GE Healthcare
Life Sciences

SOURCE™ 15Q, SOURCE 15S, and prepacked column formats

SOURCE 15Q and SOURCE 15S are high performance ion exchange chromatography (IEX) media (resins) for preparative separation of biomolecules. SOURCE 15Q and SOURCE 15S are anion and cation exchangers, respectively, and are designed for challenging separations, such as those encountered in the final stage of an industrial purification process (polishing). The media are available in bulk as well as in prepacked column formats (Fig 1). SOURCE 15Q 4.6/100 PE and SOURCE 15S 4.6/100 PE are prepacked Tricorn™ columns designed for high resolution purification at lab scale and for optimization studies when scaling up. RESOURCE™ Q and RESOURCE S are prepacked, lab-scale columns designed for fast separations and selectivity screening experiments.

SOURCE media are characterized by:

- High-resolution separations in minutes
- High sample loads
- Reproducible quality
- Proven scalability
- Maintained performance at high flow rates
- Low back pressures

SOURCE 15Q and SOURCE 15S ion exchangers

SOURCE 15 IEX media are based on 15 µm, monodisperse, rigid, polystyrene/divinyl benzene beads with an optimized pore size distribution. Emphasis during development has been on quality, reproducibility, and scalability, features that are particularly important for industrial applications where there are strict regulatory demands. Table 1 summarizes the general properties.

Fig 1. SOURCE 15Q and SOURCE 15S in media packs, prepacked Tricorn, and RESOURCE columns.

Table 1. Characteristics of SOURCE 15Q and 15S

Matrix	Polystyrene/divinyl benzene
Type of ligand	Q: -O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-$N^+(CH_3)_3$ S: -O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-SO_3^-
Bead form	Rigid, spherical, porous monodisperse
Particle size	15 µm
Maximum flow velocity	1800 cm/h
Recommended working flow velocity	150 to 900 cm/h
pH stability, working*	2 to 12
pH stability, short term†	1 to 14
Chemical stability	All commonly used buffers, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol
Operating temperature	4 to 40°C
Storage	SOURCE 15Q: 20% ethanol SOURCE 15S: 0.2 M sodium acetate in 20% ethanol

* pH interval where the medium can be operated and stored for longer periods of time without significant change in function

† pH interval where the medium can be subjected to cleaning-in-place (CIP) or sanitization-in-place without significant change in function

High resolution and high capacity

SOURCE particles have a uniform, 15 µm diameter, spherical shape (Fig 2). The beads give stable packed beds with high resolution characteristics and low back pressures. Pore size distribution is balanced to give high capacities for peptides, proteins, and oligonucleotides as well as a high degree of retained performance at high flow rates (Fig 3 and 4).

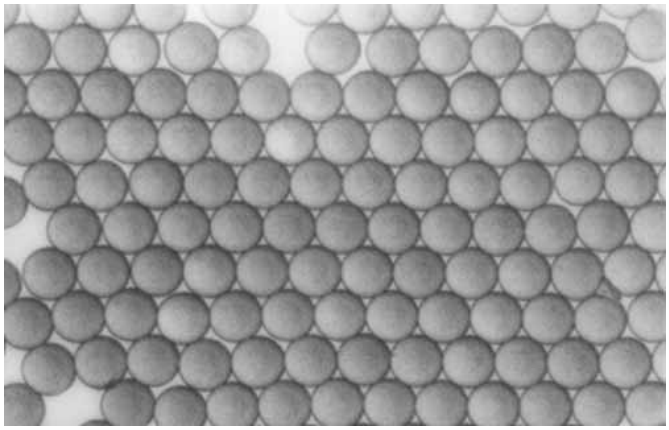


Fig 2. Light microscope photograph of SOURCE 15Q. Note the uniform size distribution.

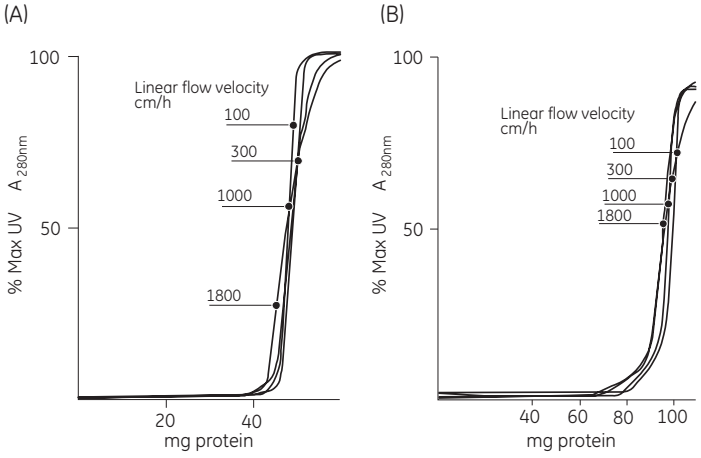


Fig 3. Breakthrough curves at different flow rates (superimposed). (A) RESOURCE Q, 1 mL (6.4 mm diameter × 30 mm bed height). Sample: bovine serum albumin (Sigma), 5 mg/mL. (B) RESOURCE S, 1 mL (6.4 mm diameter × 30 mm bed height). Sample: lysozyme (Sigma), 5 mg/mL.

