GE Healthcare
Life Sciences

SOURCE™ 30Q
SOURCE 30S

SOURCE 30Q and SOURCE 30S are high performance ion exchange chromatography (IEX) media (resins) for fast, preparative purification of biomolecules. The media are suitable for the intermediate and polishing steps of industrial purification processes where high productivity and maintained performance at large scale are important.

SOURCE 30 IEX media are characterised by:

- Exceptional pressure/flow rate characteristics
- Maintained performance at high flow rates and high sample loads
- Excellent scalability
- Reproducible quality
- High chemical stability

Chromatography media characteristics

SOURCE 30Q and 30S are based on a unique 30 µm, monosized, rigid, polystyrene/divinyl benzene matrix (Fig 1). The media are produced in a similar way to the corresponding SOURCE 15 IEX media and have been substituted with quaternary ammonium groups and sulfonate groups, respectively, and their selectivities are equivalent in most applications.

The ion exchange groups are attached to the matrix via long, hydrophilic spacer arms after hydrophilisation of the polymeric base matrix. SOURCE 30Q and 30S retain their charge over a wide pH range and give good recovery of biological activity. Emphasis during development has been on quality, reproducibility, and scalability, features which are particularly important for industrial applications where strict regulatory controls apply. Table 1 summarises the general properties of these media.

Fig 1. SOURCE 30Q and SOURCE 30S are suitable for the intermediate steps of industrial purification processes where high productivity and maintained performance at large scale are important.

SOURCE 30Q and 30S are suitable for intermediate purification steps in industrial processes when large volumes of partially purified material need to be processed. A high degree of purification can be obtained with high productivity. The sample is thereby prepared for polishing on a smaller matrix (e.g., SOURCE 15). An example of this strategy, a process for the purification of a recombinant protein, is described later under Applications. For very large-scale applications, SOURCE 30Q and 30S are excellent choices for the final polishing stage.

Table 1. Characteristics of SOURCE 30Q and 30S IEX media

Matrix	Polystyrene/divinyl benzene
Type of ligand	Q: R-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-N+$(CH_3)_3$ S: R-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-SO_3^-
Bead form	Rigid, spherical, porous, monosized
Particle size	30 µm (modal diameter 28.7–32.8 µm)
Particle size distribution	CV < 3%
Dynamic capacity[1]	SOURCE 30S: At least 80 mg lysozyme/mL medium SOURCE 30Q: At least 40 mg BSA/mL medium
Maximum flow velocity[2]	2000 cm/h
Recommended working flow velocity	300 to 1000 cm/h
pH stability working cleaning-in-place	 2 to 12 1 to 14
Chemical stability	Stable in all commonly used aqueous buffers: 1 M HCl, 1 M NaOH, 70% ethanol, 30% isopropanol with 0.5 M NaOH, 8 M urea, 6 M guanidine-HCl (all tested at 40°C for 7 d)
Autoclavable	20 min at 121°C
Operating temperature	4 to 40°C
Delivery conditions	SOURCE 30S: 0.2 M sodium acetate in 20% ethanol SOURCE 30Q: 20% ethanol

[1] Determined at 50% breakthrough by frontal analysis at flow rates up to 1800 cm/h, using a 5.0 mg/mL solution of protein in 20 mM sodium phosphate buffer, pH 6.8 (lysozyme) and 50 mM Bis-Tris propane buffer, pH 7.0 (BSA). The actual loading capacity in a real working situation will depend on the nature and concentration of the sample, and the degree of resolution required.

[2] Will depend on the pressure specification of the chromatographic system used. A linear flow velocity of 2000 cm/h will give a pressure drop of approximately 10 bar at a bed height of 10 cm.

Exceptional pressure/flow rate characteristics

The SOURCE 30 matrix has a uniform 30 µm diameter, spherical shape and is free from broken beads, fragments and fines (Fig 2). This structure results in stable beds with excellent flow properties. The back pressure from SOURCE 30Q and 30S is much lower than from other media with the same particle size range (Fig 3). This lower back pressure offers the advantage of being able to run at very high flow rates on medium pressure equipment (up to 10 bar). SOURCE 30Q and 30S can also be used with low pressure equipment (3 bar) with excellent flow rates.

