SOURCE 30Q SOURCE 30S

ION EXCHANGE CHROMATOGRAPHY

SOURCE™ 30Q and SOURCE 30S are high performance ion exchange chromatography (IEX) resins for fast, preparative purification of biomolecules. The resins 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 resins 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 resin characteristics

SOURCE 30Q and 30S are based on a ~ 30 µm, spherical and monodisperse, porous, polystyrene/divinyl benzene matrix (Fig 1). The resins are produced in a similar way to the corresponding SOURCE 15 IEX resins 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 resins.

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 under *Applications*. For very large-scale applications, SOURCE 30Q and 30S are excellent choices for the final polishing stage.

The complete range of SOURCE resins includes IEX resins based on ~ 15 µm beads (SOURCE 15Q and 15S), HIC resins (SOURCE 15ISO and 15PHE), and reversed phase chromatography resins (SOURCE 15RPC) as well as prepacked RESOURCE™ columns.

Table 1. Characteristics of SOURCE 30Q and 30S resins

Matrix	Spherical and monodisperse, porous, rigid, polystyrene/divinyl benzene particles	
Type of ion exchanger	*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-	
Mean particle diameter[1]	~ 30 µm	
Dynamic binding capacity, Q_{B50}	*SOURCE 30S:* ~ 80 mg Lysozyme/mL resin[2] *SOURCE 30Q:* ~ 45 mg BSA/mL resin[3]	
	SOURCE 30Q	*SOURCE 30S*
pH stability, operational[4]	2 to 12	2 to 13
pH stability, CIP[5]	1 to 14	1 to 14
pH ligand fully charged[6]	Entire pH range	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers, 1 M HCl, 50% acetic acid, 70% ethanol, 30% isopropanol/0.5 M NaOH, 1.0 M NaOH[7], 0.5% Tween	
Autoclavability	20 min at 121°C in H_2O, pH 7, 1 cycle	
Recommended maximum operating flow velocity[8]	2000 cm/h	
Recommended operating flow velocity	300 to 1000 cm/h	
Operating temperature	4˚C to 40˚C	
Delivery conditions	*SOURCE 30Q:* 20% ethanol *SOURCE 30S:* 20% ethanol and 0.2 M sodium acetate	
Storage	20% ethanol (SOURCE 30Q) or 20% ethanol and 0.2 M sodium acetate (SOURCE 30S), 4˚C to 30˚C	

[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 Lysozyme in 20 mM Sodium phosphate, pH 6.8.

[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 BSA in 20 mM BisTrisPropane, pH 7.0.

[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 resin is fully charged; although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges.

[7] 1.0 M NaOH should only be used for cleaning purposes.

[8] Will depend on the pressure specification of the chromatographic system used. A linear flow rate 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 resins 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 [1 MPa, 145 psi]). SOURCE 30Q and 30S can also be used with low pressure equipment (3 bar) with excellent flow rates.

