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

ION EXCHANGE CHROMATOGRAPHY

SOURCE™ 15Q and SOURCE 15S are high performance ion exchange chromatography (IEX) 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 polishing stage of an industrial purification process. The resins 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 resins 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 resins are based on 15 µm, spherical and monodisperse, porous, polystyrene/divinyl benzene particles 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 resin packs, prepacked Tricorn, and RESOURCE columns.

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).

Table 1. SOURCE 15Q and SOURCE 15S characteristics

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	
	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_{B50}	15Q: ~ 45 mg BSA/mL resin[7]	
	15S: ~ 80 mg lysozyme/mL resin[8]	
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M HCl, 100 % ethanol, 100% isopropanol, 100% acetonitrile, 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, 4˚C to 30˚C	
15S	0.2 M sodium acetate in 20% ethanol, 4˚C to 30˚C	

[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 BisTrisPropane, 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 should only be used for cleaning purposes

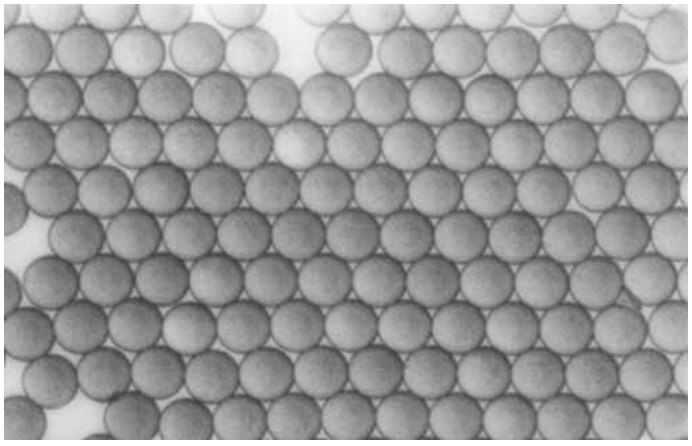

Fig 2. Light microscope photograph of SOURCE 15Q. Note the uniform size distribution

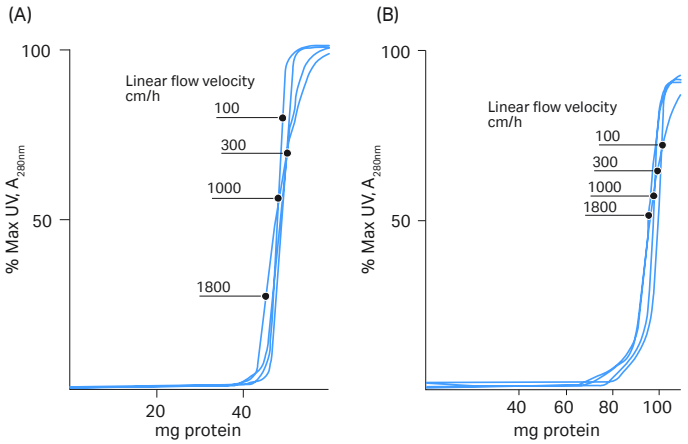

Fig 3. Breakthrough curves at different flow velocities (superimposed). (A) RESOURCE Q, 1 mL (6.4 mm diameter and 30 mm bed height), sample: bovine serum albumin (Sigma), 5 mg/mL; (B) RESOURCE S, 1 mL (6.4 mm diameter and 30 mm bed height), sample: lysozyme (Sigma), 5 mg/mL.

