

# SOURCE 5RPC ST 2.1/150

## Quick Information

SOURCE™ 5RPC ST 2.1/150 is a pre-packed stainless steel column for analytical reversed phase chromatography of peptides. The column is designed for use with ÄKTA™ design and other high performance liquid chromatography systems.

## 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:	Polystyrene/divinyl benzene	
Bonded phase:	None	
Bed form:	Rigid, spherical, porous, monodisperse	
Particle size:	5 µm	
Bed volume:	0.5 ml	
Max. capacity for peptides:	Bacitracin (Mr 1400) approx. 80 mg/ml medium	
Typical peak width at base:	0.1 ml	
pH stability*:	1–12	
Temperature:	<b>Regular use</b>	<b>Storage</b>
	+4 to +60 °C	+4 to +30 °C
Pressure over column:	Do not exceed 400 bar (5800 psi, 40 MPa)	
Flow rate:	Regular use 0.2 ml/min	

\* Extreme pH may effect the stainless steel column.

## First time use

**Equilibrate the column before initial use, after long term storage, or after changing eluent:**

1. Flush out the storage solution with at least 3.5 ml (7 CV\*\*) of eluent A.
2. Run an 18 ml (36 CV) linear gradient from 0 to 100% eluent B. Continue with 100% B until the UV signal base line is stable.
3. Run a 7.5 ml (15 CV) linear gradient from 100 to 0% eluent B.
4. Equilibrate the column with at least 3.5 ml (7 CV) of eluent A.

Eluent A: 10mM ammonium acetate, pH 7 in 2% acetonitrile

Eluent B: 70% acetonitrile

\*\*CV = Column Volume = 0.5 ml

**Note:** Before connecting the column, start the pump and remove all air in the system, including the tubes and valves.

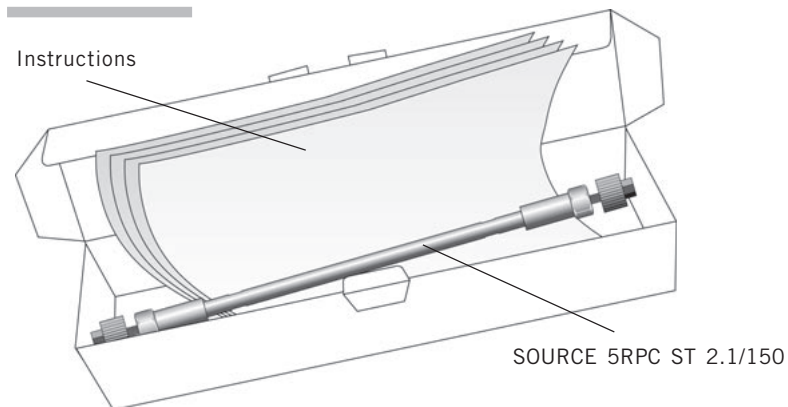
## Try these conditions first

### Equilibration before a new run:

Proceed according to steps 2 and 3 in the section “First time use”.

Eluent A:	10mM ammonium acetate, pH 7 in 2% acetonitrile
Eluent B:	70% acetonitrile
Flow rate:	0.2 ml/min
Gradient:	0–100% B
Gradient volume:	10 ml (20 CV)

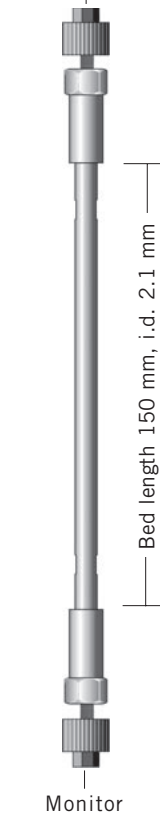
The pH of the eluents will affect selectivity. For other eluent systems, please refer to the section “Choice of eluent” on the reverse of these instructions, or to the handbook “Reversed phase chromatography, Principles and Methods” available from Amersham Biosciences, (see Accessories).



## Solutions and solvents

The water used for the eluent should be Milli-Q™ or corresponding quality. Use HPLC grade solvents and salts. Filter all solutions containing salts through a 0.45 µm filter.

Injection valve



Monitor

### Daily use/Cleaning

Aqueous solutions pH 2–12,  
Trifluoroacetic acid (TFA), up to 0.3%  
Pentafluoropropionic acid, up to 0.3%  
Heptafluorobutyric acid, up to 0.3%  
Perchloric acid, up to 0.3%  
Formic acid, up to 0.3%  
Acetic acid, up to 60%  
Ammonium acetate 10–50 mM  
Phosphoric acid 10–50 mM

Water miscible organic solvents,  
Methanol  
Ethanol (will create high back-pressure, reduce flow rate)

Acetonitrile  
1-Propanol (will create high back pressure, reduce flow rate)  
2-Propanol

Ion-pairing agents,  
Trifluoroacetic acid  
Tributylphosphate  
Triethylammonium phosphate  
Tetrabutylammonium salts  
Hexylsulphate

### Avoid

Detergents  
Solutions < pH 1  
Solutions > pH 12  
Oxidising agents

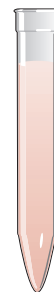


## Sample requirements/recommendations

Preparation 0.45 µm filter or centrifugation at 10 000 g for 10 min.

Recommended sample load See max capacity for peptides.

Dissolve the sample in eluent A.



## In Depth Information

### Delivery/storage

The column is supplied in 87% acetonitrile. At the end of the working day, flush the column with at least 2 ml of eluent B.

