# SOURCE™ 15Q and SOURCE 15S

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

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

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Read these instructions carefully before using the product.

## Safety

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

# 1 Description

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

BioProcess chromatography resins are developed and supported for production-scale chromatography. 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.

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

| | | |
|---|---|---|
| **Matrix** | Spherical and monodisperse, porous, rigid polystyrene/divinyl benzene particles | |
| **Ion exchange type** | | |
| **Q (anion)** | $–CH_2–O–CH_2–CHOH–CH_2–O–CH_2–CHOH–CH_2–N^+(CH_3)_3$ | |
| **S (cation)** | $–O–CH_2–CHOH–CH_2–O–CH_2–CHOH–CH_2–SO_3$ | |
| **Mean particle diameter**[1] | ~ 15 µm | |
| **Particle loading range (proteins)** | ≤ 25 mg/mL resin | |
| | 15Q | 15S |
| **pH stability, operational**[2] | 2 to 12 | 2 to 13 |
| **pH stability, CIP**[3] | 1 to 14 | 1 to 14 |
| **pH ligand fully charged**[4] | Entire pH range | Entire pH range |
| **Recommended maximum operating flow velocity** | 1800 cm/h[5] | |
| **Recommended operating flow velocity** | 150 to 900 cm/h[6] | |
| **Dynamic binding capacity,** $Q_{B10}$ | 15Q: ~ 45 mg BSA/mL resin[7]<br>15S: ~ 80 mg lysozyme/mL resin[8] | |
| **Chemical stability** | Stable to commonly used aqueous buffers, 1.0 M HCl, 100% ethanol, 100% isopropanol, 100% acetonitril, 100% methanol, 1.0 M NaOH[9] | |
| **Operating temperature** | 4°C to 40°C | |
| **Autoclavability** | 20 min at 121°C in $H_2O$, pH 7, 1 cycle | |
| **Delivery conditions** | 15Q: 20% ethanol<br>15S: 0.2 M sodium acetate in 20% ethanol | |

[1] Monodisperse size distribution

[2] pH range where resin can be operated without significant change in function

[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

[4] pH range where ligand is fully charged

[5] 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

[6] 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

[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 BSA in 20 mM Bis-Tris propane, pH 7.0

[8] 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

[9] 1.0 M NaOH must only be used for cleaning purposes.

# 2 Packing columns

Treat SOURCE carefully and especially avoid "digging" or stirring directly in sedimented resin or packed beds, which can generate fines. Resuspend SOURCE 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, 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.

Since SOURCE 15Q and SOURCE 15S are usually used for difficult "polishing" applications, to separate very similar molecules and trace contaminants, well designed columns are essential to avoid loss of performance. Filters which prevent particle passage are also essential.

When packing the column, adjustment of the adapter on top of the bed requires larger forces than typically used for softer resins. For columns larger than ca 30 mm diameter this will be difficult to achieve satisfactorily 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 put additional demands on design.

Special columns with hydraulically controlled adapter 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 GE 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) |
|---|---|---|---|
| **Lab-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 |
| 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 µm | 350 | 14 400 | 30 to 150 |
| FineLINE 350PL, EDPM, 10 µm | 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 which column 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

## Material needed

Column Tricorn 10/100, packing equipment Tricorn 10/100, pump, 5 mL syringe, glass rod, graduated glass filter or laboratory beaker and 20% ethanol (20% ethanol with 0.2 M NaAc for SOURCE 15S).

## Preparing the resin and material

1. Equilibrate all material to room temperature.

   SOURCE 15Q are supplied in 20% ethanol and SOURCE 15S is supplied in 20% ethanol with 0.2 M NaAc.

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 breakage of the particles.

3. Add 20% ethanol (20% ethanol with 0.2 M NaAc for SOURCE 15S) to give a final slurry equivalent to approximately 50% settled resin for Tricorn 10/100 columns.

## Assembling 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.

1. Attach the packing reservoir to the column.

2. Connect the column bottom end piece to a syringe. Submerge the end piece in 20% ethanol (20% ethanol with 0.2 M NaAc 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 put the end piece on the column.