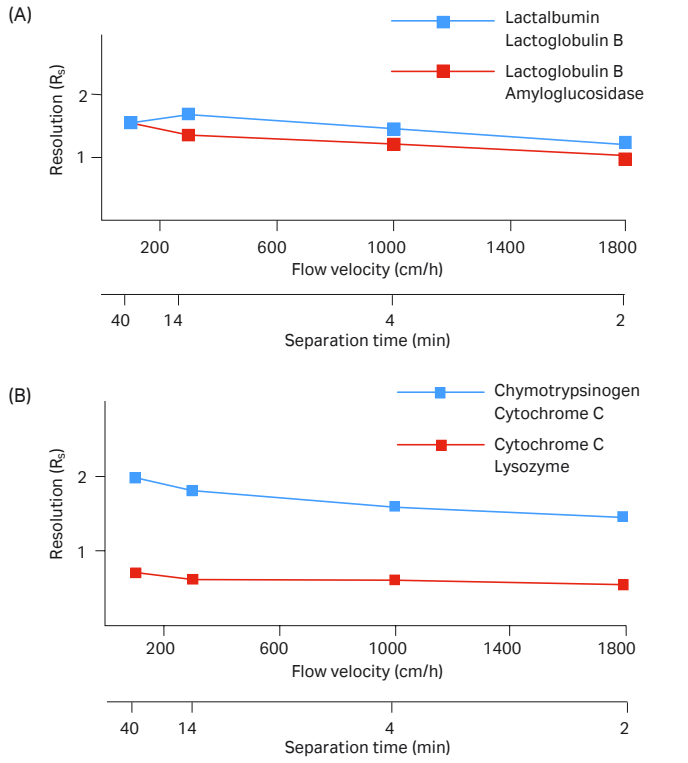

Fig 4. Resolution versus flow for model proteins. Resolution is maintained at high flow velocities. (A) RESOURCE Q, 1 mL (6.4 mm diameter and 30 mm bed height), sample: lactalbumin, lactoglobulin B, and amyloglucosidase (Sigma), total load: 12 mg; (B) RESOURCE S, 1 mL (6.4 mm diameter and 30 mm bed height), 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 cleaning. SOURCE 15Q and 15S have been substituted with quaternary ammonium 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.

Resin:	SOURCE 15Q, 4 separate batches
Column:	7.5 mm i.d., 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 propane 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

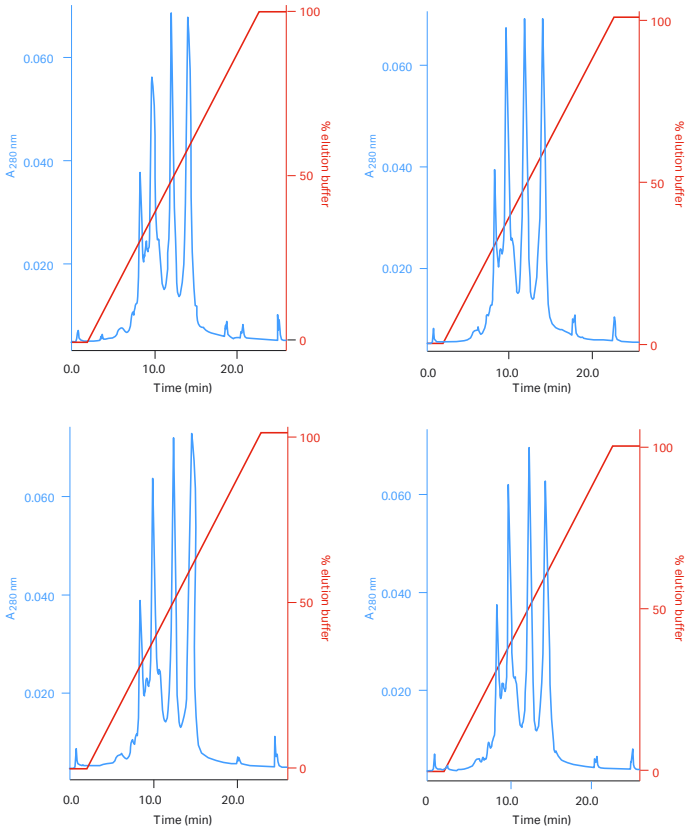

Fig 5. Results of selectivity tests on four batches of SOURCE 15Q show reproducible quality

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 lab scale because of the high capacity of the resin 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 0.1 MPa [1 bar, 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 operating flow rate (mL/min)	1 to 10	1 to 60	0.5 to 2.5
Maximum operating flow rate (mL/min)	10	60	5.0
Maximum operating pressure (MPa [bar, psi])	1.5 (15, 220)	0.6 (6, 87)	4 (40, 580)

Operation

SOURCE 15 IEX resins 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 resins 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 with salt gradients up to 0.5 M or 1 M NaCl. The strong ion exchange groups allow operatation between pH 2 and 12 (SOURCE 15Q) and 2 to 13 (SOURCE 15S).

Wide pH stability not only allows cleaning with harsh agents like 1.0 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.