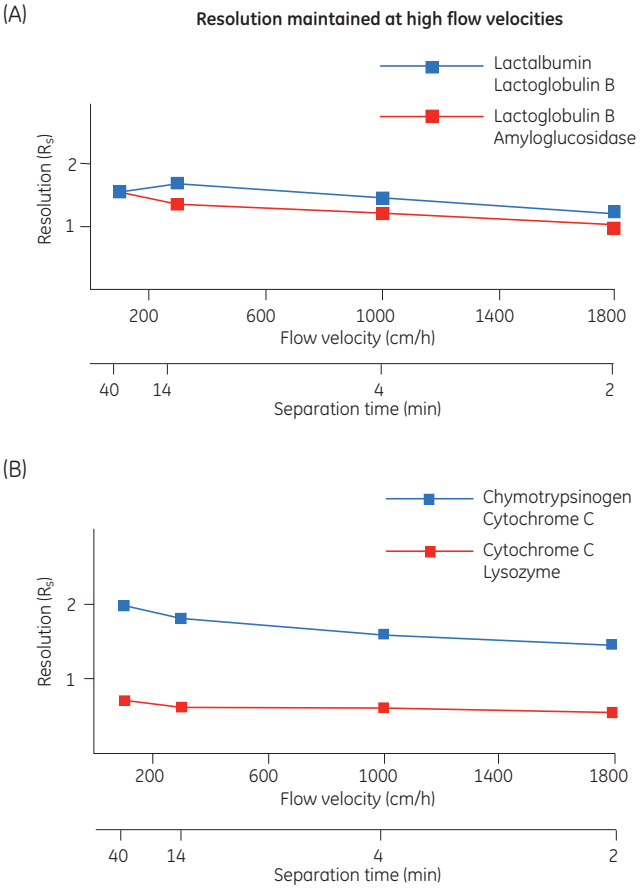


Fig 4. Resolution versus flow for model proteins. (A) RESOURCE Q, 1 mL (6.4 mm diameter × 30 mm bed height). Sample: lactalbumin, lactoglobulin B, and amyloglucosidase (Sigma). Total load 12 mg. (B) RESOURCE S, 1 mL (6.4 mm diameter × 30 mm bed height). Sample: chymotrypsinogen, cytochrome C, lysozyme (Sigma). Total load 16 mg.

Chemical and pH stability

The hydrophilized polymeric matrix has high chemical stability and can be used over a wide pH range allowing flexibility in the choice of conditions for separation and for cleaning. SOURCE 15Q and 15S have been substituted with quaternary ammonium groups and sulphonate groups, respectively, to form strong anion and cation exchangers. Both groups are attached to the matrix via long, hydrophilic spacer arms. SOURCE 15Q and 15S retain their charge over a wide pH range and give good recovery of biological activity.

Batch-to-batch reproducibility

Through the combination of high quality standards and a patented manufacturing process, particle structure is consistent both bead-to-bead and batch-to-batch. As shown in Figure 5, chromatographic performance reflects these reproducible qualities.

Reproducible quality

Medium:	SOURCE 15Q, 4 separate batches
Column:	7.5 mm diam × 50 mm bed height
Sample:	200 µL mixture of ovalbumin (3 mg/mL) and β-lactoglobulin (3 mg/mL)
Start buffer:	20 mM Bis-Tris propan buffer, pH 7.0
Elution buffer:	Start buffer + 0.35 M NaCl
Flow rate:	2.2 mL/min (300 cm/h)
Gradient:	Start buffer: 2 column volumes (CV), then a linear gradient 0% to 100% elution buffer, 21 CV