3. Flush the column with 20% ethanol (20% ethanol with 0.2 M NaAc for SOURCE 15S), leaving a few mL at the bottom. Put the column vertically on a laboratory stand.

## Packing the column

These instructions are for packing SOURCE 15 ion exchange resins in the recommended Tricorn Columns. To modify these instructions for columns of different dimensions, refer to Appendix A.

1. Pour the resin slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps 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 the packing by pumping 20% ethanol (20% ethanol with 0.2 M NaAc 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.
3. Take the column from the stand and remove the packing reservoir over a sink. Remount the column vertically and fill to the top with 20% ethanol (20% ethanol with 0.2 M NaAc for SOURCE 15S).
4. Wet the column adapter by submerging the plunger end in 20% ethanol (20% ethanol with 0.2 M NaAc 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 a further 15 min. Reposition the adapter on the resin surface if necessary.

The column is now ready for equilibration. If required, the quality of packing can be checked using the testing procedure described in Section 5.

# 3 Evaluation of packing

To check the quality of packing and to monitor this during the working life of the column, we recommend that you determine the number of plates per meter (N/m) and the peak asymmetry factor ($A_S$). A well packed column can be expected to give values in the following range, 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 is proposed below. Acetone is commonly used at small scale but salt is preferred at large scale to avoid the use of large volumes of ethanol as eluent. In your laboratory you maybe 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. will influence the result.

## 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 conc.: 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 per metre (N/m) as follows:

$$N/m = 5.54\,(V_e / W_{1/2})^2 \times 1000/L$$

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.

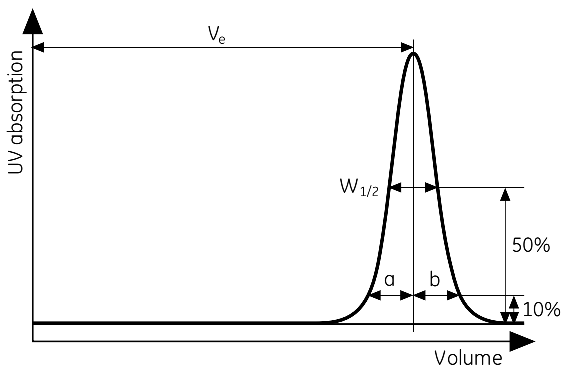


**Fig 1.** Example showing results obtained from the column evaluation method described above.

# 4 Equilibration

Before starting a run, the ion exchanger has to be charged with counter-ions and then equilibrated with the starting 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 starting buffer (low ionic strength buffer) through the column until the conductivity and/or pH of the effluent is the same as that of the ingoing eluent.

# 5 Binding

The most common procedure is to let the molecules of interest bind to the ion exchanger and separate them by developing a salt gradient. This is particularly useful if one of the aims is to concentrate the product of interest from a large volume of diluted sample. In some cases, it is useful to choose conditions where a particular contaminant is bound and the product of interest flows through.

For efficient adsorption, it is critical to choose a buffer at an appropriate pH (at least 1 pH unit above for Q, or 1 pH unit below for S, the pI of the molecule of interest when binding is desired) and with buffering capacity at that pH. Refer to Table 3 and Table 4 and operate within 0.5 pH units of the buffer's $pK_a$.

**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 |

Although simplicity suggests that buffering ions that bind to the exchanger had better be avoided, in large-scale applications, economic considerations often limit the choice to acetate, citrate, phosphate, or other inexpensive components even for anion exchange.

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 anion concentration if the substance of interest 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 |
| Formate | 50 mM | 3.8 |
| Acetate | 50 mM | 4.8 |
| Malonate | 50 mM | 5.7 |
| MES | 50 mM | 6.2 |
| Phosphate | 50 mM | 7.2 |
| HEPES | 50 mM | 7.6 |
| BICINE | 50 mM | 8.4 |

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

# 6 Flow rates

SOURCE resins have excellent flow properties. For example, RESOURCE prepacked 1 mL and 6 mL columns can be used at very high flow rates (e.g., 300 to 1800 cm/h) with ÄKTA™ chromatography systems. This is useful for lab preparative 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 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 desired resolution, productivity, and product yield with convenient large-scale separation times of a few minutes to one hour.

