SOURCE 15PHE 4.6/100 PE

Instructions for Use

Quick information

SOURCE™ 15PHE 4.6/100 PE is a Tricorn™ high performance column. The column is a pre-packed PEEK column for rapid, preparative hydrophobic interaction chromatography of proteins and oligonucleotides.

Column data

Matrix	Polystyrene/divinyl benzene
Ligand	Phenyl
Bead form	Rigid, spherical, porous monodisperse
Particle size	15 µm
Column dimensions	4.6 x 100 mm
Bed volume	1.7 ml
Loading capacity	At least 40 mg albumin
(will vary depending on sample and loading conditions)	
(will vary depending on sample and loading conditions)	
pH stability range	
regular use	2 to 12
cleaning	1 to 14
Temperature	
operating	4 °C to 40 °C
storage	4 °C to 30 °C
Flow rate (water at room temperature)	
recommended	0.5–2.5 ml/min
maximum	5 ml/min
Pressure over column	
maximum	4 MPa, 40 bar, 580 psi

First-time use

Equilibrate the column for first-time use or after long term storage as follows:

Step	Action
1	8 ml distilled water at 1 ml/min at room temperature.
2	8 ml elution buffer at 2 ml/min at room temperature.
3	8 ml start buffer at 2 ml/min at room temperature.
	Note: *Before connecting the column to a chromatography system, start the pump to remove all air and debris from the system, particularly in the tubing and valves.*

Try these conditions first

Flow rate:	2 ml/min at room temperature
Gradient:	0-100% elution buffer in 20 column volumes (CV)
Start buffer[1]:	50 mM phosphate buffer, 1.5 M (NH4)2SO4, pH 7.0
Elution buffer:	50 mM phosphate buffer, pH 7.0

[1] Use a lower concentration of ammonium sulfate if the target protein begins to precipitate at this concentration.

Equilibration between runs:

Proceed according to steps 2 and 3 in the section *First-time use, on page 1*. Extended equilibration may be needed if detergents are included in the eluent. Read the section *Optimization, on page 2* for information about how to optimize a separation.

Buffers and solvent resistance

Install an on-line filter upstream of the injection valve. Buffers and solvents with increased viscosity will affect the backpressure and flow rate. De-gas and filter all solutions through a 0.22 µm filter.

Daily use

All commonly used aqueous buffers, pH 2–12

Urea, up to 8 M

Acetonitrile, up to 30% in aqueous buffers

Cleaning

Acetonitrile, up to 30%

Sodium hydroxide, up to 2 M

Ethanol, up to 100%

Methanol, up to 100%

Acetic acid, up to 50%

Isopropanol, up to 100 %

Hydrochloric acid, up to 1 M

Guanidine hydrochloride, up to 6 M

Anionic, cationic and non-ionic detergents

Avoid

Unfiltered solutions

Oxidizing agents

Sample recommendations

Recommended initial sample load	≤ 40 mg
Preparation	Dissolve the sample in start buffer, filter through a 0.22 µm filter or centrifuge at 10 000 x g for 10 min
Temperature[1]	Ambient

[1] Hydrophobic interactions increase with increased temperature. Results achieved at room temperature may therefore not be reproduced in a cold room, or vice versa.

In-depth information

Delivery/storage

The column is delivered in 20% ethanol. If the column is to be stored for more than two days after use, wash the column with 8 ml distilled water and then equilibrate with at least 8 ml of 20% ethanol.

Choice of eluent

In general, the adsorption process is often more selective than the desorption process. It is therefore important to optimise the binding buffer (start buffer) conditions with respect to:

- pH
- salt concentration
- type of salt

The combination of salt and pH can be manipulated to give optimum selectivity. Optimal conditions differ from application to application and are best established by running linear gradients and by varying the salt concentration and pH in the start buffer. The buffers given in the section *Try these conditions first, on page 1* are recommended as the first choice. The Hofmeister series, see Table below, gives guidelines in choosing the type of salt to use. The most efficient salts are normally ammonium sulfate (up to 2 M) and sodium sulfate (up to 1 M), but "weaker" salts such as sodium chloride (up to 4 M) can also be considered.

Table 1. The Hofmeister series.

← Increasing binding effect									
Anions:	PO_4^{3-} >	SO_4^{2-} >	CH_2COO^-	Cl^- >	Br^- >	NO^{3-} >	ClO_4^- >	I^- >	SCN^-
Cations:	NH_4^+ >	Rb^+ >	K^+ >	Na^+ >	Cs^+ >	Li^+ >	Mg^+ >	Ca^{2+} >	Ba^{2+}
← Increasing binding effect									

Optimization

Perform a first run as described in the section *Try these conditions first, on page 1*. If the obtained results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (see *Choice of eluent, on page 2*)	Gives weaker/stronger binding.
Change salt (see *Choice of eluent, on page 2*)	Changes selectivity.
Decrease the sample load	Improves resolution.
Decrease the flow rate	Improves resolution.
Change gradient slope	Shallower gradients improve selectivity but broadens peaks (decreased efficiency). A steeper gradient will sharpen peaks, but move them closer together.