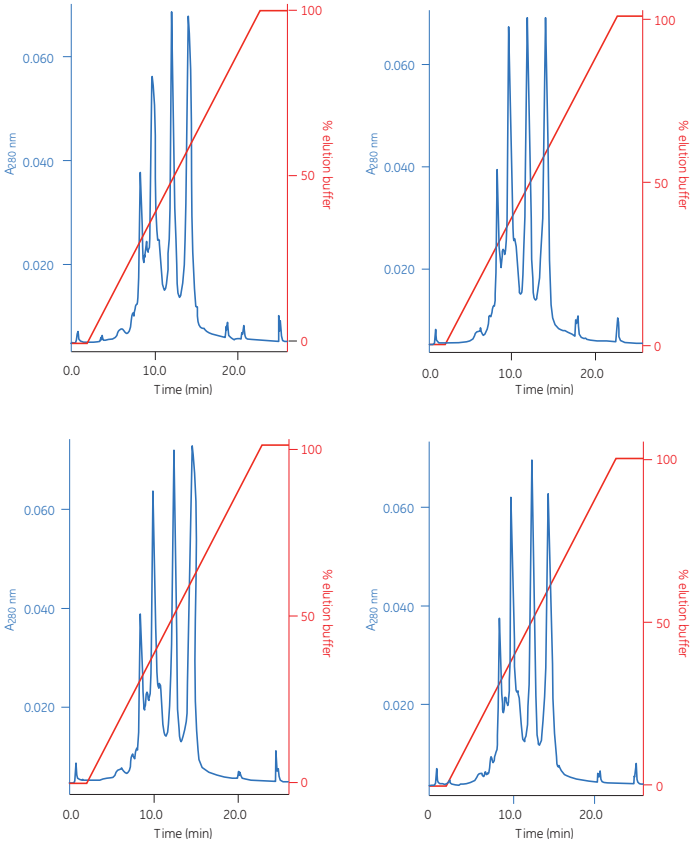


Fig 5. Results of selectivity tests on four batches of SOURCE 15Q.

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

SOURCE 15Q 4.6/100 PE and SOURCE 15S 4.6/100 PE are Tricorn columns prepacked with SOURCE 15Q and SOURCE 15S, respectively. The columns are an excellent choice for preparative IEX purification at laboratory scale because of the high capacity of the media and the relatively long bed height (100 mm). The columns are also useful for optimization studies before scale-up.

The design of the Tricorn columns gives high performance without compromising user friendliness and reliability. The distribution system creates an even liquid distribution over the entire column cross section to enable high resolution separations. The columns are simple to use, with Valco fittings for uncomplicated connection to ÄKTA™ chromatography systems and other high-performance liquid chromatography (LC) systems, and can be run according to their specifications where the systems have the appropriate pressure capacity.

The column material is polyetheretherketone (PEEK), which is chemically resistant and has a high pressure tolerance. The main chromatographic properties of SOURCE 15Q 4.6/100 PE and SOURCE 15S 4.6/100 PE columns are listed in Table 2.

RESOURCE Q and RESOURCE S columns

RESOURCE Q and RESOURCE S columns are prepacked with SOURCE 15Q and SOURCE 15S, respectively. The columns give fast, high-capacity, and high-resolution separation of biomolecules with ÄKTA systems and other high-performance LC systems. These columns generate low back pressures (typically around 1 bar, 0.1 MPa, 15 psi at flow rates of 1 mL/min), making high resolution separations achievable in 20 min, even with a system based on a peristaltic pump.

RESOURCE Q and RESOURCE S columns are made of PEEK and are available in two dimensions, 1 mL and 6 mL. Table 2 lists the main chromatographic properties of the columns.

Table 2. Main chromatographic properties of columns prepacked with SOURCE 15 Q and 15S

	RESOURCE Q RESOURCE S		SOURCE 15Q SOURCE 15S
	1 mL	6 mL	4.6/100 PE
Column dimensions i.d. × bed height (mm)	6.4 × 30	16 × 30	4.6 × 100
Bed volume (mL)	1	6	1.7
Recommended flow rate (mL/min)	1 to 10	1 to 60	0.5 to 2.5
Max. flow rate (mL/min)	10	60	5.0
Max. back pressure (MPa, bar, psi)	1.5, 15, 220	0.6, 6, 87	4, 40, 580

Operation

SOURCE 15 IEX media combine excellent capacity (Fig 3), outstanding resolution over a wide range of flow rates (Fig 4), and exceptional pressure/flow characterisitics (Fig 7), making rapid separations (Fig 6) and high resolution possible at large scale (Fig 11 and 12).

SOURCE 15 IEX media and prepacked Tricorn and RESOURCE columns can be used with standard methods for IEX. Typically these methods involve aqueous buffers in the pH range where the sample is stable, and salt gradients up to 0.5 M or 1 M NaCl. The strong ion exchange groups allow operatation between pH 2 and 12.