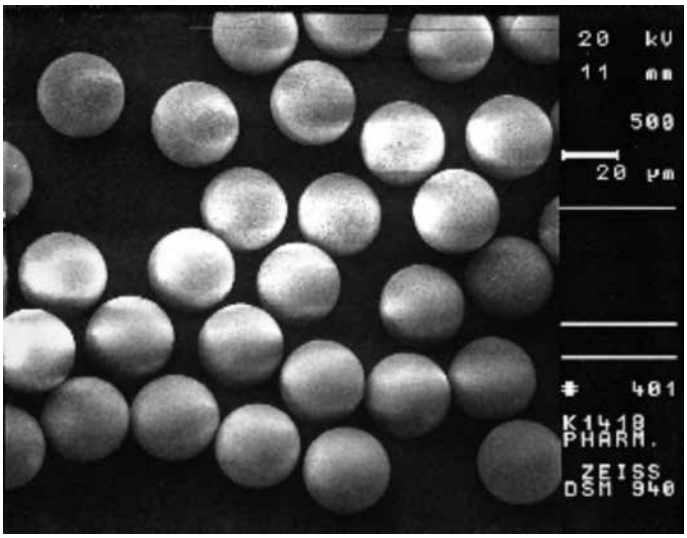
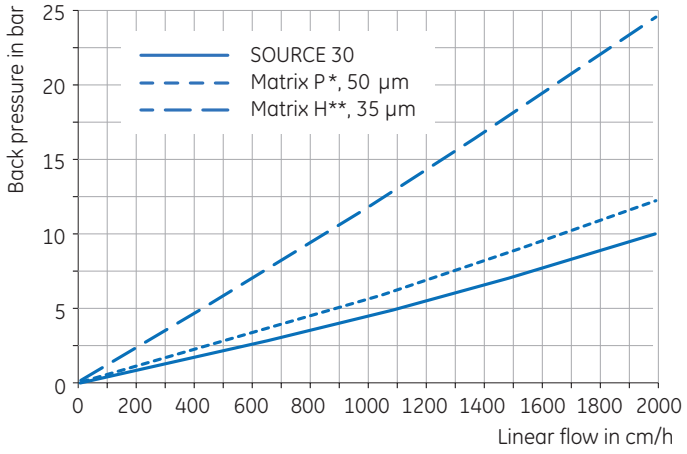


Fig 2. Electron micrograph of SOURCE 30 beads. Note the uniform size and absence of fines, fragments, and broken beads.



* POROS™ 50 HS, Lot No. 250-135, Perseptive Biosystems.

** S HyperD™ F, Lot No. 3412, BioSepra.
Both were handled according to the manufacturers' recommendations.

Fig 3. Pressure/flow rate characteristics of the monosized SOURCE 30 matrix compared with polysized media with mean diameters of 35 and 50 µm, respectively. The pressure/flow rate data were determined in a 100 mm i.d. column with 10 cm bed height.

Maintained performance at high flow rate and high sample load

The wide, pore-size distribution and the large, specific surface area of the media offer excellent resolution and capacity for a wide range of molecules, from small peptides and oligonucleotides up to large proteins. The performance is well maintained at high flow rates and high sample loads.

This is illustrated in Figures 4 and 5, which show separations of model protein mixtures.

Influence of increasing flow rate

Column:	SOURCE 30Q, 10 mm i.d. × 50 mm (4 mL)
Sample:	Mixture of lactalbumin, lactoglobulin B, and amyloglucosidase
Sample load:	1 mg/mL bed volume
Start buffer:	20 mM Bis-Tris propane, pH 7.0
Elution buffer:	0.5 M sodium chloride, 20 mM Bis-Tris propane, pH 7.0
Flow rate:	(A) 4 mL/min (300 cm/h) (B) 13 mL/min (1000 cm/h)
Gradient:	0–100% elution buffer, 20 column volumes