For more information, please refer to the handbook *"Hydrophobic Interaction Chromatography, Principles & Methods"*, which can be ordered from Cytiva, or to the *"Method Handbook"* supplied with each ÄKTA™ system.

Cleaning-in-place (CIP)

Regular cleaning:

Wash the column with 8 ml distilled water after each run. Re-equilibrate the column with at least 8 ml start buffer until the UV base-line and pH/conductivity values are stable.

More rigorous cleaning:

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

1. 5 ml 30% isopropanol
2. 5 ml 1 M NaOH

Note: *To avoid precipitation, rinse with 5 ml of distilled water between each wash.*

Do not store the column in 1 M NaOH.

Depending on the nature of the contaminants, the following cleaning solutions may also be appropriate:

30% Acetonitrile

2 M NaOH including 1 M NaCl

70% Ethanol

0.5% non-ionic detergent in 1 M acetic acid

Note: *Always rinse with at least 3 ml distilled water when any of the above cleaning solutions has been used. If detergents have been used, rinse with at least 8 ml 70% ethanol followed by 5 ml distilled water before equilibrating the column.*

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 one hour at 37 °C. Depending on the contaminant, other enzymes may also be used, e.g. DNase. After enzymatic treatment, repeat steps 1–2 in the section *More rigorous cleaning:, on page 2* described above. After cleaning, equilibrate the column in the normal flow direction before use.

DO NOT OPEN THE COLUMN!

Troubleshooting

Symptom	Remedy
Increased back-pressure over the column	Reverse the flow direction and pump 8 ml elution buffer at a flow rate of 0.5 ml/min through the column. Return to normal flow direction and run for 5 minutes at a flow rate of 2 ml/min. If high back-pressure persists, clean the column.
Loss of resolution and/or decreased sample recovery	Clean the column according to the procedure described in the section *More rigorous cleaning:, on page 2*.
Air in the column	Reverse the flow direction and pump 20 ml of well de-gassed start buffer through the column at a flow rate of 0.5 ml/min.

Column performance control

Check the function of the column when new by running the separation described in *Fig. 1, on page 3*. Figure 1 shows a typical chromatogram on an optimised system. Since the system can profoundly effect the resolution it is most meaningful to compare runs done on the same system. Check the column at regular intervals and whenever you suspect a problem.

Column SOURCE 15PHE 4.6/100 PE

Sample:	1. Myoglobin (2 mg/ml)	SIGMA M-0630
	2. Ribonuclease A (8 mg/ml)	SIGMA r-5000
	3. Lysozyme (2 mg/ml)	SIGMA L-6876
	4. α-chymotrypsiogen A (3.2 mg/ml)	SIGMA C-4879
Sample volume:	200 µL	
Gradient:	0–100% elution buffer in 20 CV	
Start buffer:	100 mM phosphate buffer, 2 M $(NH_4)_2SO_4$, pH 7.0	
Elution buffer:	100 mM phosphate buffer, pH 7.0	
Flow rate:	0.5 ml/min	

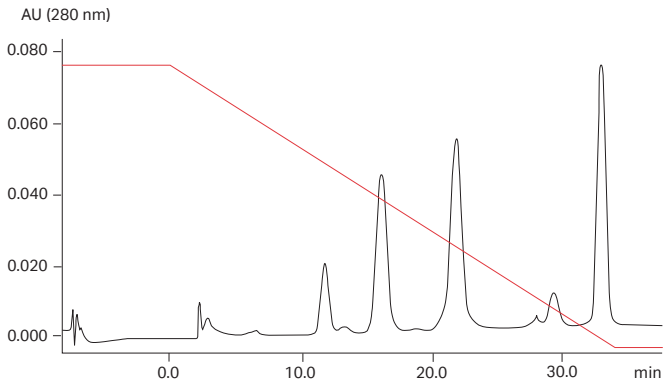

Fig 1. Typical chromatograms from a function test of SOURCE 15PHE 4.6/100 PE

Ordering information

Designation	No. per pack	Code No.
SOURCE 15PHE 4.6/100 PE	1	17518601

Related products

Designation	No. per pack	Code No.
RESOURCE™ ETH	1	17118401
RESOURCE ISO	1	17118501
RESOURCE PHE	1	17118601
HiTrap™ Desalting	5 x 5ml	17140801

Accessories

Designation	No. per pack	Code No.
Tubing connectors:		
Fingertight connector 1/16" male	10	18111255
Union M6 female/1/16" male	8	18111258
Handbook:		
Hydrophobic Interaction Chromatography, Principles & Methods	1	18102090

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