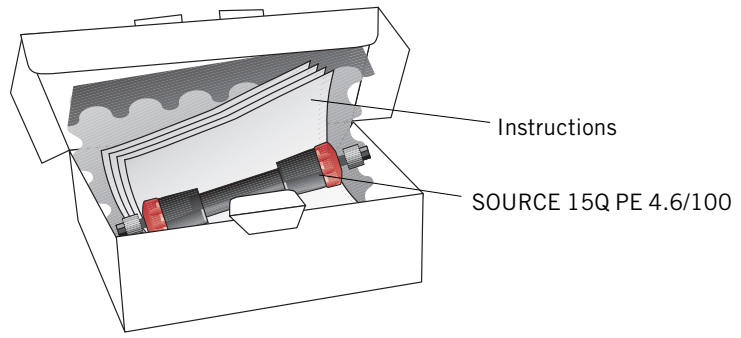
Flow rate:	0.5 ml/min, room temperature
Gradient:	0–100% elution buffer in 20 min
Starting buffer*:	20mM Tris-HCl, pH 7.0
Elution buffer*:	20mM Tris-HCl + 0.25 M NaCl, pH 7.0

Equilibration before a run:

Proceed according to steps 2 and 3 in the section "First time use". Read the back of this instruction for information on optimising a separation.



* If you are using ÄKTA™design system, select one of the buffer recipes recommended for anion exchange chromatography at pH 7.0.



Solutions and solvents

De-gas and filter all buffers through a 0.45 µm filter.

Daily use

Aqueous solutions pH 2–12
Urea, up to 8 M
Acetonitrile, up to 30% in aqueous buffers
Cationic and non-ionic detergents

Cleaning

Acetonitrile, up to 100%
Ethanol, up to 70%
Methanol, up to 100%
2-propanol, up to 30%
Hydrochloric acid, up to 1 M
Sodium hydroxide, up to 2 M
Acetic acid, up to 50%
Guanidine hydrochloride, up to 8 M
Cationic and non-ionic detergents

Avoid

Unfiltered solutions
Oxidising agents
Anionic detergents

Sample requirements/recommendations

Net charge of target molecule:	negative
Recommended sample load:	≤ 35 mg protein/column
Sample preparation:	Filtered through a 0.45 µm filter or centrifuged at 10 000 g for 10 min

The sample should be dissolved in starting buffer.

In depth information

Delivery/storage

The gel is supplied in 20% ethanol. If the column is to be stored for more than two days after use, wash the column with 10 ml distilled water and then equilibrate with at least 10 ml 20% ethanol.

Choice of eluent

Aqueous buffers: Standard aqueous buffer solutions; refer to Fig. 1.
Volatile buffers: Table 1 lists examples of volatile buffer systems.

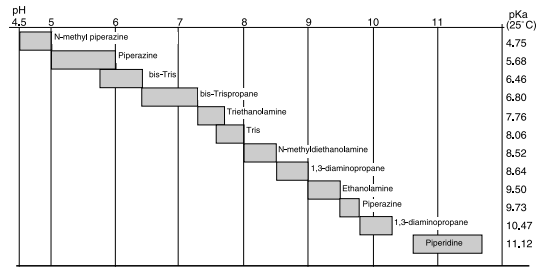


Fig 1. Recommended buffer substances for anion exchange chromatography.