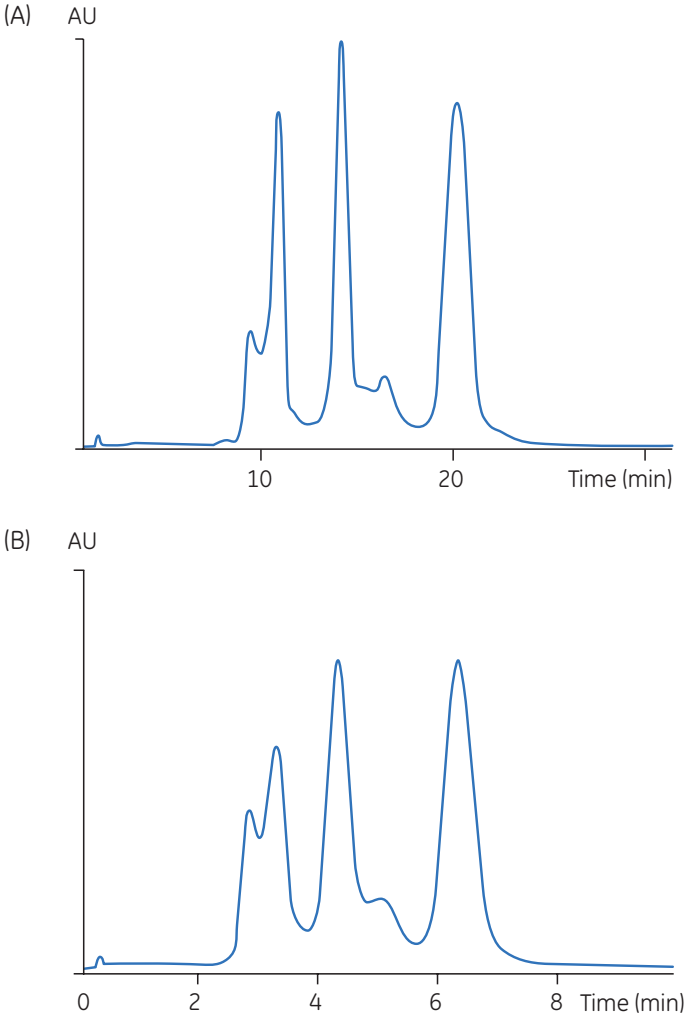


Fig 4. The influence of increasing flow rate on resolution.

Influence of increasing sample load

Column:	SOURCE 30S, 5 mm i.d. × 50 mm (1 mL)
Sample:	Mixture of chymotrypsinogen, cytochrome C, and lysozyme
Sample load:	(A) 1 mg (B) 10 mg
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	0.5 M sodium chloride, 20 mM sodium phosphate, pH 6.8
Flow rate:	1 mL/min (300 cm/h)
Gradient:	0–100% elution buffer, 20 column volumes

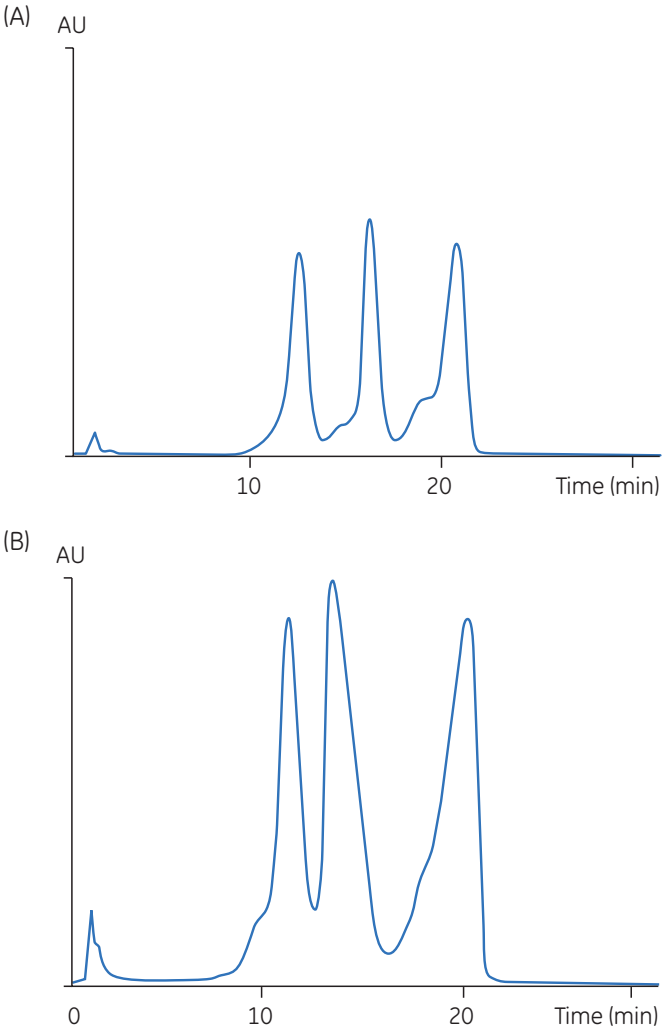


Fig 5. The influence of increasing sample load on resolution.

Excellent scalability

SOURCE 30Q and 30S are easy to pack at both laboratory and large scale and they maintain performance during scale-up. Figure 6 shows a 700-fold scale up of a model protein separation on SOURCE 30S going from a 2.2 mL column to a 1.6 L column in one step.

Figure 7 shows a scale up on SOURCE 30S from a 105 mL small-scale column to a 50 L custom-designed production column.