Wide pH stability not only allows cleaning with harsh agents like 1 M NaOH, but also enables the use of high pH to prevent aggregation in applications such as purification of synthetic oligonucleotides.

Optimal running conditions differ between applications and are preferably established by first varying pH (Fig 8) and then other parameters such as gradient and flow rate.

Medium:	RESOURCE Q 1 mL
Column:	6.4 mm diam × 30 mm bed height
Sample:	Pancreatin 5 mg/mL, 0.2 mL
Flow rate:	9.6 mL/min (1800 cm/h)
Start buffer:	20 mM Bis-Tris propane, pH 7.5
Elution buffer:	Start buffer + 0.5 M NaCl
Gradient:	0 to 80% elution buffer, 20 mL (20 CV)

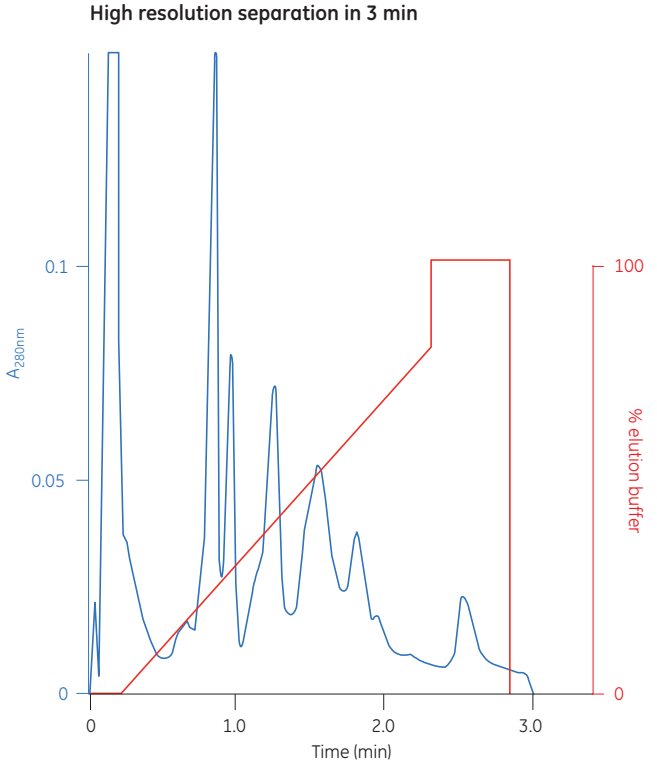
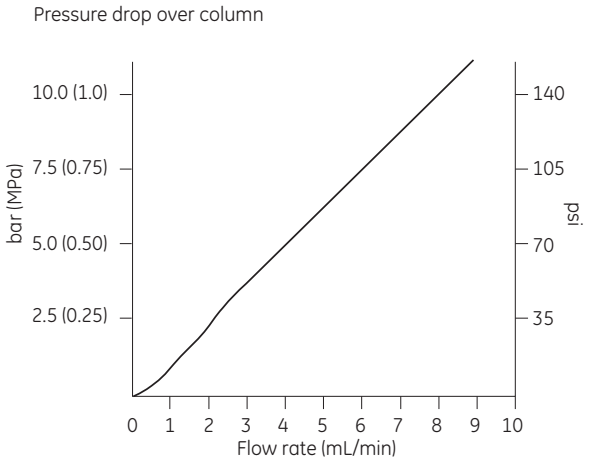


Fig 6. Rapid separation and high resolution using RESOURCE Q 1 mL.

(A) RESOURCE 1 mL



(B) FineLINE™ 100 column

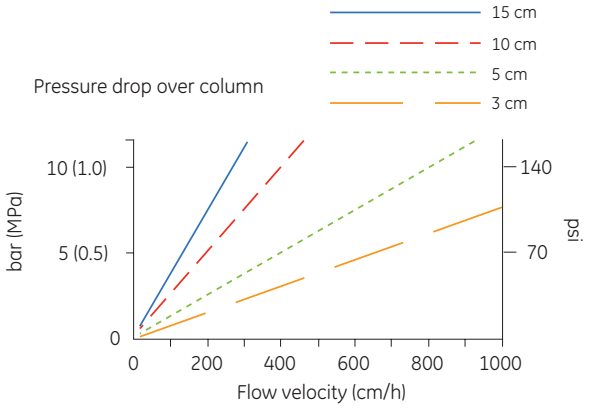


Fig 7. Pressure/flow curves that can be expected with SOURCE 15Q and SOURCE 15S. (A) RESOURCE 1 mL column (6.4 mm diameter × 30 m bed height) can be used with high pressure equipment or peristaltic pumps. (B) FineLINE 100 columns packed with SOURCE 15 to four different bed heights.