Resin:	RESOURCE Q 1 mL
Column:	6.4 mm i.d., 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)

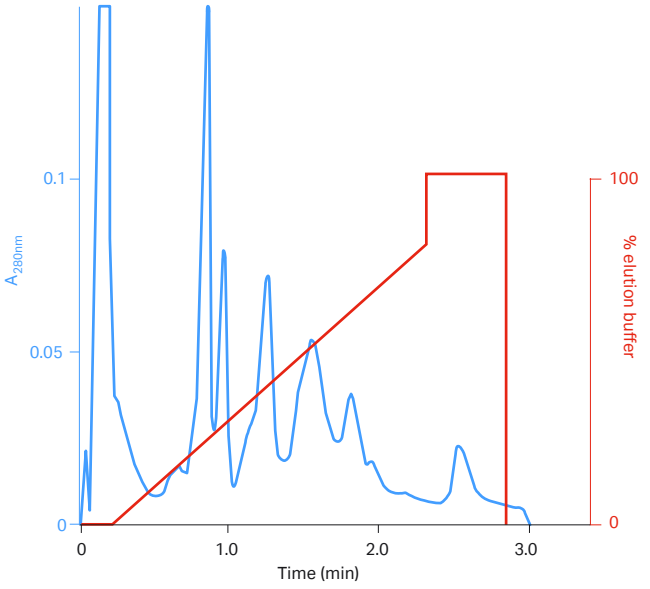

Fig 6. Rapid and high resolution separation in 3 minutes using RESOURCE Q 1 mL

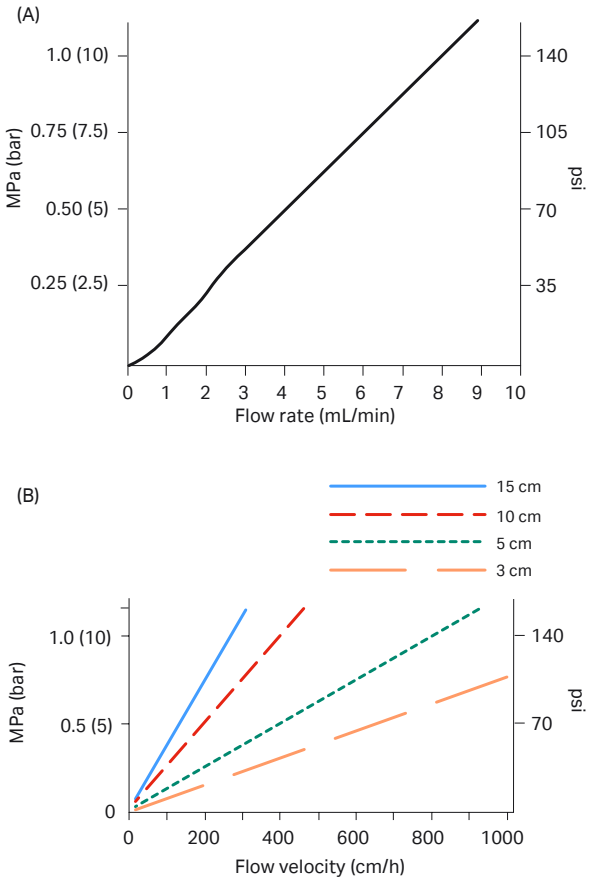

Fig 7. Pressure/flow curves that can be expected with SOURCE 15Q and SOURCE 15S. (A) Pressure drop over RESOURCE 1 mL column (6.4 mm diameter and 30 m bed height). Column can be used with high pressure equipment or peristaltic pumps; (B) Pressure drop over FineLINE 100 columns packed with SOURCE 15 to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

Equipment and flow rates

The excellent flow properties of SOURCE 15Q and SOURCE 15S make these resins suitable to use in lab-scale columns (Table 3) 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 lab equipment at back pressures of less than 0.1 MPa (1 bar, 15 psi).