700-fold scale up

Column: SOURCE 30S
(A) 7.5 mm i.d. × 50 mm (2.2 mL)
(B) 200 mm i.d. × 50 mm (1.57 L)
Sample: Mixture of chymotrypsinogen, cytochrome C, and lysozyme
Sample load: 0.32 mg/mL bed volume
Start buffer: 20 mM sodium phosphate, pH 6.8
Elution buffer: 0.5 M sodium chloride, 20 mM sodium phosphate, pH 6.8
Flow rate: (A) 2.2 mL/min (B) 1.57 L/min (300 cm/h)
Gradient: 0–100% elution buffer, 20 column volumes

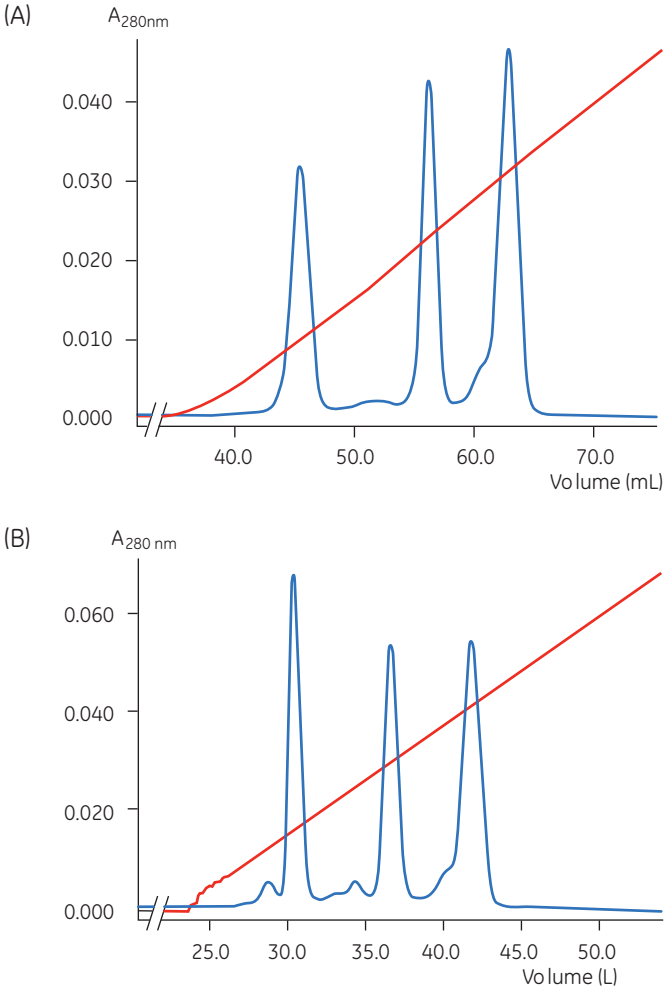


Fig 6. 700-fold scale up from a 2.2 mL lab-scale column to a 1.6 L production-scale FineLINE™ 200 column.

Scale up to production

Column: SOURCE 30S: (A) FineLINE Pilot 35, 35 mm i.d. × 109 mm (105 mL)
SOURCE 30S: (B) FineLINE 100, 100 mm i.d. × 100 mm (0.78 L)
SOURCE 30S: (C) FineLINE 800, 800 mm i.d. × 100 mm (50 L)
Sample: Ribonuclease A, cytochrome C, and lysozyme (3.75:1:1)
Sample load: 0.32 mg total protein/mL media
Start buffer: 20 mM sodium phosphate, pH 6.8
Elution buffer: 20 mM sodium phosphate + 0.4 M NaCl, pH 6.8
Flow rate: (A) 48 mL/min (B) 0.39 L/min, (C) 25 L/min
Gradient: 0–100% elution buffer, 20 column volumes