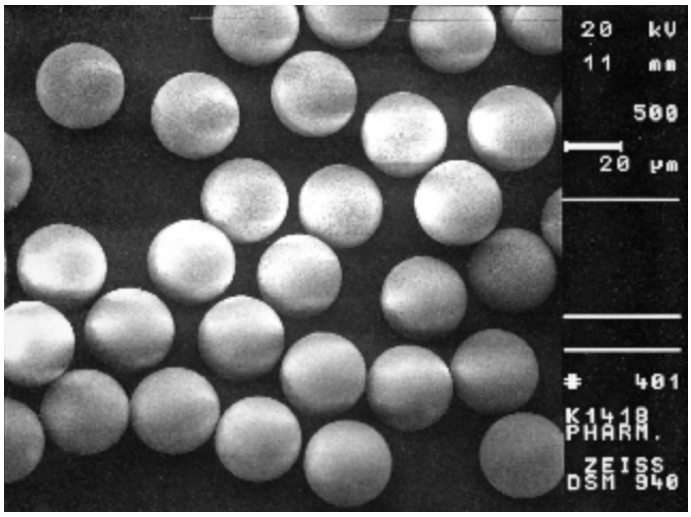
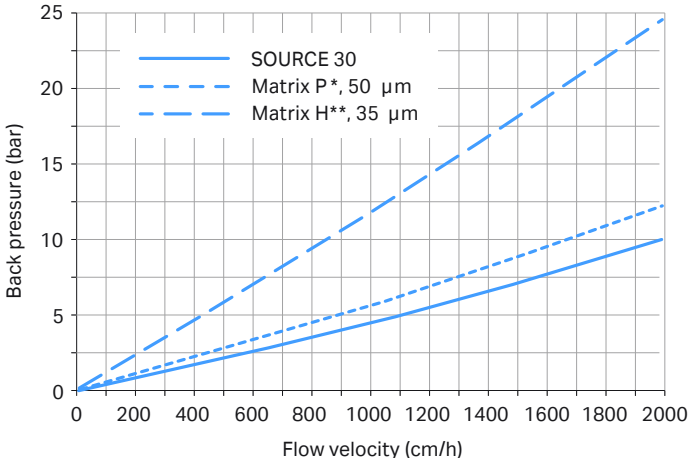


Fig 2. Electron micrograph of SOURCE 30 beads. Note the uniform size and absence of fines, fragments, and broken beads.



* 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 characteristics of the monosized SOURCE 30 matrix compared with polysized resins with mean diameters of ~ 35 and ~ 50 µm, respectively. The pressure/flow 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 resins 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

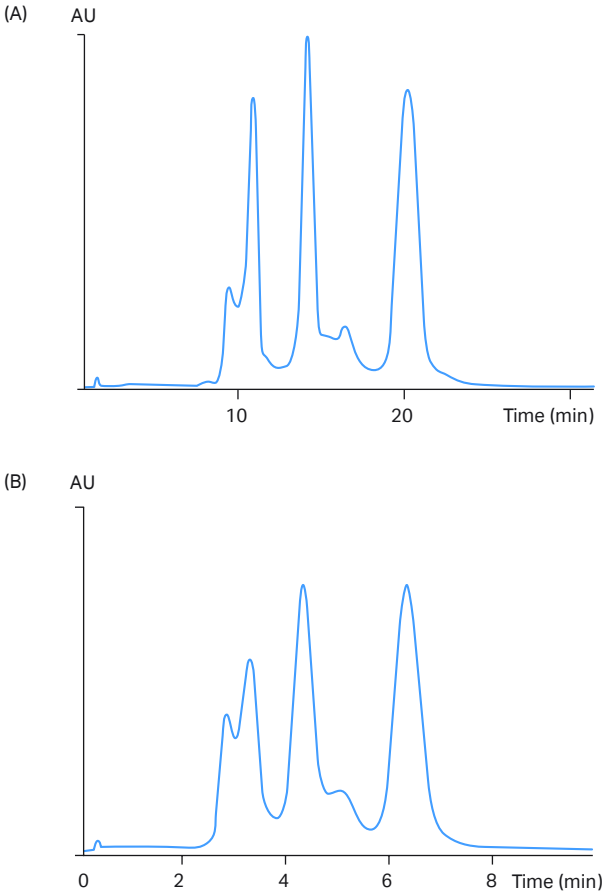


Fig 4. The influence of increasing flow rate on resolution.

