



SOURCE™ 15Q

SOURCE™ 15S

Ion exchange chromatography resins

Instructions for Use

SOURCE™ 15Q and SOURCE 15S are strong anion and cation exchange chromatography resins based on spherical and monodisperse, porous, rigid polystyrene/divinyl benzene monodisperse particles of ~ 15 µm diameter. The resins yield stable beds, low back pressures, and separations at high flow rates.

SOURCE 15Q and SOURCE 15S are also available in prepacked laboratory-scale columns, RESOURCE™ Q and RESOURCES, for convenience in method scouting and small-scale optimization.

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Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 BioProcess resins

BioProcess™ chromatography resins are developed and supported for production-scale chromatography. All BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin properties

SOURCE 15Q and SOURCE 15S are BioProcess resins with a high chromatographic performance, high physical and chemical stability, and high batch-to-batch reproducibility. These properties make them ideal for separation of closely related protein variants at all stages of an operation – from research and process development through scale-up to production.

Table 1. Characteristics of SOURCE 15Q and SOURCE 15S

Matrix	Spherical and monodisperse, porous, rigid, polystyrene/divinyl benzene particles
Type of ion exchanger	Q (anion): $-\text{CH}_2-\text{O}-\text{CH}_2-\text{CHOH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{CHOH}-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$ S (cation): $-\text{O}-\text{CH}_2-\text{CHOH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{CHOH}-\text{CH}_2-\text{SO}_3^-$
Mean particle diameter¹	~ 15 µm
Particle loading range (proteins)	≤ 25 mg/mL resin

Dynamic binding capacity, QB_{50%}	SOURCE 15Q: ~ 45 mg BSA/mL resin ² SOURCE 15S: ~ 80 mg lysozyme/mL resin ³	
	SOURCE 15Q	SOURCE 15S
pH stability, operational⁴	2 to 12	2 to 13
pH stability, CIP⁵	1 to 14	1 to 14
pH ligand fully charged⁶	Entire pH range	Entire pH range
Recommended maximum operating flow velocity	1800 cm/h ⁷	
Recommended operating flow velocity	150 to 900 cm/h ⁷	
Chemical stability	Stable in commonly used aqueous buffers, 1.0 M HCl, 100% ethanol, 100% isopropanol, 100% acetonitril, 100% methanol, 1.0 M NaOH ⁸	
Operating temperature	4°C to 40°C	
Autoclavability	20 min at 121°C in H ₂ O, pH 7, 1 cycle	
Delivery conditions	SOURCE 15Q: 20% ethanol SOURCE 15S: 0.2 M sodium acetate in 20% ethanol	

¹ Monodisperse size distribution.

² 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 Bis-Tris propane, pH 7.0.

³ 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.

⁴ pH range where resin can be operated without significant change in function.

⁵ pH range where resin can be subjected to cleaning- or sanitization- in-place without significant change in function.

⁶ pH range where ligand is fully charged.

⁷ In a FineLINE™ 100 column with 10 cm diameter and 10 cm bed height using buffers with the same viscosity as water at room temperature.

⁸ 1.0 M NaOH should only be used for cleaning purposes.

3 Packing columns

Treat SOURCE resins carefully and especially avoid digging or stirring directly in sedimented resin or packed beds, which can generate fines. Resuspend SOURCE resin stored in its container by shaking. A packed bed in a column is best removed by dismantling the upper endpiece and pumping in liquid from the other end. Suspend SOURCE 15Q in 20% ethanol, and SOURCE 15S in 20% ethanol and 0.2 M sodium acetate for packing.

Recommended columns

Typically for ion exchange separations of large biomolecules, short columns (3 to 15 cm) are used, which minimizes back pressures and allows high throughput.

As SOURCE 15Q and SOURCE 15S are normally used for challenging polishing applications to separate very similar molecules and trace impurities or contaminants, well-designed columns are essential to avoid loss of performance. Filters that prevent particle passage are also essential.

When packing the column, adjustment of the top adapter requires larger forces than typically used for softer resins. For columns larger than approximately 30 mm diameter this is difficult to achieve with conventional designs.