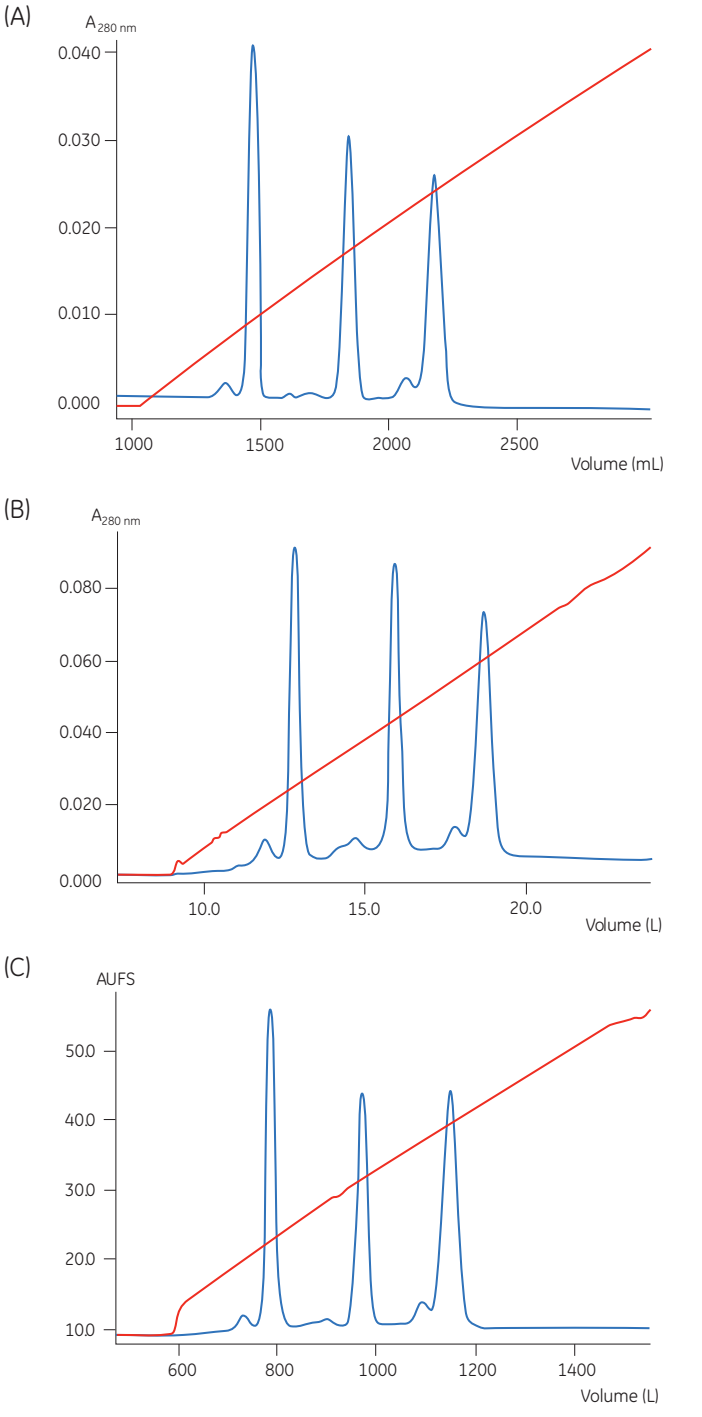


Fig 7. Scale-up from FineLINE Pilot 35 column via FineLINE 100 column (7x) to FineLINE 800 custom designed column (64x). Total scale up factor = 476x.

Batch-to-batch reproducibility

The combination of a unique manufacturing process and high-quality assurance standards results in consistent batch-to-batch quality. Chromatograms from QC analyses of four production batches of SOURCE 30S are shown in Figure 8.

Evaluation of 4 production batches

Column:	SOURCE 30S, 4 separate batches, 7.5 mm i.d. × 50 mm (2.2 mL)
Sample:	Mixture of chymotrypsinogen, cytochrome C, and lysozyme
Sample load:	0.32 mg/mL bed volume
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	0.5 M sodium chloride, 20 mM sodium phosphate, pH 6.8
Flow rate:	2.2 mL/min (300 cm/h)
Gradient:	0–100% elution buffer, 20 column volumes

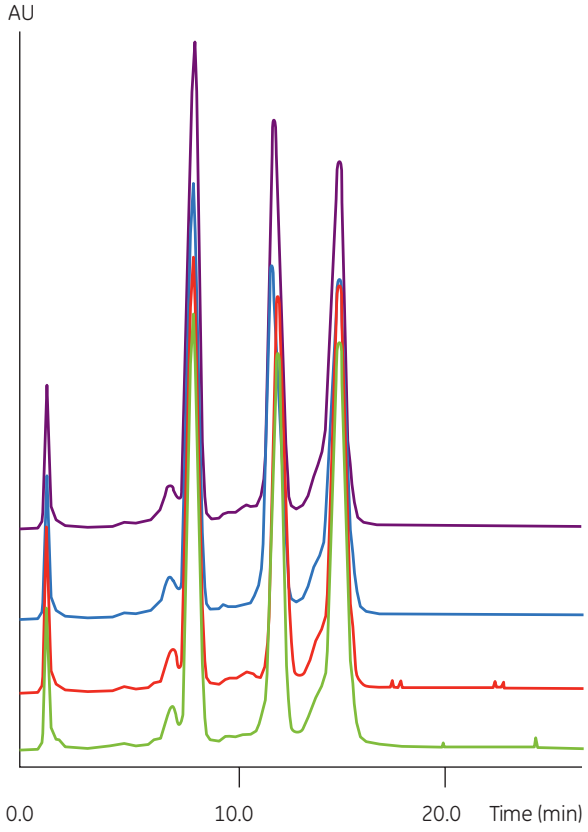


Fig 8. QC evaluation of 4 production batches of SOURCE 30S.

