SOURCE 15Q 4.6/100 PE and SOURCE 15S 4.6/100 PE

High performance columns Instructions for Use

Quick information

SOURCE™ 15Q 4.6/100 PE and SOURCE 15S 4.6/100 PE are Tricorn™ high performance columns. The columns are prepacked PEEK columns for high performance ion exchange chromatography of proteins, peptides, polynucleotides, and other biomolecules.

Column data

Matrix	Spherical and monodisperse, porous, rigid, polystyrene/divinyl benzene particles	
Mean particle diameter[1]	~ 15 µm	
Column dimensions	4.6 x 100 mm	
Bed volume	1.7 mL	
Maximum loading capacity (will vary depending on sample and loading conditions)	40 mg	
Recommended operating flow rate[2]	0.5 to 2.5 mL/min	
Maximum operating flow rate[2]	5 mL/min	
Maximum pressure over column	4 MPa, 40 bar, 580 psi	
	SOURCE 15Q	**SOURCE 15S**
pH stability, operational[3]	2 to 12	2 to 13
pH stability, CIP[4]	1 to 14	1 to 14
pH ligand fully charged[5]	Entire pH range	Entire pH range
Dynamic binding capacity, Q_{B50}	~ 45 mg BSA/mL resin[6]	~ 80 mg Lysozyme/mL resin[7]
Temperature		
operating	4 °C to 40 °C	4 °C to 40 °C
storage	20% ethanol, 4 °C to 30 °C	20% ethanol with 0.2 M sodium acetate, 4 °C to 30 °C
Type of exchanger	Strong anion	Strong cation
Charged group	$O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}N^+(CH_3)_3$	$\text{-}O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}SO_3$

1 Monodisperse size distribution.

2 At room temperature using buffers with the same viscosity as water.

3 pH range where resin can be operated without significant change in function.

4 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

5 pH range where ligand is fully charged; although ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges.

6 Dynamic binding capacity at 50% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/50 column at 5 cm bed height (1 min residence time) for BSA in 20 mM BisTrisPropane, pH 7.0.

7 Dynamic binding capacity at 50% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/50 column at 5 cm bed height (1 min residence time) for Lysozyme in 20 mM Sodium phosphate, pH 6.8

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 start buffer at 1 mL/min at room temperature.
3	8 mL elution buffer at 2 mL/min at room temperature.
4	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 (SOURCE 15Q)[1]:	20 mM Tris-HCl, pH 8.0
Elution buffer (SOURCE 15Q)[1]:	20 mM Tris-HCl + 1.0 M NaCl, pH 8.0
Start buffer (SOURCE 15S)[1]:	20 mM 2-[N-morpholino] ethanesulphonic acid (MES), pH 6.0
Elution buffer (SOURCE 15S)[1]:	20 mM MES + 1.0 M NaCl, pH 6.0

[1] Users of ÄKTA™ design system can select one of the buffer recipes recommended for anion exchange chromatography at pH 8 or cation exchange chromatography at pH 6.

Equilibration between runs:

Proceed according to steps 3 and 4 in the section *First-time use, on page 1*. Extended equilibration might be needed if detergents are included in the eluent. Read the section *Optimization, on page 3* 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. Degas and filter all solutions through a 0.22 µm filter.

Daily use

Stable to commonly used aqueous buffers, pH 2 to 12

Urea, up to 8 M

Acetonitrile, up to 30% in aqueous buffers

Nonionic detergents

Cationic detergents (SOURCE 15Q)

Anionic detergents (SOURCE 15S)

Cleaning

Acetonitrile, up to 100%

Sodium hydroxide, up to 1 M

Ethanol, up to 100%

Methanol, up to 100%

Isopropanol, up to 100%

Hydrochloric acid, up to 1 M

Guanidine hydrochloride, up to 6 M

Avoid

Unfiltered solutions

Oxidizing agents

Anionic detergents (SOURCE 15Q)

Cationic detergents (SOURCE 15S)

Sample recommendations

Net charge of target molecule	negative (SOURCE 15Q), positive (SOURCE 15S)
Recommended initial sample load	≤ 35 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.