Flow distribution at the top and bottom of the column must be effective without resulting in large mixing volumes. In many production situations, stringent hygiene requirements place additional demands on design.

Special columns with hydraulically controlled adapters for large-scale applications (see below) have been designed. These adapters not only meet all of the requirements, but also allow you to pack columns in about 10 minutes with excellent performance and reproducibility. It is strongly recommended to contact your local Cytiva representative about suitable columns for optimal performance of SOURCE resins.

Table 2. Recommended columns for SOURCE 15Q and SOURCE 15S

Column	Inner diameter (mm)	Bed volume (mL)	Bed height (mm)
Laboratory-scale			
FineLINE Pilot 35	35	29 to 140	30 to 150
Tricorn™ 5/20	5	0.0 to 0.5	0 to 26
Tricorn 5/50	5	0.2 to 1.1	8 to 56
Tricorn 10/20	10	0.0 to 2.1	0 to 26
Tricorn 10/50	10	0.0 to 4.4	0 to 56
Tricorn 10/100	10	3.6 to 8.4	46 to 106
Tricorn 10/150	10	7.6 to 12.3	96 to 156
Tricorn 10/200	10	11.5 to 16.2	146 to 206
Tricorn 10/300	10	19.4 to 24.1	246 to 306
Production-scale			
FineLINE 70	70	580	30 to 150
FineLINE 70L	70	1200	50 to 300
FineLINE 100P	100	1200	30 to 150

Column	Inner diameter (mm)	Bed volume (mL)	Bed height (mm)
FineLINE 100PL	100	240	50 to 300
FineLINE 200P	200	470	30 to 150
FineLINE 200PL	200	940	50 to 300
FineLINE 350P, PFR, 2 μ m	350	14 400	30 to 150
FineLINE 350PL, EDPM, 10 μ m	350	28 800	50 to 300

Equipment

A pump capable of delivering a flow velocity of at least 600 to 1900 cm/h (depending on what column is used) through the column is needed. It is recommended to include a manometer and pressure reduction valve for larger scale columns to avoid exceeding the maximum pressure of the column.

Packing Tricorn 10/100 columns

Materials needed

- column Tricorn 10/100
- packing equipment Tricorn 10/100
- pump
- 5 mL syringe
- glass rod

- graduated glass filter or laboratory beaker
- 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S)

Prepare the materials and resin

Step	Action
1	Equilibrate all materials to room temperature. SOURCE 15Q is supplied in 20% ethanol, and SOURCE 15S is supplied in 20% ethanol with 0.2 M NaOAc.
2	Suspend the resin by shaking, and pour into a graduated glass filter, or a graduated laboratory beaker. Avoid using a spatula or glass rod, which can cause fines.
3	Add 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S) to give a final slurry equivalent to approximately 50% settled resin for Tricorn 10/100 columns.

Assemble the column

Details of the column parts and packing equipment are found in the instructions supplied. Before packing, make sure that all parts, particularly the nets, net fasteners, and glass tube, are clean and intact.

Step	Action
1	Attach the packing reservoir to the column.

Step	Action
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- | | |
|---|--|
| 2 | Connect the column bottom end piece to a syringe. Submerge the end piece in 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S) and fill it using the syringe. Make sure that no air bubbles are trapped under the net. Close the tubing with a stopper and place the end piece on the column. |
| 3 | Flush the column with 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S), leaving a few mL at the bottom. Attach the column vertically to a laboratory stand. |

Packing the column

The instructions below are for packing SOURCE 15 resins in the recommended Tricorn columns.