High chemical stability

The hydrophilised polymeric matrices of SOURCE 30Q and 30S have high chemical stability and can be used over a wide pH range (Table 1), allowing good flexibility in choosing conditions for separations as well as for efficient cleaning and sanitization.

Operation

SOURCE 30Q and 30S can be used with standard methods for IEX. Typical binding conditions are aqueous buffers in the pH range where the sample is stable and where optimal selectivity and capacity are obtained. Elution is normally achieved with salt gradients up to 0.5 or 1.0 M sodium chloride.

Efficient cleaning and sanitization can often be obtained by using 0.5 to 1 M NaOH.

The excellent flow properties of SOURCE 30Q and 30S make them suitable to use in lab-scale columns at flow velocities up to at least 2000 cm/h with ÄKTA™ systems. This is useful for lab-scale, preparative applications and for scouting different separation conditions at small-scale.

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.

Typically, linear flow velocities in the range 300 to 1000 cm/h will provide the desired resolution, productivity, and product yield with convenient, large-scale separation times from a few minutes to one hour.

Equipment

Table 2. Recommended columns for labratory scale

Column i.d.	Bed height	Bed volume
HiScale™ 16/20	0 to 20	0 to 40
HiScale 26/20	0 to 20	0 to 106
HiScale 50/20	0 to 20	0 to 393

For scale-up, the recommendation is the AxiChrom™ column range, visit www.gelifesciences.com/bioprocess for more information or see Ordering information “Related literature”.

Applications

Recombinant protein

In the application described in detail below, SOURCE 30Q was used in one of the intermediate steps, giving a high degree of purification with excellent productivity. This intermediate step was followed by a polishing step using SOURCE 15PHE to remove the final contaminants.

Recombinant *Pseudomonas aeruginosa* exotoxin A, produced as a periplasmic protein in *Escherichia coli*, was initially purified with STREAMLINE™ DEAE expanded bed adsorption (step 1), followed by hydrophobic interaction chromatography (HIC) on Phenyl Sepharose™ 6 Fast Flow (high sub) (step 2). The initial purification was followed by IEX on SOURCE 30Q (step 3). Final polishing was performed by HIC on SOURCE 15PHE (step 4).

The SOURCE 30Q step was scaled up to a FineLINE 100 column, i.d. 100 mm (chromatogram in Fig 9). The sample load was 4.8 mg protein/mL bed volume and the flow velocity during sample loading and elution was 600 cm/h. The purities of the product pools in steps 2 to 4, estimated by native PAGE, are shown in Figure 10. The recovery of exotoxin A was 91% in the SOURCE 30Q step and 44% in the overall process*.

Intermediate purification on SOURCE 30Q

Column:	SOURCE 30Q, FineLINE 100, 100 mm i.d. × 48 mm (375 mL)
Sample:	Partially purified recombinant *P. aeruginosa* exotoxin A, pool from step 2 diluted 1:3 with water
Sample load:	1.8 g total protein (0.29 g exotoxin A) in 1.5 L
Start buffer:	20 mM sodium phosphate, pH 7.4
Elution buffer:	1.0 M sodium chloride, 20 mM sodium phosphate, pH 7.4
Flow rate:	785 mL/min (600 cm/h)
Gradient:	0–50% elution buffer, 20 column volumes

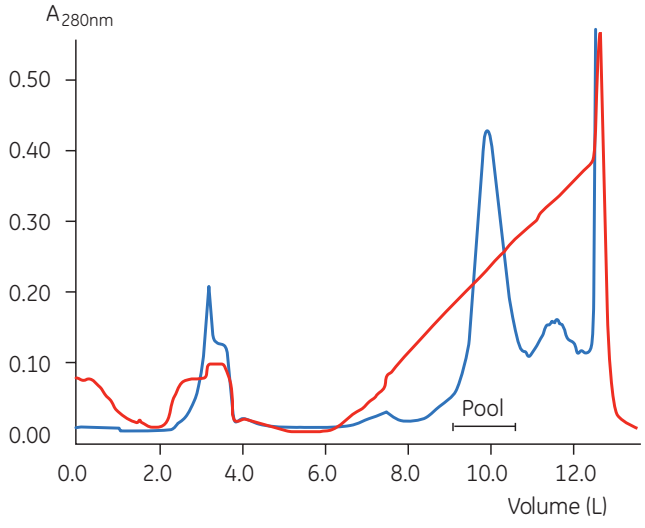
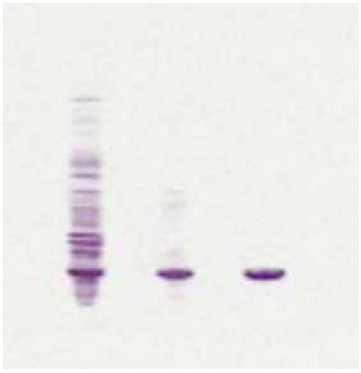


Fig 9. Intermediate purification of recombinant *P. aeruginosa* exotoxin A on SOURCE 30Q.