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

Table 3. Recommended lab-scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
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

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

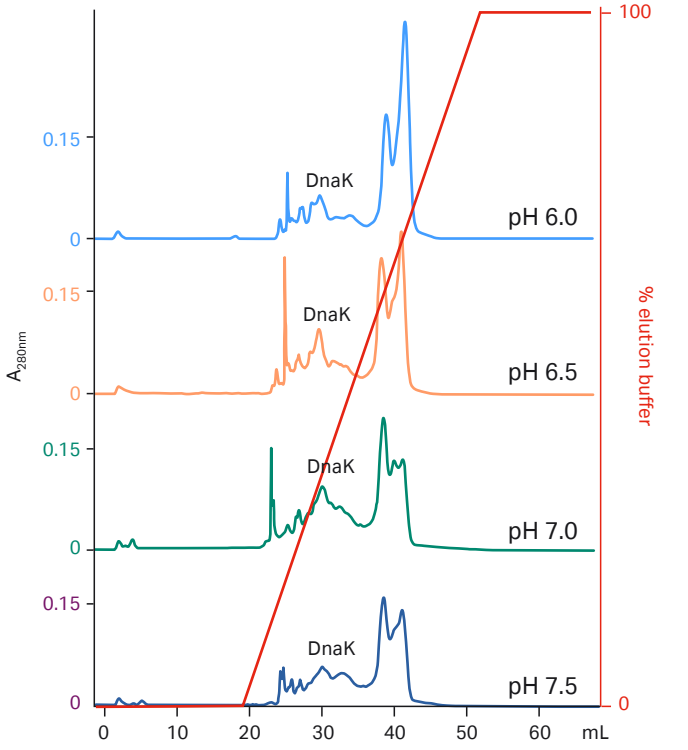

Fig 8. To find an optimal pH for the separation of the chaparone protein DnaK, four buffers with different pH values were tested during pH scouting: pH 6.0, 6.5, 7.0, 7.5. The chromatograms show that the DnaK-containing peak is most defined and concentrated when purifying at pH 6 and 6.5. The purity 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.

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

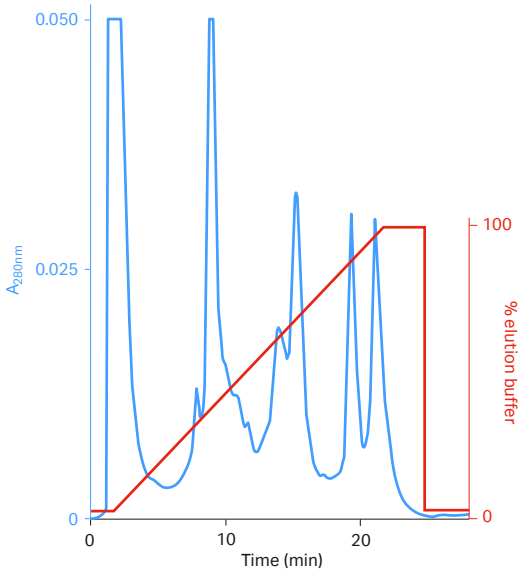

Fig 9. Separation of snake venom (Sigma) on RESOURCE S, 1 mL at 1 mL/min (180 cm/h) shows high performance using a peristaltic pump. Although high-performance separations with RESOURCE Q and S do not put special demands on the pump, resolution obtained on the column can be lost through mixing in dead 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 resins 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 lab column to a 390 mL FineLINE 100 production column.

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

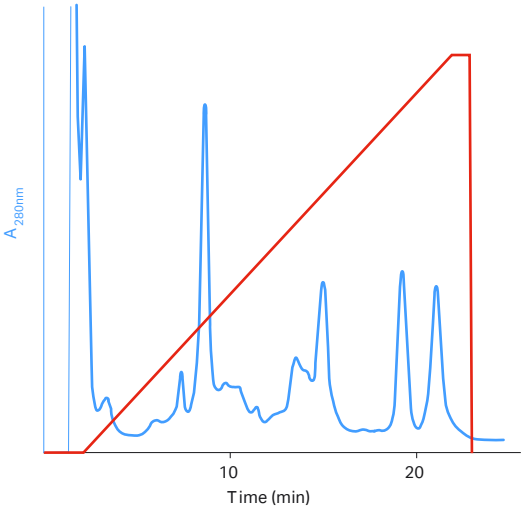

Fig 10. Linear scale-up on RESOURCE S 6 mL column. Separation of snake venom (Sigma) at 6 mL/min (180 cm/h).