### Choice of eluent

SOURCE 5RPC ST 2.1/150 is a reversed phase chromatography column with excellent performance over a wide pH range, 1–12. This gives increased control of selectivity and in many cases, improved solubility and yield of active sample components. The column shows particularly good resolution at basic pH, and is superior to most other

media for the separation of peptides in the range pH 8–10. This latter aspect makes SOURCE 5RPC ST 2.1/150 especially useful for peptides that are insoluble or difficult to dissolve at lower pH and also adds another selectivity range for peptides normally separated at lower pH.

### Acidic pH

Eluent A: 0.065% trifluoroacetic acid (TFA) in 2% acetonitrile  
 Eluent B: 0.050% TFA in 80% acetonitrile

### Basic pH

Eluent A: 0.125% ammonium solution pH 10 in 2% acetonitrile  
 Eluent B: 80% acetonitrile

Note: Eluent A must always contain at least 2% acetonitrile.

Note: The recommended equilibration volume for the column is at least 7 CV.

### Optimization

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	Alters selectivity
Change organic solvent	Alters selectivity/alters retention time. Ranking of elution power: 2-propanol > acetonitrile > ethanol > methanol
Change ion-pairing agent	Alters selectivity
Lower flow rate	Increases resolution
Use a shallower gradient	Improves resolution Broader peaks

For more information, please refer to the handbook “Reversed Phase Chromatography. Principles and Methods” available from Amersham Biosciences, or the “Method handbook” supplied with each ÄKTAdesign system.

### Alternative buffer suggestions for SOURCE 5RPC columns

Eluent A	pH 2.0	0.1% TFA, 2% acetonitrile in water
	pH 4.5	0.01 M Na-acetate, 2% acetonitrile buffer
	pH 7.0	0.01 M Potassium phosphate, 2% acetonitrile buffer
	pH 9.0	0.01 M Tris-HCl, 2% acetonitrile buffer
	pH 12	0.01 M NaOH, 2% acetonitrile buffer
Eluent B	70% acetonitrile	

### Column cleaning

#### Regular cleaning:

Clean the column regularly, we recommend the following procedure:

1. Run a 10 CV (5 ml) gradient from 10 to 80% acetonitrile.
2. Continue with 80% acetonitrile for 10 CV.
3. Run a 5 CV gradient from 80 to 5% acetonitrile.

#### Rigorous cleaning

For more rigorous cleaning, we recommend the following procedure:

1. Wash with 10 CV (5 ml) 90% acetic acid, at 0.1 ml/min.
2. Wash with 10 CV 50 mM NaOH, at 0.1 ml/min.
3. Equilibrate with 10 CV 5% acetonitrile.

#### After cleaning

Before the next run, equilibrate the column according to the procedure in the section “First time use”.

### Trouble shooting

Symptom	Remedy
Increased back pressure	Reverse the column, pump 2 ml of eluent B at a flow rate of 0.1 ml/min. Return to normal flow direction and run for 5 minutes at 0.2 ml/min.
Loss of resolution	Follow the procedure described in the section “Column cleaning”.
Decreased sample recovery	Follow the procedure described in the section “Column cleaning”.

### DO NOT OPEN THE COLUMN!

### Column performance control

We recommend checking column function at regular intervals.

Use the following procedure:

Sample mixture:	(Ile <sup>7</sup> ) Angiotensin III	0.125 mg/ml	(SIGMA A-0911)
	(Val <sup>4</sup> ) Angiotensin III	0.125 mg/ml	(SIGMA A-6277)
	Angiotensin III	0.125 mg/ml	(SIGMA A-0939)
	Angiotensin I	0.125 mg/ml	(SIGMA A-9650)
Sample volume:	2 µl		
Eluent A:	10 mM NaOH		
Eluent B:	70% acetonitrile		
Flow rate:	0.2 ml/min		
Gradient:	20–50% B over 6 CV		
Detection:	214 nm		

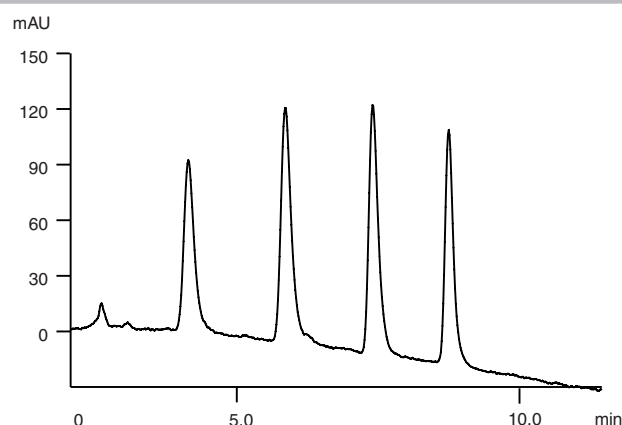


Figure 1. Typical chromatogram from a function test of: SOURCE 5RPC ST 2.1/150.

### Ordering information

Designation	No. per pack	Code No.
SOURCE 5RPC ST 2.1/150	1	17-5208-01
SOURCE 5RPC ST 4.6/150	1	17-5116-01

### Accessories

Designation	No. per pack	Code No.
Fingertight connector 1/16"	10	18-1112-55
Union 1/16 male/male I.D. 0.25 mm	2	18-1120-92
PEEK tubing i.d. 0.25 mm o.d. 1/16"	2 m	18-1120-95
Handbook: “Reversed Phase Chromatography, Principles and Methods”	1	18-1134-16

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