Step	Action
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- | | |
|---|---|
| 1 | Pour the slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps to prevent the introduction of air bubbles. Immediately fill the remainder of the column and reservoir with 20% ethanol. Attach the lid on the packing reservoir and connect it to the pump. |
| 2 | Open the column outlet and start packing by pumping 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S) through the column at a flow velocity of approximately 1900 cm/h for Tricorn 10/100. This should generate a pressure of 25 bar. Switch off and disconnect the pump. Close the column outlet. |

Step	Action
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- | | |
|---|---|
| 3 | Take the column from the stand and remove the packing reservoir over a sink. Reattach the column vertically to the stand and fill to the top with 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S). |
| 4 | Wet the column adapter by submerging the plunger end in 20% ethanol (20% ethanol with 0.2 M NaOAc for SOURCE 15S) and drawing through with a syringe. Make sure that all bubbles have been removed. Insert the adapter into the top of the column, taking care not to trap air under the net. |
| 5 | With the adapter outlet open, push the adapter into the column and down approximately 2 mm into the resin bed, allowing the ethanol to displace any air remaining in the tubing. |
| 6 | Lock the adapter in position, connect it to the pump, open the column outlet, and continue packing for an additional 15 min. If necessary, reposition the adapter on the resin surface. |

The column is now ready for equilibration. If required, packing quality can be checked using the testing procedure in [Chapter 4 Evaluation of packing, on page 11](#).

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

4 Evaluation of packing

To check the quality of packing and to monitor this during the working life of the column, it is recommended that you determine the number of theoretical plates per meter (N/m) and the peak asymmetry factor (A_s). A well-packed column can be expected to give values in the range shown below, but note that results are dependent on many factors related to measuring procedure and equipment.

$$N/m > 20\,000$$

$$0.8 < A_s < 1.5$$

A method to evaluate the packing is proposed below. Acetone is commonly used at small scale, but salt is preferred at large scale to avoid the use of organic solvents. In your laboratory you might find another solute or eluent to be more convenient. The most important thing is that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, flow rate, liquid pathway, temperature, etc. influence the results.

Method

To avoid dilution of the sample, apply it as close to the column inlet as possible.

Conditions

Sample volume:	1% of bed volume
Sample concentration:	1% to 2% (v/v) acetone in ethanol or 0.8 M NaCl in water
Eluent:	Ethanol for acetone, or 0.4 M NaCl for NaCl.
Flow velocity:	60 cm/h
Detection:	Acetone: UV 280 nm. NaCl: conductivity

Calculate the plate number $N/m = 5.54 (V_e / W_{1/2})^2 \times 1000 / L$
per meter (N/m) as follows:

Calculate the peak
asymmetry factor as
follows:

$$A_s = b/a$$

V_e = peak elution distance

$W_{1/2}$ = peak width at half peak height

L = bed height in mm

a and b are measured at 10% of peak height.

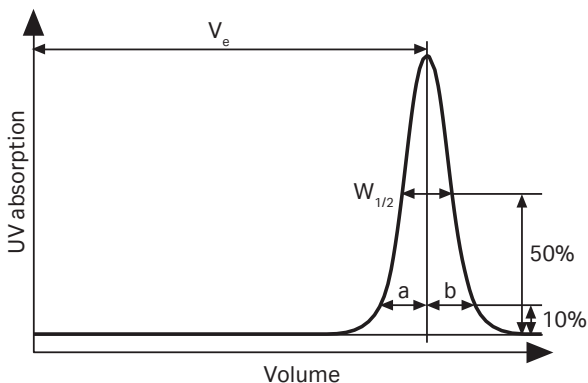


Fig 1. Example showing results obtained from the column evaluation method described above.

5 Equilibration

After packing and CIP, and before starting a run, the ion exchanger has to be charged with counter-ions and then equilibrated with the start buffer to be used during sample application. Pump one column volume of a high ionic strength buffer followed by 5 to 10 column volumes of start buffer (low ionic strength buffer) through the column until the conductivity and/or pH of the effluent is the same as that of the incoming eluent.

6 Binding

The most common procedure is to let the target molecules bind to the ion exchanger and separate them by applying a salt gradient. This is particularly useful if one of the aims is to concentrate the target product from a large volume of diluted sample. In some cases, it is useful to select conditions where particular impurities or contaminants are bound, and the target product flows through. However, a resin with a larger particle size might be better suited.