Equipment and flow rates

The excellent flow properties of SOURCE 15Q and SOURCE 15S make these media suitable to use in lab-scale columns at flow velocities up to 2000 cm/h with ÄKTA systems.

With LC equipment, which can be conducted at pressures around and above 1 MPa (10 bar, 150 psi), high flow velocities of up to 1800 cm/h can be used to give rapid separations with RESOURCE columns (Fig 7). Figures 9 and 10 illustrate separations with low-pressure laboratory equipment at back pressures of less than 1 bar (0.1 MPa, 15 psi).

In large-scale applications, equipment pressure specifications may restrict flow velocities to below 600 cm/h. However, outstanding resolution can still be obtained in 10 to 40 min (Fig 11 and 12).

Optimization studies on SOURCE 15Q 4.6/100 PE

Column:	SOURCE 15Q 4.6/100 PE
Sample:	Clarified *E. coli* extract expressing a recombinant chaperone protein, DnaK
Sample load:	3 mg
Start buffer:	25 mM MES, pH 6.0 and pH 6.5, 25 mM HEPES, pH 7.0, and pH 7.5
Elution buffer:	Start buffer + 1 M NaCl
Flow rate:	2 mL/min
Gradient:	Linear gradient from 0 to 100% elution buffer, 20 CV

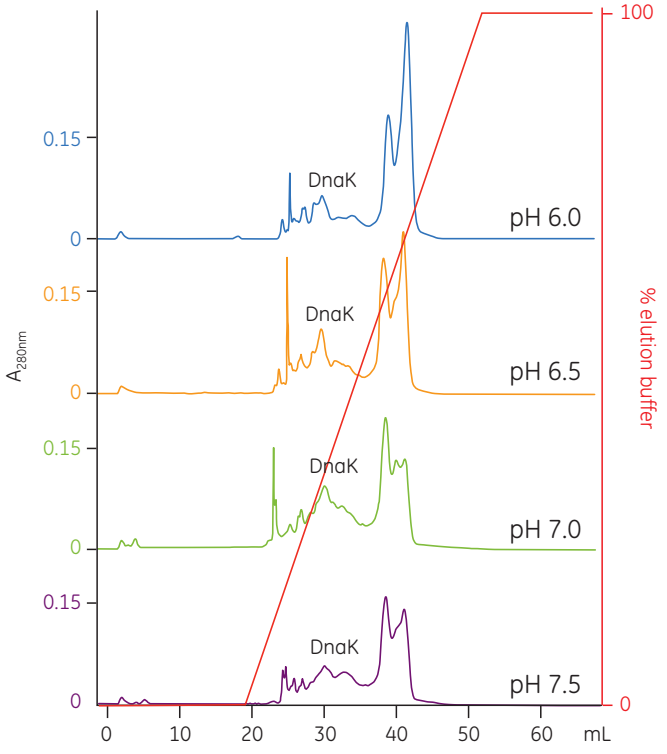


Fig 8. To find an optimal pH for the separation of the chaparone protein DnaK, four buffers with different pH were tested during pH scouting: pH 6.0, 6.5, 7.0, 7.5. The chromatograms show that the DnaK-containing peak is more well defined and concentrated when purifying at pH 6 and 6.5. The purity in each eluate was analyzed by SDS-PAGE and found to be higher at pH 6.5. Considering the results, pH 6.5 was chosen for the separation of DnaK on SOURCE 15Q 4.6/100 PE.

High performance using a peristaltic pump

Column:	RESOURCE S 1 mL
Sample:	Snake venom 4 mg/mL, 0.1 mL
Flow rate:	1 mL/min (180 cm/h)
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	Start buffer + 0.4 M NaCl
Gradient:	0 to 100% elution buffer, 20 CV

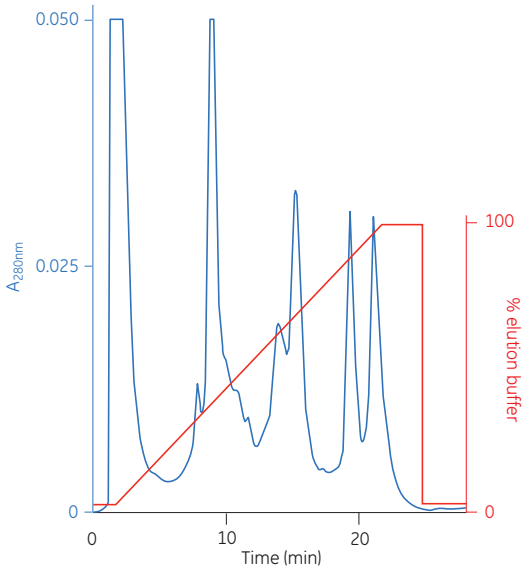


Fig 9. Separation of snake venom (Sigma) on RESOURCE S, 1 mL at 1 mL/min (180 cm/h). Although high-performance separations with RESOURCE Q and S do not put special demands on the pump, resolution obtained on the column can be lost through mixing in dead spaces. Low dead volumes, accurate gradient generation, and a good detector and fraction collector are also essential for good results.