Amersham Biosciences

Table 1. Volatile buffer systems.

pH	Substance
2.3-3.5	Pyridine/formic acid
3.0-5.0	Trimethylamine/formic acid
4.0-6.0	Trimethylamine/acetic acid
6.8-8.8	Trimethylamine/HCl
7.0-8.5	Ammonia/formic acid
8.5-10.0	Ammonia/acetic acid
7.0-12.0	Trimethylamine/CO_2
8.0-9.5	Ammonium carbonate/ammonia
8.5-10.5	Ethanolamine/HCl

Optimisation

Standard protocol

A first run can be performed as described in the section "Try these conditions first". If the results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer (see Fig. 1 for buffers)	Selectivity change, weaker/stronger binding
Change salt, counter-ion and/or co-ion	Selectivity change
Decrease the sample load	Improved resolution
Decrease the flow rate	Improved resolution
Use a shallower gradient	Improved resolution, but broader peaks and decreased concentration in fractions

Fore more information, please refer to the handbook "Ion Exchange Chromatography, Principles and Methods" available from Amersham Biosciences or to the "Method Handbook" supplied with each ÄKTAdesign system.

Column cleaning

Regular cleaning:

After each run, inject 5 ml 2 M NaCl to elute material still bound to the column.

If detergents have been used, rinse the column with 5 ml distilled water followed by 2 ml 2 M NaCl.

Before the next run, re-equilibrate the column with at least 5 ml starting buffer until the UV base-line and pH/conductivity values are stable.

More rigorous cleaning:

Reverse the flow direction and run at a flow rate of 0.2 ml/min with the following sequence of washing solutions:

Rinse with 5 ml distilled water between each step.

1. 5 ml 1 M NaCl
2. 5 ml 1 M NaOH
3. 5 ml 1 M HCl
4. 5 ml 1 M NaCl

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

Depending of the nature of the contaminants, the following cleaning solutions may also be appropriate:
30% 2-propanol
30% acetonitrile
2 M NaOH including 1 M NaCl
50% acetic acid
1% trifluoroacetic acid

Always rinse with 5 ml distilled water when any of the above cleaning solutions has been used.

If column performance is still not restored, inject a solution of 1 mg/ml pepsin in 0.1 M acetic acid including 0.5 M NaCl and leave overnight at room temperature or 1 hour at 37 °C. Depending on the contamination, other enzymes can also be used, e.g. DNase. After enzymic treatment, repeat steps 1-4 in the rigorous cleaning procedure described above. Wash with elution buffer before equilibration with starting buffer and new sample application.

Trouble-shooting

Symptom	Remedy
Increased back-pressure over the column	Using reverse flow at 0.5 ml/min, pump 15 ml elution buffer through the column. Then return to normal flow direction and run for 10 min at 2.0 ml/min.
Loss of resolution and/or decreased sample recovery	Follow the procedure described in the section "More rigorous cleaning"
Air in the column	Reverse the flow direction and pump 20 ml of well de-gassed starting buffer at a flow rate of 0.5 ml/min.

DO NOT OPEN THE COLUMN!

Column performance control

We recommend checking the column performance at regular intervals. The function of the column can be checked as described in Figure 2.

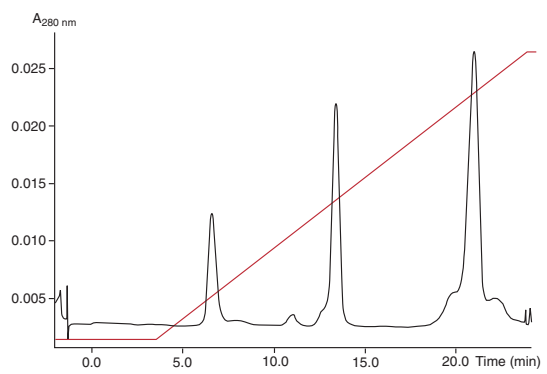


Fig 2. Typical chromatogram from a function test of SOURCE 15Q PE 4.6/100.

Ordering information

Designation	No. per pack	Code No.
SOURCE 15Q PE 4.6/100	1	17-5065-01
Related products		
SOURCE 15S PE 4.6/100	1	17-5067-01

Accessories

Designation	No. per pack	Code No.
Union M6 female/1/16" male	8	18-1112-58
On-line Filter	1	18-1118-01
Handbook: "Ion Exchange Chromatography, Principles and Methods"	1	18-1114-21

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