For efficient adsorption, it is critical to select a buffer with good buffering capacity at an appropriate pH. For SOURCE 15Q, the pH of the buffer should be at least 1 pH unit above the pI of the target molecule. For SOURCE 15S, the pH of the buffer should be at least 1 pH unit below the pI of the target molecule. See the tables below and operate within 0.5 pH units of the pK_a of the buffer.

Table 3. Suggested buffers for use with SOURCE 15Q

Buffer	Concentration	pK _a (25°C)
N-methylpiperazine	20 mM	4.8
Piperazine	20 mM	5.7
Bis-Tris	20 mM	6.5
Triethanolamine	20 mM	7.8
Tris	20 mM	8.2
Diethanolamine	20 mM	8.9
Ethanolamine	20 mM	9.5
Glycine	20 mM	9.9
1,3-diamino- propane	20 mM	10.5
Piperidine	20 mM	11.1

A basic principle is to avoid the use of buffering ions that bind to the exchanger, but in large-scale applications, economic considerations often limit the choice to acetate, citrate, phosphate, or other inexpensive components, even for anion exchangers. Chloride is the most commonly used counter-ion for elution. A suggested gradient is 0 to 0.5 M NaCl over 20 column volumes. Increase the NaCl concentration if the target substance is not eluted in the gradient.

Table 4. Suggested buffers for use with SOURCE 15S

Buffer	Concentration	pK _a (25°C)
Citrate	50 mM	3.1, 4.8, 6.4
Formate	50 mM	3.8
Acetate	50 mM	4.8
Malonate	50 mM	5.7
MES	50 mM	6.2
Phosphate	50 mM	2.1, 7.2, 12.3
HEPES	50 mM	7.6
BICINE	50 mM	8.4

Sodium is the most commonly used counter-ion for elution. A suggested gradient is 0 to 0.5 M NaCl over 20 column volumes. Increase the NaCl concentration if the target substance is not eluted in the gradient.

7 Flow rates

SOURCE resins have excellent flow properties. For example, prepacked RESOURCE 1 mL and 6 mL columns can be used at very high flow velocities (e.g., 300 to 1800 cm/h) with ÄKTA™ chromatography systems. This is useful for preparative laboratory applications, for scouting different separation conditions, and for purification of many different samples.

When scaling up, it is important to consider practical issues, such as pressure limitations of large-scale equipment, difficulties of liquid handling and process control at very high volumetric flow rates. Demands on process robustness and potentially expensive consequences of reductions in product

yield also inhibit very high flow rates. Serious method development on a small scale (i.e., after the initial scouting) is best performed at the same flow velocities as the envisaged full-scale process. Typically, flow velocities in the range 150 to 900 cm/h will provide the target resolution, productivity, and product yield with convenient large-scale separation times of a few minutes to one hour.

See [Fig. 2, on page 17](#) for a guide to back pressures at different flow velocities and different bed heights.

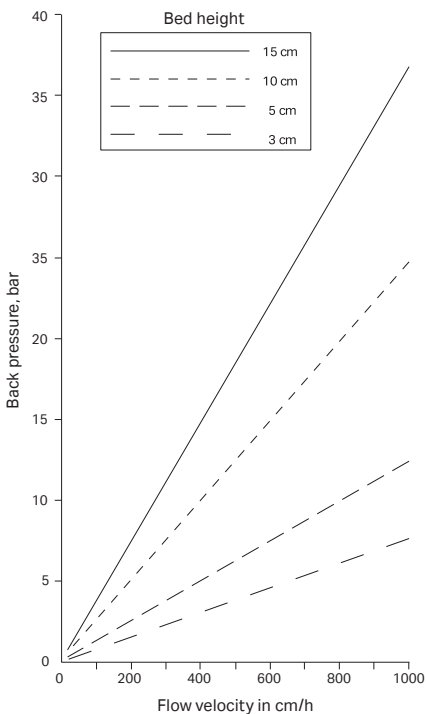


Fig 2. Pressure-flow curves that can be expected with SOURCE 15Q and SOURCE 15S.