Scaling up

SOURCE 15Q and 15S allow separations achieved with Tricorn columns and RESOURCE columns to be scaled up. By keeping the same linear flow rate, sample load per column volume, and bed height, scale-up is very predictable. Figures 9 and 10 illustrate scale up from a RESOURCE 1 mL to a RESOURCE 6 mL column. SOURCE 15Q and 15S media allow you to pack your own column in dimensions suitable for your resolution and capacity needs.

Figure 11 shows retained resolution upon scale-up from a 2.2 mL laboratory column to a 390 mL FineLINE 100 production column.

Linear scale up on RESOURCE S 6 mL

Column:	RESOURCE S 6 mL
Sample:	Snake venom 4.8 mg/mL, 0.5 mL
Flow rate:	6 mL/min (180 cm/h)
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	Start buffer + 0.4 M NaCl
Gradient:	0 to 100% elution buffer, 20 CV

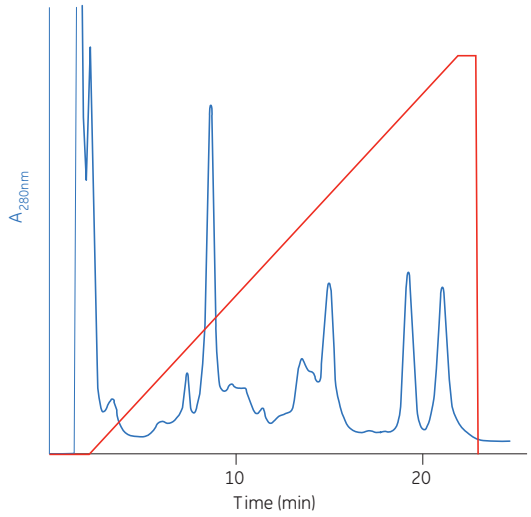


Fig 10. Separation of snake venom (Sigma) on RESOURCE S, 6 mL at 6 mL/min (180 cm/h).

GE Healthcare has designed a range of columns, FineLINE, for optimal performance of SOURCE media in scale-up and production (see Table 3). These have hydraulically controlled adapters that allow packing to be completed in about 10 min with excellent performance and reproducibility. For more information about this column family, visit www.gelifesciences/bioprocess

Table 3. Dimensions of FineLINE columns. Other dimensions are also available on request

	Column i.d. (mm)	Bed heights range (cm)	Bed volume range (mL)
FineLINE Pilot	35	3–15	50– 145
FineLINE 100	100	3–15	235–1180
FineLINE 100 L	100	5–30	390–2350
FineLINE 200	200	3–15	940–4710
FineLINE 200 L	200	5–30	1570–9420

The examples in Figures 6, 8, 9, 10, and 11 show protein separations. Typically loadings up to 25 mg/mL medium will still give adequate resolution. Figure 13 shows a separation of partially purified bacitracin from *Bacillus subtilis*. Figures 12 and 14 show one step purifications of a synthetic DNA oligonucleotide and of a synthetic phosphorothioate DNA oligonucleotide, respectively.

Reproducible scale up

Column:	A) SOURCE 15S, 2.2 mL (7.5 mm diam. × 50 mm bed height) B) SOURCE 15S, FineLINE 100, 390 mL (100 mm diam. × 50 mm bed height)
Sample:	A) 200 µL containing ribonuclease, cytochrome C, and lysozyme (all from Sigma). Total protein load 0.46 mg B) 350 mL containing ribonuclease, cytochrome C, and lysozyme (all from Sigma). Total protein load 80.5 mg
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	Start buffer + 0.4 M NaCl
Flow rate:	A) 2.2 mL/min (300 cm/h) B) 385 mL/min (300 cm/h)
Gradient:	2 CV start buffer, then 0% to 100% elution buffer, 21 CV