See Figure 2 for a guide to back pressures at different flow velocities and different bed heights.

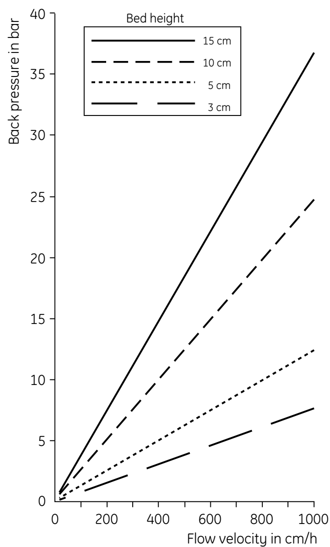


**Fig 2.** Pressure/flow curves that can be expected with SOURCE 15Q and SOURCE 15S

# 7 Elution

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

# 8 Regeneration

Normally a separation is followed by washing with high ionic strength salt (e.g., 1 to 2 M NaCl) and/or changing pH, followed by re-equilibration in starting buffer.

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

# 9 Cleaning-In-Place (CIP)

- Remove ionically bound proteins by washing the column with 0.5 bed volumes of a 2 M NaCl solution at a flow velocity of approximately 90 cm/h, contact time 10 to15 minutes, reversed flow direction.
- Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 1.0 M NaOH solution at a flow velocity of approximately 40 cm/h, contact time 1 to 2 hours, reversed flow direction.
- Remove strongly hydrophobically bound proteins, lipoproteins, and lipids by washing the column with four bed volumes of 70% ethanol or 30% isopropanol at 10 cm/h, reversed flow direction. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents. Alternatively, wash the column with two bed volumes of 0.1 to 0.5% nonionic detergent in a basic or acidic solution. After treatment with detergent always remove residual detergent by washing with five bed volumes of 70% ethanol.

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

# 10 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.

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

# 11 Storage

Store SOURCE 15Q resin in 20% ethanol, store SOURCE 15S in 20% ethanol and 0.2 M sodium acetate, storage temperature 4°C to 30°C to avoid microbiological growth.

# 12 Ordering information

| Resin | Product code |
|---|---|
| SOURCE 15Q, 10 mL | 17094720 |
| SOURCE 15Q, 50 mL | 17094701 |
| SOURCE 15Q, 200 mL | 17094705 |
| SOURCE 15Q, 500 mL | 17094702 |
| SOURCE 15Q, 1 liter | 17094703 |
| SOURCE 15S, 10 mL | 17094410 |
| SOURCE 15S, 50 mL | 17094401 |
| SOURCE 15S, 200 mL | 17094405 |
| SOURCE 15S, 500 mL | 17094402 |
| SOURCE 15S, 1 liter | 17094403 |

| Columns and accessories | Product code |
|---|---|
| Tricorn 5/200 | 28406408 |
| Tricorn 5/50 | 28406409 |
| Tricorn 10/20 | 28406413 |
| Tricorn 10/50 | 28406414 |
| Tricorn 10/100 | 28406415 |
| 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 |

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| SOURCE 15Q and 15S in prepacked columns | 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 |
| RESOURCE S, 1 mL | 17117801 |
| RESOURCE Q, 6 mL | 17117901 |
| RESOURCE S, 6 mL | 17118001 |

| Literature | Product code |
| --- | --- |
| Handbook, Ion Exchange Chromatography Principles and Methods | 11000421 |
| Selection Guide, Ion Exchange columns and media | 18112731 |
| Data file, SOURCE 15Q, SOURCE 15S and prepacked column formats | 18112365 |

# Appendix A

Converting to columns of different dimensions

## Flow rates

Column cross section area shall be in $cm^2$ in the equations below.
To convert from linear flow (cm/h) to volumetric flow (mL/min):

$$\text{Volumetric flow} = \frac{\text{Linear flow}}{60} \times \text{Column cross-sectional area}$$

To convert from volumetric flow (mL/min) to linear flow (cm/h):

$$\text{Linear flow} = \frac{\text{Volumetric flow} \times 60}{\text{Column cross-sectional area}}$$

## Volumes

To convert volumes for columns of different dimensions, increase or decrease in proportion to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

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