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

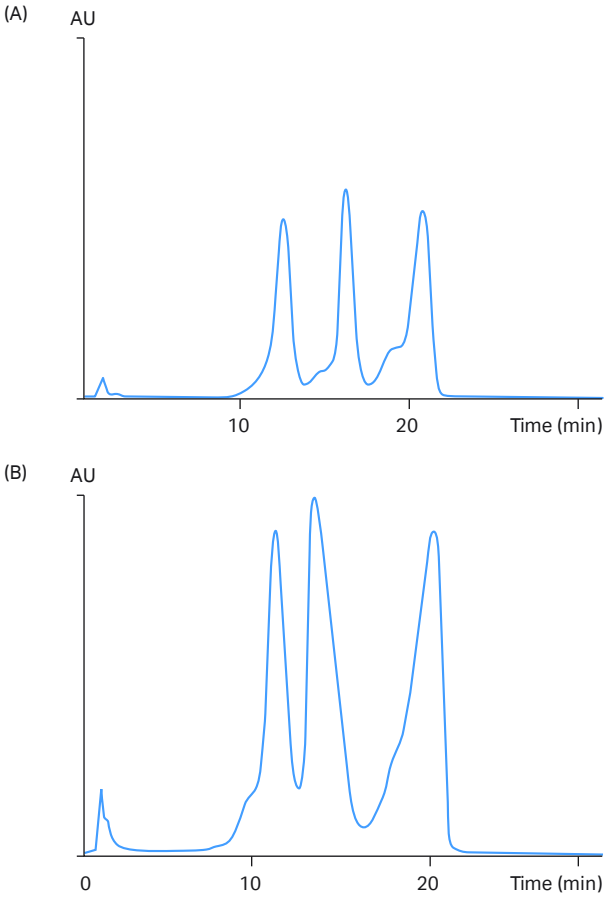


Fig 5. The influence of increasing sample load on resolution.

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

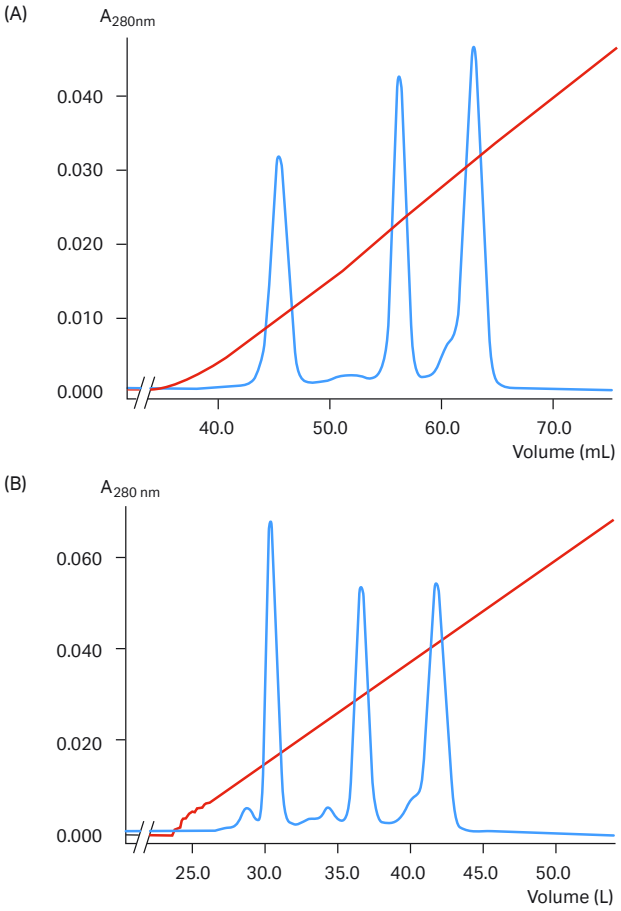


Fig 6. 700-fold scale up from a 2.2 mL lab-scale column to a 1.6 L production-scale FineLINE™ 200 column.