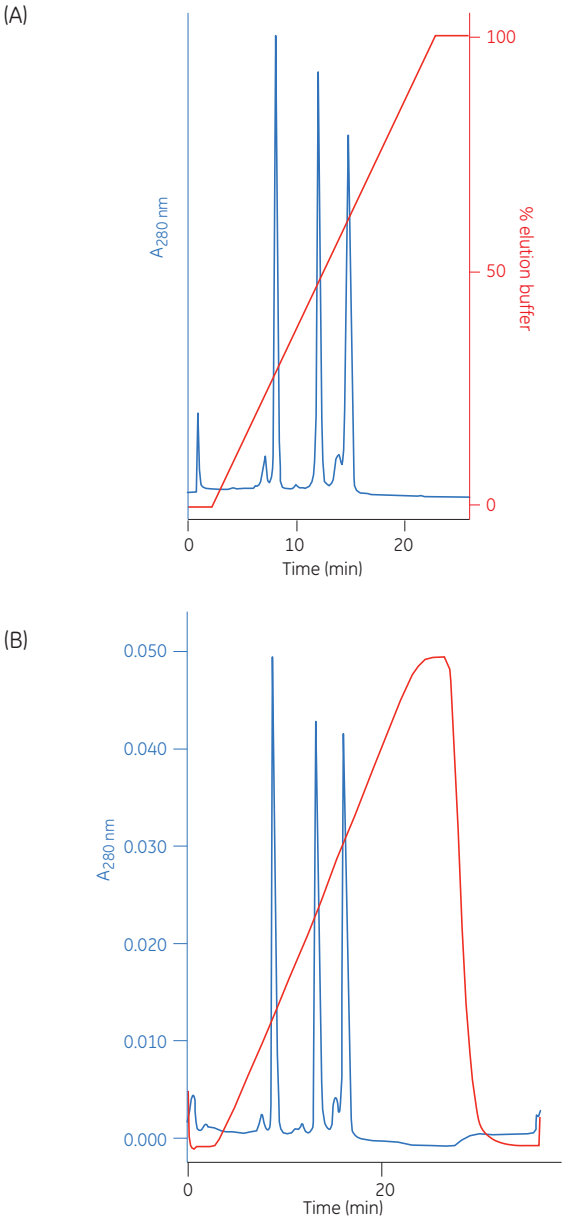


Fig 11. Separation of proteins scaled up to 100 mm diameter column. Note that the excellent resolution is retained.

Synthetic oligonucleotide purification

Column:	A) RESOURCE Q 1 mL (6.4 mm diam. × 30 mm bed height) B) SOURCE 15Q in FineLINE 100, 240 mL (100 mm diam. × 30 mm bed height).
Sample:	A) 800 µmol synthesis of 19-mer DNA oligo, load 5 mg B) Same oligo as in A), load 820 mg
Sequence:	ATACCGATTAAGCAAGTTT
Start buffer:	10 mM NaOH, pH 12
Elution buffer:	Start buffer + 1.5 M NaCl
Flow rate:	A) 1.6 mL/min (300 cm/h). B) 385 mL/min (300 cm/h)
Gradient:	0.25 to 0.75 M NaCl in 30 CV