Native PAGE results

Lane 1 Pool from step 2 on Phenyl Sepharose Fast Flow (high sub)

Lane 2 Pool from step 3 on SOURCE 30Q

Lane 3 Pool from step 4 on SOURCE 15PHE

Fig 10. Native PAGE of pools from steps 2 to 4 of the purification of recombinant *P. aeruginosa* exotoxin A.

Antisense phosphorothioate oligonucleotide

A method for purifying phosphorothioate oligonucleotides using SOURCE 30Q has been developed. The method includes adsorption of trityl-on oligonucleotide on SOURCE 30Q, washing with 10 mM NaOH and 2 M sodium chloride to remove nontritylated failure sequences, on-column cleavage of the trityl groups using 0.4% trifluoroacetic acid (TFA), washing with 10 mM NaOH, and eluting the oligonucleotide with a sodium chloride gradient to further purify it from shorter sequences. After elution, SOURCE 30Q is regenerated with 30% isopropanol in 2 M sodium chloride to wash away the adsorbed trityl-groups.

A 25-mer phosphorothioate oligonucleotide produced on OligoPilot™ II DNA/RNA Synthesizer was purified with this method. A 25% ammonia solution containing the crude oligonucleotide mixture obtained after synthesis was applied directly onto a 0.8 L SOURCE 30Q column. The chromatogram from the gradient elution is shown in Figure 11. Analysis of the pool revealed a yield of 1.56 g product with a purity of 97% as determined by capillary electrophoresis (Fig 12). The overall recovery was approximately 70%. The complete process (cleavage and purification) took less than three hours.

The complete range of SOURCE media includes IEX media based on 15 µm beads (SOURCE 15Q and 15S), HIC media (SOURCE 15ETH, 15ISO and 15PHE), and reversed phase chromatography media (SOURCE 15RPC) as well as pre-packed RESOURCE™ columns.

* (Work by GE Healthcare in co-operation with Dr. J. Shiloach, National Institutes of Health, Bethesda, USA.)

Preparative purification on SOURCE 30Q

Column: SOURCE 30Q, FineLINE 100, 100 mm i.d. × 100 mm (785 mL)
Sample: Trityl-on 25-mer phosphorothioate oligonucleotide, (washed with 2.0 M sodium chloride and subsequently treated with 0.4% trifluoroacetic acid before gradient elution).
Sample load: Approximately 2.3 g of full length product
Start buffer: 0.8 M sodium chloride, pH 12
Elution buffer: 2.0 M sodium chloride, pH 12
Flow rate: 390 mL/min (300 cm/h)
Gradient: 0–80% elution buffer, 25 column volumes

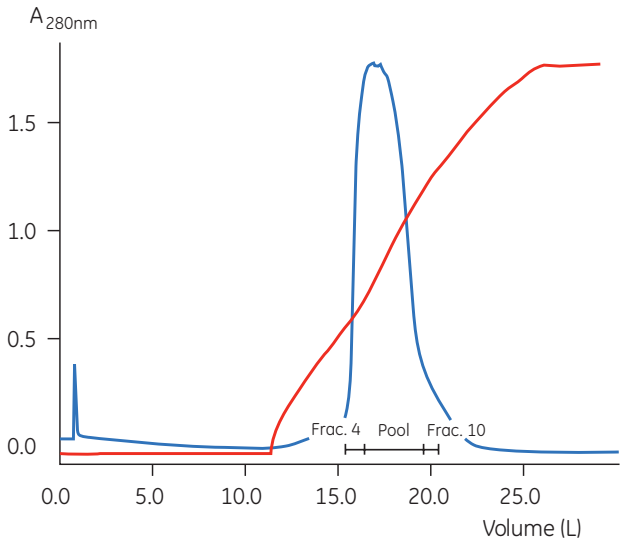


Fig 11. Preparative purification of 25-mer phosphorothioate oligonucleotide on SOURCE 30Q.