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 resin
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

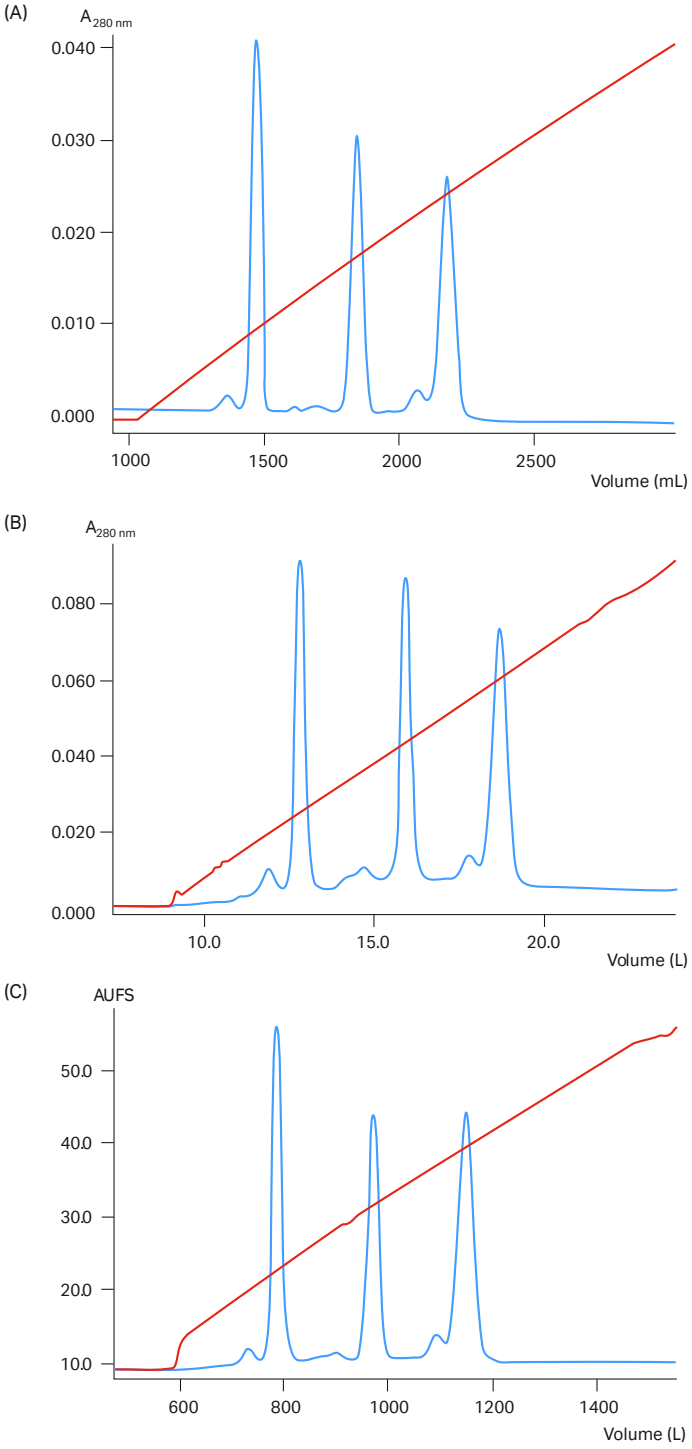


Fig 7. Scale-up from FineLINE Pilot 35 column via FineLINE 100 column (7×) to FineLINE 800 custom designed column (64×). Total scale up factor was 476.

Batch-to-batch reproducibility

The combination of a specific 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.

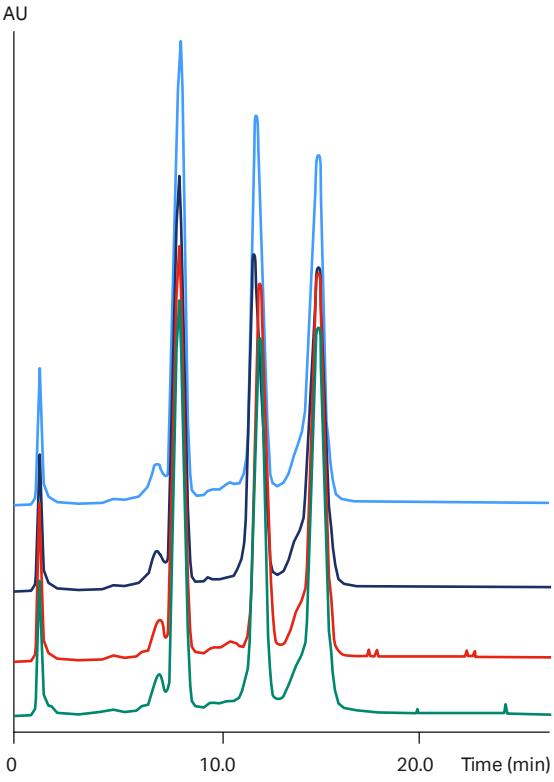


Fig 8. QC evaluation of 4 production batches of SOURCE 30S.