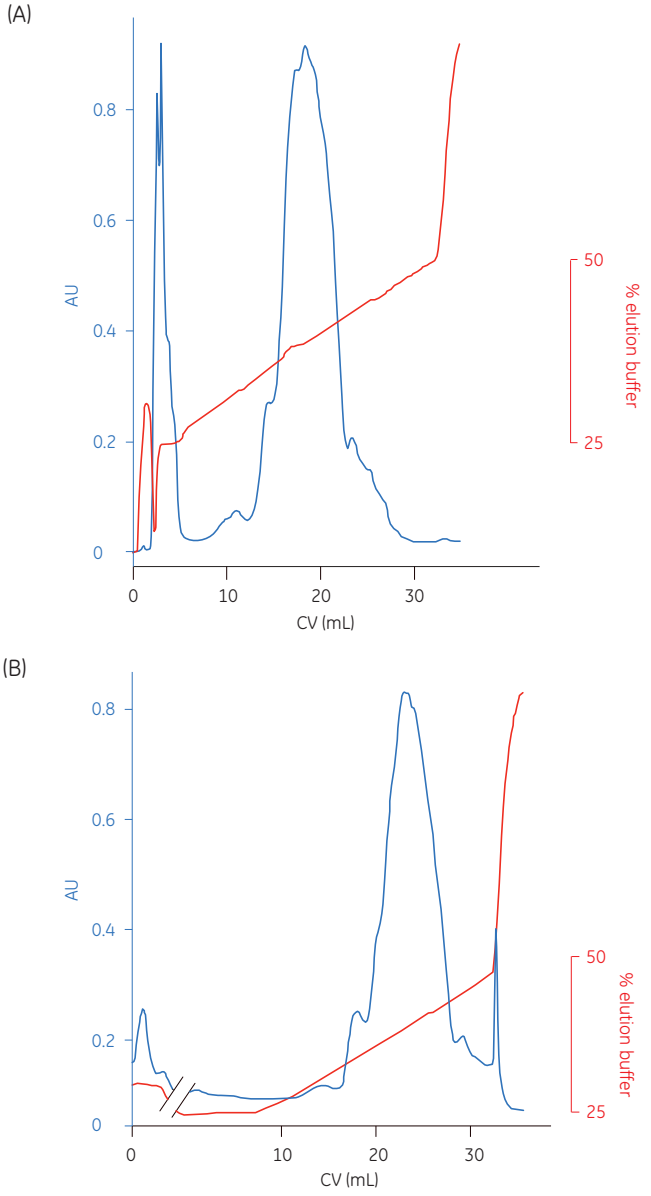


Fig 12. Purification of 19-mer DNA oligonucleotide on RESOURCE Q 1 mL scaled up to FineLINE 100 column. Separation optimized for sample load, yield, and purity of product.

Partially purified peptide

Column:	RESOURCE S 1 mL
Sample:	100 µL partially purified bacitracin, 5 mg/mL
Start buffer:	5 mM potassium phosphate, pH 2.8, 30% acetonitrile
Elution buffer:	Start buffer + 0.4 M KCl
Flow rate:	1 mL/min (180 cm/h)
Gradient:	0% to 100% elution buffer in 10 min

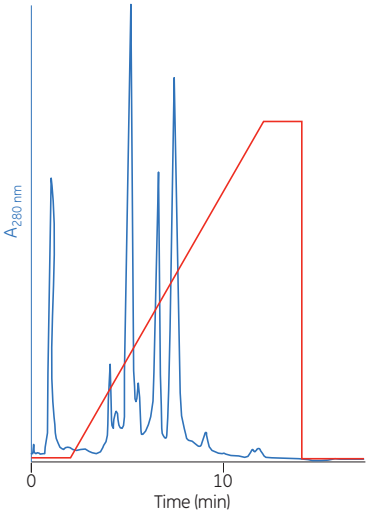


Fig 13. Separation of partially purified bacitracin from *Bacillus subtilis*.

Phosphorothioate DNA

Column:	RESOURCE Q 1 mL
Sample:	Fully thiolated DNA 20-mer, ATA CCG ATT-AAG CGA AGT TT
Sample load:	Orange curve: 0.66 mg/mL medium Green curve: 3.3 mg/mL medium Blue curve: 6.6 mg/mL medium
Start buffer:	10 mM NaOH, pH 12, 0.8 M NaCl
Elution buffer:	10 mM NaOH, pH 12, 1.8 M NaCl
Flow rate:	300 cm/h (1.67 mL/min)
Gradient:	0% to 80% elution buffer, 32 CV

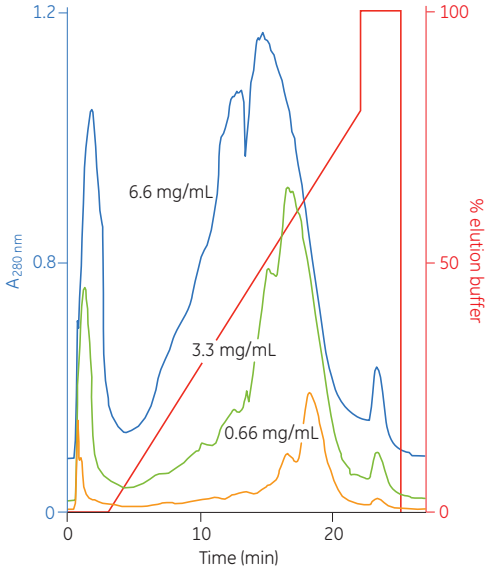


Fig 14. Purification of a synthetic phosphorothioate DNA oligonucleotide.

Ordering information

Prepacked columns	Pack size	Code number
SOURCE 15Q 4.6/100 PE		17-5181-01
SOURCE 15S 4.6/100 PE		17-5182-01
RESOURCE Q	1 mL	17-1177-01
	6 mL	17-1179-01
RESOURCE S	1 mL	17-1178-01
	6 mL	17-1180-01

Media	Pack size	Code number
SOURCE 15Q	10 mL	17-0947-20
	50 mL	17-0947-01
	200 mL	17-0947-05
	500 mL	17-0947-02
	1 L	17-0947-03
	5 L	17-0947-04
SOURCE 15S	10 mL	17-0944-10
	50 mL	17-0944-01
	200 mL	17-0944-05
	500 mL	17-0944-02
	1 L	17-0944-03

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