Cytiva has designed a range of columns, FineLINE, for optimal performance of SOURCE resins in scale-up and production (see Table 4). 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 cytiva.com/bioprocess.

Table 4. Recommended large-scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
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, EPDM, 10 µm	350	28 800	50 to 300

The examples in Figures 6, 8, 9, 10, and 11 show protein separations. Typically loadings up to 25 mg/mL resin 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.

Column: (A) SOURCE 15S, 2.2 mL (7.5 mm i.d., 50 mm bed height)
(B) SOURCE 15S, FineLINE 100, 390 mL (100 mm i.d., 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

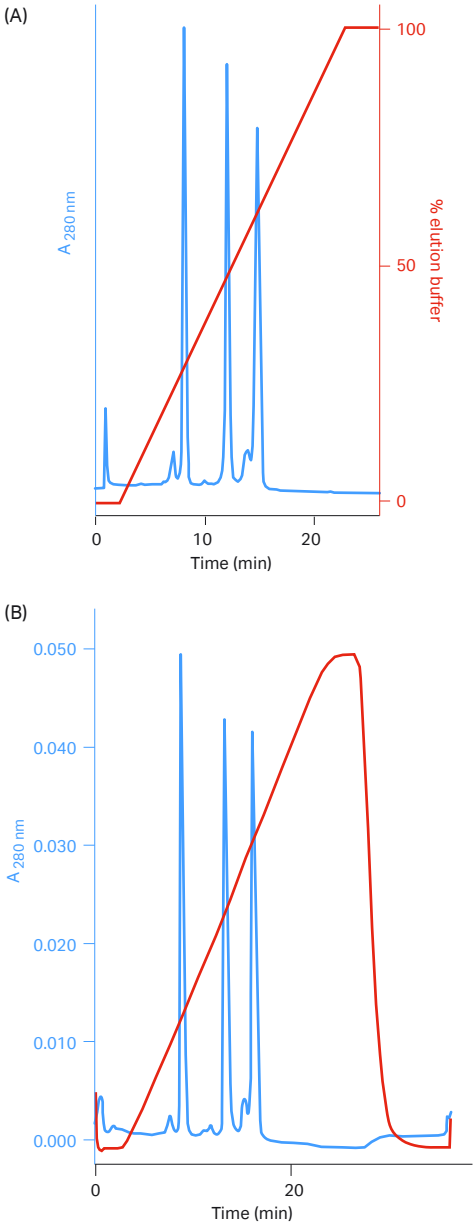

Fig 11. Separation of proteins scaled up to 100 mm diameter column. Note that the excellent resolution is retained, showing reproducibility.

Column:	(A) RESOURCE Q 1 mL (6.4 mm i.d., 30 mm bed height) (B) SOURCE 15Q in FineLINE 100, 240 mL(100 mm i.d., 30 mm bed height)
Sample:	(A) 800 µmol synthesis of 19-mer DNA oligo, load 5 mg (B) 800 µmol synthesis of 19-mer DNA oligo, 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