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 1.0 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, 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

Lab-scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
FineLINE Pilot 35	35	29-140	30-150
Tricorn™ 5/20	5	0.0-0.5	0-26
Tricorn 5/50	5	0.2-1.1	8-56
Tricorn 10/20	10	0.0-2.1	0-26
Tricorn 10/50	10	0.0-4.4	0-56
Tricorn 10/100	10	3.6-8.4	46-106
Tricorn 10/150	10	7.6-12.3	96-156
Tricorn 10/200	10	11.5-16.2	146-206
Tricorn 10/300	10	19.4-24.1	246-306

Production Scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
FineLINE 70	70	580	3-15
FineLINE 70L	70	1200	5-30
FineLINE 100P	100	1200	3-15
FineLINE 100PL	100	240	5-30
FineLINE 200P	200	470	3-15
FineLINE 200PL	200	940	5-30
FineLINE 350P, PFR, 2 µm	350	14 400	3-15
FineLINE 350PL, EPDM, 10 µm	350	28 800	5-30

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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 (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

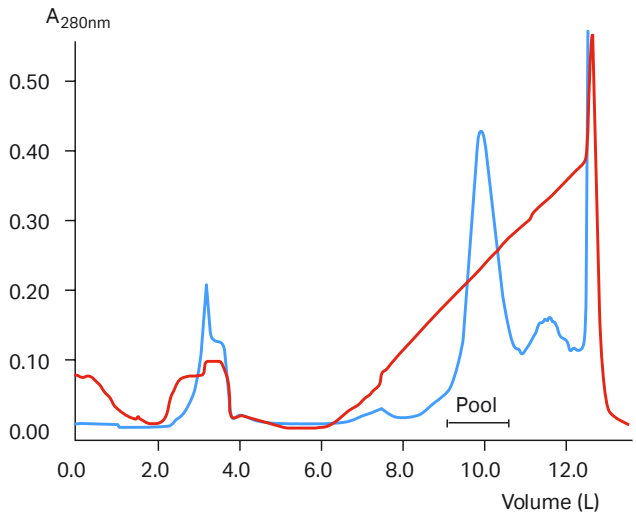

Fig 9. Intermediate purification of recombinant *P. aeruginosa* exotoxin A on SOURCE 30Q.

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.

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

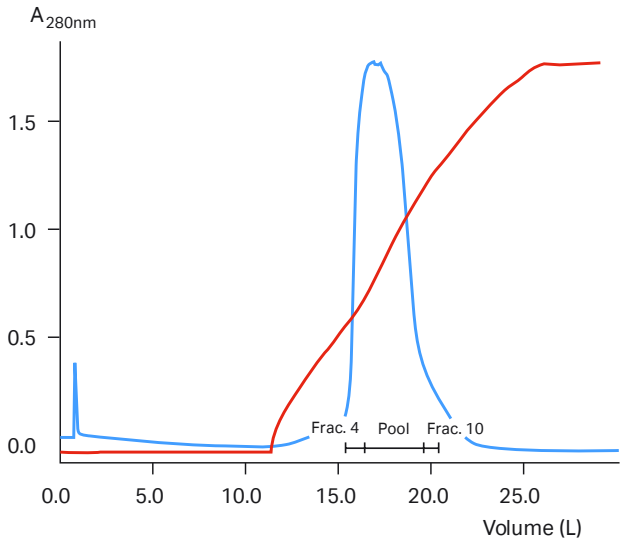

Fig 11. Preparative purification of 25-mer phosphorothioate oligonucleotide on SOURCE 30Q.