Capillary electrophoresis results

Samples: All samples were desalted on NAP 10 Columns.
(A) Pool
(B) Fraction 4
(C) Fraction 10
Capillary: µPAGE (5% T, 5% C), capillary length: 40 cm (J&W Scientific, FISON)
Buffer: Tris-borate and urea buffer, (J&W Scientific, FISON)
Running conditions: 8 kV, 10 s (sample) 16 kV, 30 min (run)
System: Waters Quanta 4000 E

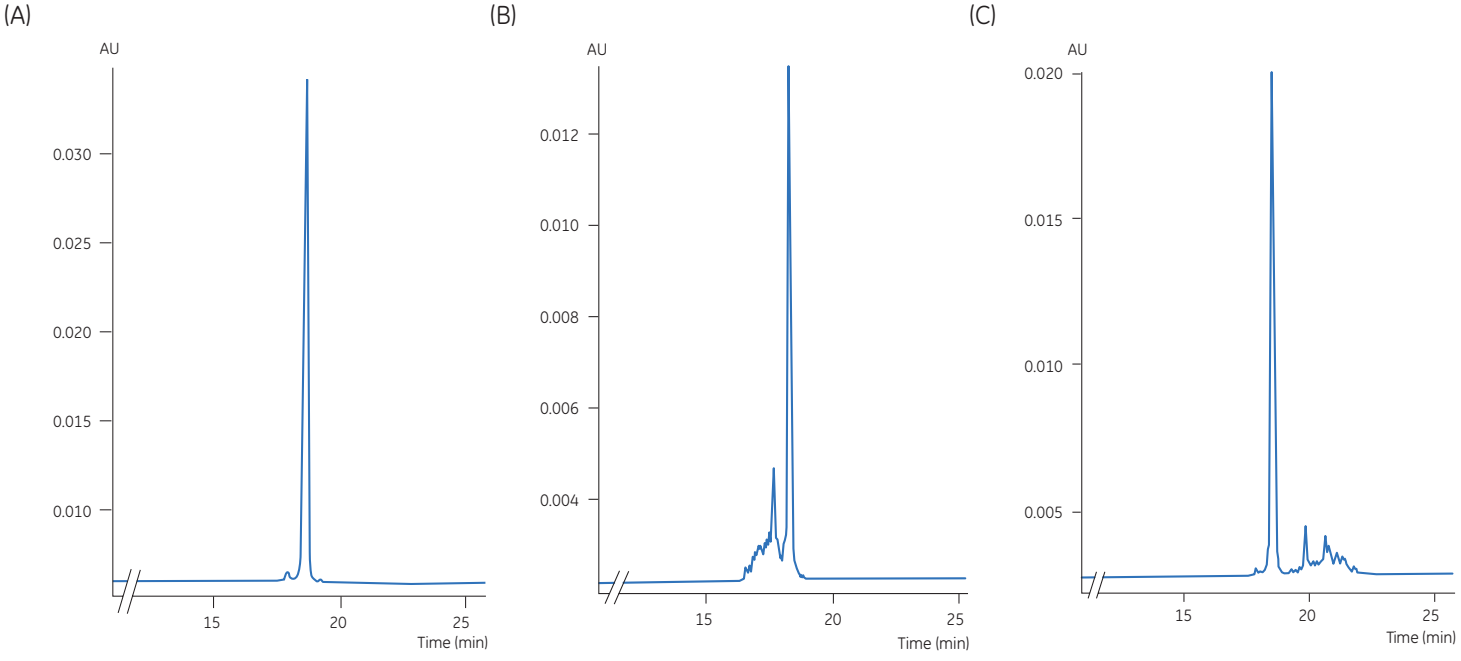


Fig 12. Capillary electrophoresis of the pool and side fractions from the preparative purification of a 25-mer phosphorothioate oligonucleotide.

Ordering information

Product	Pack size	Code number
SOURCE 30Q	50 mL	17-1275-01
	200 mL	17-1275-02
	1 L	17-1275-03
	5 L	17-1275-04
SOURCE 30S	50 mL	17-1273-01
	200 mL	17-1273-02
	1 L	17-1273-03
	5 L	17-1273-04

Empty columns

HiScale 16/20	28-9644-41
HiScale 16/40	28-9644-24
HiScale 26/20	28-9645-14
HiScale 26/40	28-9645-13
HiScale 50/20	28-9644-45
HiScale 50/40	28-9644-44

* Each HiScale column is delivered with two adapters

Related literature

Data File	Code number
AxiChrom columns	28-9290-41

Handbook

Ion Exchange Chromatography, Principles and Methods	18-1114-21

Selection guides

Ion exchange columns and media	18-1127-31
Prepacked chromatography columns for AKTA systems	28-9317-78

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