In-depth information

Delivery/storage

SOURCE 15Q 4.6/100 PE is delivered in 20% ethanol. SOURCE 15S 4.6/100 PE is delivered in 20% ethanol containing 0.2 M sodium acetate. 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 SOURCE 15Q 4.6/100 PE with at least 8 mL 20% ethanol or SOURCE 15S 4.6/100 PE with at least 8 mL 20% ethanol containing 0.2 M sodium acetate.

Choice of eluent

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select an eluent with buffering ions of the same charge as the substituent groups on the ion exchanger.

Choose the start buffer pH so that substances to be bound to the ion exchanger are charged, e.g., at least 1 pH unit above the isoelectric point for anion exchangers and at least 1 pH unit below the isoelectric point for cation exchangers. The figures below list a selection of standard aqueous buffers. *Table 1, on page 3* lists suggested volatile buffers that can be used in cases where the purified substance has to be freeze-dried.

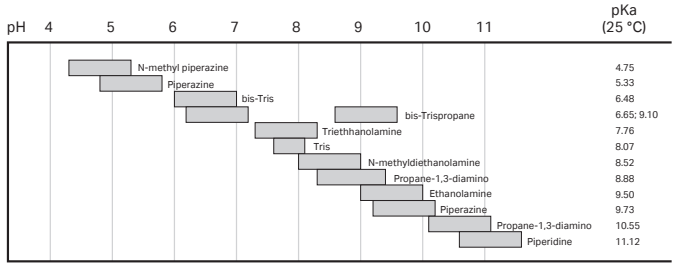

Fig 1. Recommended buffers for anion exchange chromatography

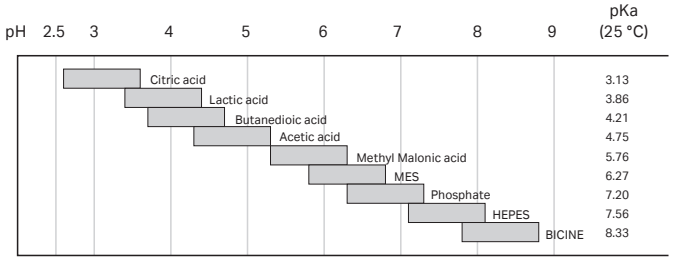

Fig 2. Recommended buffers for cation exchange chromatography

Table 1. Volatile buffer systems

pH	Substance
3.3–4.3; 4.8–5.8	Pyridine/formic acid
3.3–4.3; 9.3–10.3	Trimethylamine/formic acid
4.3–5.8	Pyridine/acetic acid
3.3–4.3; 8.8–9.8	Ammonia/formic acid
4.3–5.3; 8.8–9.8	Ammonia/acetic acid
5.9–6.9; 9.3–10.3	Trimethylamine/carbonate
5.9–6.9; 8.8–9.8	Ammonium carbonate/ammonia
4.3–5.3; 7.2–8.2	N-ethylmorpholine/acetate

Optimization

Perform a first run as described in the section *Try these conditions first, on page 2*. If the results obtained are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (see *Fig. 1, on page 2* and *Fig. 2, on page 2* for buffers)	Changes selectivity, gives weaker/stronger binding.
Change salt, counter ions and/or co-ions	Changes selectivity.
Decrease the sample load	Improves resolution.
Decrease the flow rate	Improves resolution.
Change gradient slope	Shallower gradients improve selectivity but broaden peaks (decrease efficiency). A steeper gradient will sharpen peaks, but move them closer together.

For more information, please refer to the handbook *"Ion Exchange Chromatography, Principles and 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 3 mL 2 M NaCl after each run to elute material still bound to the column. If detergents have been used, rinse the column with 8 mL distilled water followed by 3 mL 2 M NaCl. Re-equilibrate the column until the UV baseline and pH/ conductivity values are stable (usually at least 10 CV).

More rigorous cleaning:

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

Step	Action
1	7 mL 1 M NaCl
2	7 mL 1 M NaOH
3	7 mL 1 M HCl
4	7 mL 1 M NaCl
	Note: *Always rinse with at least 3 mL distilled water between each step.*

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

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

30% Acetonitrile

30% Isopropanol

Note: *Always rinse with at least 3 mL distilled water when any of the above cleaning solutions has been used.*

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 backpressure 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 3*.
Air in the column	Reverse the flow direction and pump 20 mL of well degassed start buffer through the column at a flow rate of 0.5 mL/min.