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

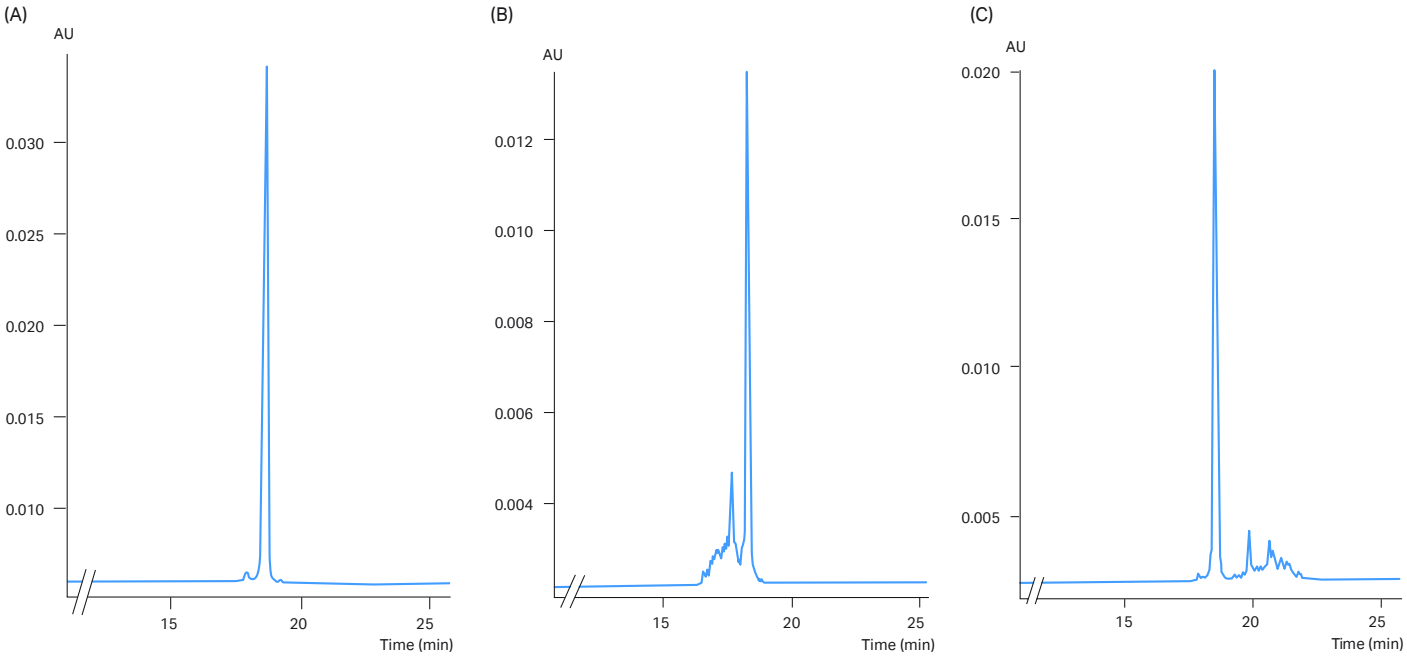

Fig 12. Capillary electrophoresis of the pool and side fractions from the preparative purification of a 25-mer phosphorothioate oligonucleotide.

Ordering information

Product	Pack size	Code number
SOURCE 30Q	50 mL	17127501
	200 mL	17127502
	1 L	17127503
	5 L	17127504
SOURCE 30S	50 mL	17127301
	200 mL	17127302
	1 L	17127303
	5 L	17127304

Lab-scale columns

Column	Product Code
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

Production Scale columns

Column	Product Code
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

Related literature

Handbook

Ion Exchange Chromatography, Principles and Methods	11000421

Selection guides

Ion exchange columns and media	18112731
Prepacked chromatography columns for ÄKTA systems	28931778

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