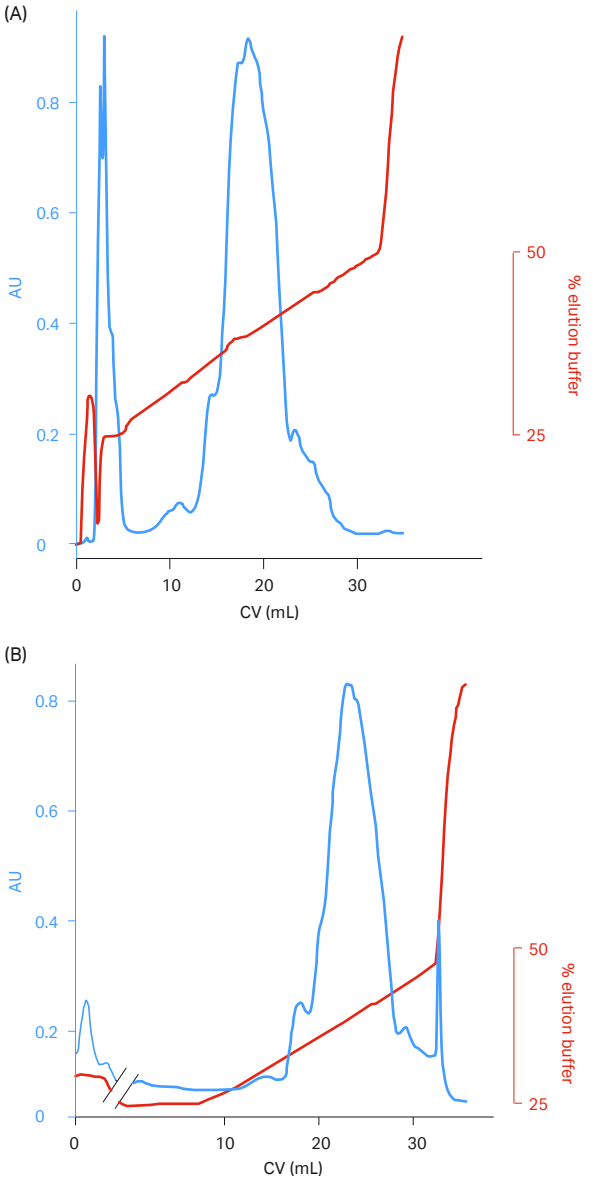

Fig 12. Purification of synthetic 19-mer DNA oligonucleotide on RESOURCE Q 1 mL scaled up to FineLINE 100 column. Separation optimized for sample load, yield, and purity of product.

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

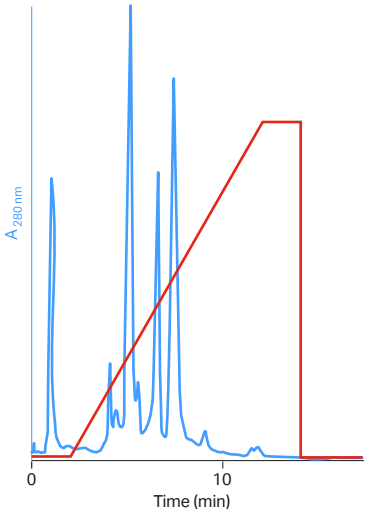

Fig 13. Separation of partially purified bacitracin from *Bacillus subtilis*.

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 resin Green curve: 3.3 mg/mL resin Blue curve: 6.6 mg/mL resin
Start buffer:	10 mM NaOH, pH 12, 0.8 M NaCl
Elution buffer:	10 mM NaOH, pH 12, 1.8 M NaCl
Flow rate:	1.67 mL/min (300 cm/h)
Gradient:	0% to 80% elution buffer, 32 CV

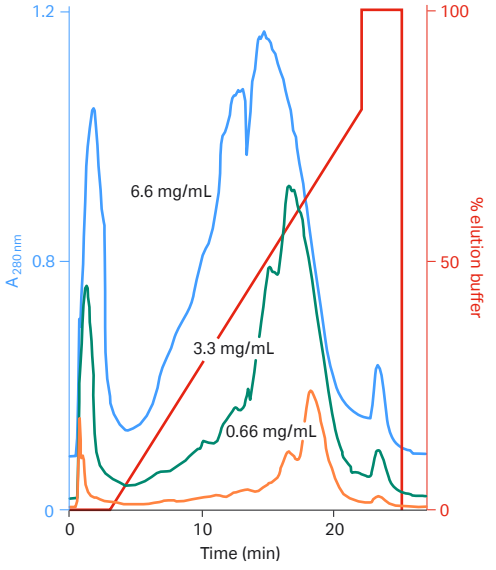

Fig 14. Purification of a synthetic phosphorothioate DNA oligonucleotide

Ordering information

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

Lab-scale columns		
FineLINE Pilot 35		18110202
Tricorn 5/20		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

Large-scale columns		
FineLINE 70		18115298
FineLINE 70L		18115299
FineLINE 100P		11002798
FineLINE 100PL		11002799
FineLINE 200P		11003114
FineLINE 200PL		11003115
FineLINE 350P, PFR, 2µm		11002792
FineLINE 350PL, EPDM, 10µm		11002785

Resin		Product code
SOURCE 15Q	10 mL	17094720
	50 mL	17094701
	200 mL	17094705
	500 mL	17094702
	1 L	17094703
	5 L	17094704
SOURCE 15S	10 mL	17094410
	50 mL	17094401
	200 mL	17094405
	500 mL	17094402
	1 L	17094403

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