Column performance control

Check the performance of the column when new by running the separation described in the figure below and *Fig. 4, on page 4*. The figures show a typical chromatogram on an optimized system. Since the system can profoundly affect the resolution it is more meaningful to compare runs done on the same system. Check the column at regular intervals and whenever you suspect a problem.

Column SOURCE 15Q 4.6/100 PE

Sample:	1. α-Amylase (3 mg/mL) 2. α-Lactalbumin (4 mg/mL) 3. Trypsin inhibitor (6 mg/mL)
Sample volume:	200 µL
Gradient:	0–100% elution buffer in 20 CV
Start buffer:	20 mM Tris-HCl, pH 7.0
Elution buffer:	20 mM Tris-HCl+ 0.25 M NaCl, pH 7.0
Flow rate:	0.5 mL/min (room temperature)

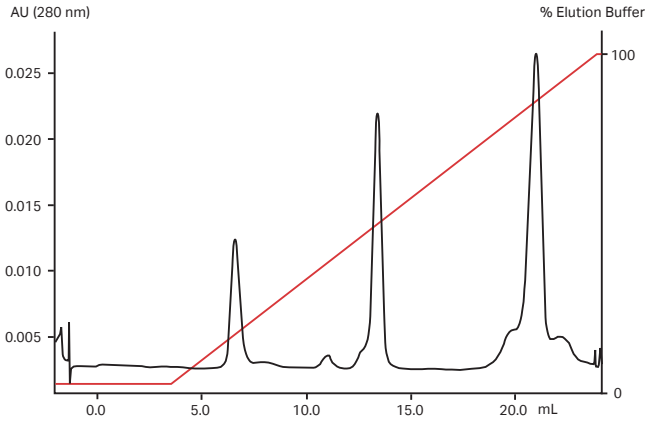

Fig 3. Typical chromatograms from a function test of SOURCE 15Q 4.6/100 PE

Column SOURCE 15S 4.6/100 PE

Sample:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.4 mg/mL) 3. Lysozyme (0.4 mg/mL)
Sample volume:	200 µL
Gradient:	0–100% elution buffer in 12 CV
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	20 mM sodium phosphate + 0.4 M NaCl, pH 6.8
Flow rate:	0.5 mL/min (room temperature)

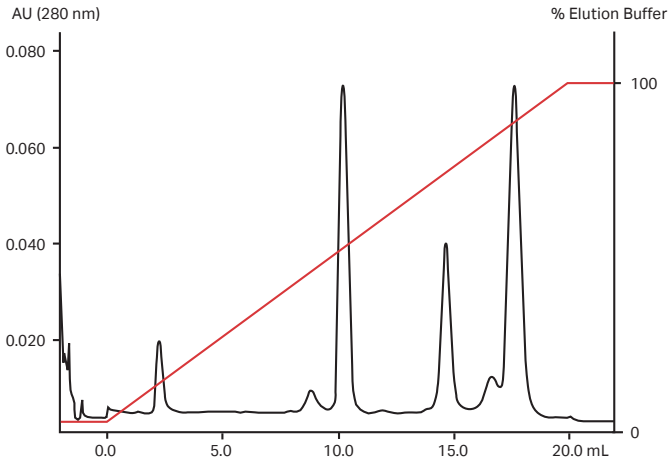

Fig 4. Typical chromatograms from a function test of SOURCE 15S 4.6/100 PE

Ordering information

Designation	No. per pack	Code No
SOURCE 15Q 4.6/100 PE	1	17518101
SOURCE 15S 4.6/100 PE	1	17518201

Related products

Designation	No. per pack	Code No
RESOURCE™ Q, 1 mL	1	17117701
RESOURCE Q, 6 mL	1	17117901
RESOURCE S, 1 mL	1	17117801
RESOURCE S, 6 mL	1	17118001
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:		
Ion Exchange Chromatography, Principles & Methods	1	11000421

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