8 Elution

Elution can be achieved using an increasing salt concentration gradient (linear, curved or step) or a pH gradient.

9 Regeneration

Normally, a separation cycle is followed by washing the column with high ionic strength buffer (e.g., containing 1 to 2 M NaCl), by changing the pH, or both, followed by re-equilibration in start buffer.

In some applications, substances such as denatured proteins or lipids, do not elute in the regeneration process and require Cleaning-In-Place procedures.

10 Cleaning-In-Place (CIP)

CIP is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the buildup of impurities or contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of the resins. A specific CIP protocol must be designed for each process according to the type of impurities or contaminants present.

It is recommended to perform a CIP:

- before first-time use – especially after packing the column – or after long-term storage
- between cycles

- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches

Recommended protocols for removing specific contaminants or impurities are described below.

CIP protocols	
Ionically bound substances	Wash with 0.5 column volumes of 2 M NaCl at a flow velocity of 90 cm/h with reversed flow direction. Contact time 10 to 15 minutes.
Precipitated substances, hydrophobically bound substances, and lipoproteins	Wash with at least 3 column volumes of 1.0 M NaOH at a flow velocity of 40 cm/h with reversed flow direction. Contact time 1 to 2 hours.

CIP protocols	
Strongly hydrophobically bound proteins, lipoproteins, and lipids	Wash with 70% ethanol or 30% isopropanol at a flow velocity of 10 to 40 cm/h with reversed flow direction. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents. Alternatively, wash the column with 2 to 4 bed volumes of 0.1% to 0.5% of a non-ionic detergent in a basic or acidic solution. After treatment, always remove detergent with 70% ethanol or 30% isopropanol. Contact time 1 to 2 hours.

Note: *After washing the column, always equilibrate with at least three bed volumes of start buffer before use.*

11 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum.

Wash the column with 0.5 to 1.0 M NaOH at a flow velocity of approximately 40 cm/h, contact time 30 to 60 minutes, reversed flow direction. For more information about the use of NaOH for sanitization, refer to *Application note (CY13951)*.

Re-equilibrate the column with 3 to 5 bed volumes of sterile start buffer.

12 Storage

Store SOURCE 15Q resin in 20% ethanol and SOURCE 15S in 20% ethanol and 0.2 M sodium acetate at 4°C to 30°C to avoid microbiological growth. After storage, equilibrate with at least 3 bed volumes of start buffer before use.

13 Ordering information

Product	Pack size	Product code
SOURCE 15Q	10 mL	17094720
	50 mL	17094701
	200 mL	17094705
	500 mL	17094702
	1 L	17094703
SOURCE 15S	10 mL	17094410
	50 mL	17094401
	200 mL	17094405
	500 mL	17094402
	1 L	17094403

Columns and accessories	Product code
Tricorn 5/20	28406408
Tricorn 5/50	28406409
Tricorn 10/20	28406413
Tricorn 10/50	28406414
Tricorn 10/100	28406415

Columns and accessories	Product code
Tricorn 10/150	28406416
Tricorn 10/200	28406417
Tricorn 10/300	28406418
FineLINE Pilot 35	18110202
FineLINE 70	18115298
FineLINE 70L	18115299
FineLINE 100P	11002798
FineLINE 100PL	11002799
FineLINE 200P	11003114
FineLINE 200PL	11003115
FineLINE 350P, PFR, 2 µm	11002792
FineLINE 350PL, EPDM, 10 µm	11002785
Packing equipment 10/100	18115325

Prepacked columns	Pack size	Product code
SOURCE 15Q 4.6/100 PE	1.7 mL	17518101
SOURCE 15S 4.6/100 PE	1.7 mL	17518201
RESOURCE Q	1 mL	17117701
	6 mL	17117901
RESOURCES	1 mL	17117801
	6 mL	17118001

Related literature	Reference
Application note	
Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951
Data file	
SOURCE 15Q, SOURCE 15S, and preppacked column formats	CY13331
Handbook	
Ion Exchange Chromatography Principles and Methods	CY13983
Selection guide	
Ion Exchange columns and resins	18112731

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