High Performance Reversed Phase Chromatography

SOURCE™ 15RPC
SOURCE 15RPC ST 4.6/100
RESOURCE™ RPC

SOURCE 15RPC is designed for reversed phase chromatography (RPC) of peptides, proteins and oligonucleotides. It is part of the SOURCE resin range developed for rapid, high resolution preparative separations. The range is characterized by a monodisperse, polymeric matrix with a controlled and reproducible pore size distribution.

SOURCE 15RPC ST 4.6/100, and RESOURCE RPC 1 mL and 3 mL columns are prepacked with SOURCE 15RPC resin. SOURCE 15RPC ST 4.6/100 is intended for lab-scale separations and for optimization studies when scaling up. RESOURCE RPC columns are designed for fast, lab-scale separations and selectivity screening experiments.

SOURCE 15RPC resin is characterized by:

- High resolution separations in minutes
- High performance at low back pressures
- High capacity at high flow rates
- Wide pH stability range
- Excellent scalability
- Reproducible quality

Fig 1. SOURCE 15RPC, SOURCE 15RPC ST, and RESOURCE RPC columns are members of the SOURCE/RESOURCE family.

SOURCE 15RPC

SOURCE 15RPC is based on rigid, monodisperse, ~ 15 µm diameter polystyrene/divinyl benzene beads (Table 1). The matrix has unique selectivity for RPC. With its controlled pore size distribution, batch reproducibility and scalability, SOURCE 15RPC offers outstanding properties superior to those of other polymeric matrices.

SOURCE 15RPC is ideally suited for difficult preparative separations at all scales, from the laboratory to the final stages of an industrial purification process.

Table 1. Characteristics of SOURCE 15RPC

Matrix	Spherical and monodisperse, porous, rigid, polystyrene/divinyl benzene particles
Mean particle diameter[1]	~15 µm
Dynamic binding capacity, Q_{B10}[2]	~18 mg BSA/mL resin ~14 mg Bacitracin/mL resin ~45 mg insulin/mL resin
pH stability, operational[3]	2 to 12
pH stability, CIP[4]	1 to 14
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M HCl 1.0 M HCl/90% Methanol 90% Acetic acid 6 M Guanidine hydrochloride 100% n-propanol 100% Ethanol 100% Methanol 100% Acetone 0.45 M NaOH/40% isopropanol 1.0 M NaOH[5] 0.1% TFA in water 0.1% TFA in acetonitrile 100% isopropanol 100% Tetrahydrofuran
Pressure/flow characteristics	400 cm/h at < 1 MPa in a FineLine 100 column with 10 cm diameter and 10 cm bed height (at room temperature using buffers with the same viscosity as water)[6]
Operating temperature	4˚C to 40˚C
Delivery conditions	20% ethanol
Storage	20% ethanol, 4˚C to 30˚C
Autoclavability	20 min at 121°C in H_2O pH 7, 1 cycle

1 Monodisperse size distribution.
2 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a HR 10/10 column at 10 cm bed height (2 min residence time) for BSA/bacitracin/insulin in 0.1 % TFA in water.
3 pH range where resin can be operated without significant change in function.
4 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function should only be used for cleaning purposes.
5 1.0 M NaOH should only be used for cleaning purposes
6 The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

High resolution and high capacity

SOURCE monodisperse particles yield high resolution at high flow rates (Fig. 3). Pore size distribution is balanced to give high capacities for peptides, proteins and oligonucleotides (Fig. 4). Furthermore, mass recoveries, as illustrated in the applications in this data file, are typically over 85%.

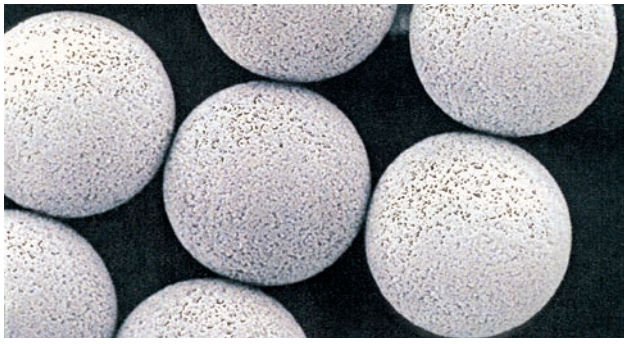


Fig. 2. Electron microscope photograph of SOURCE 15RPC. Note the uniform size distribution and the absence of broken beads and bead fragments.

Column: RESOURCE RPC 1 mL
Sample: 100 µl of
Angiotensin III, 1 mg/mL
Insulin, 1 mg/mL
Lysozyme, 1 mg/mL
Albumin, 1 mg/mL
Solvent A: 0.1% TFA in water
Solvent B: 60% acetonitrile + 0.1% TFA in water
Flow rates: 0.3, 1, 2, 5 and 10 mL/min (60, 180, 360, 900 and 1800 cm/h)
Gradient: 10–90% B, 20 mL
System: FPLC
Detection: 214 nm

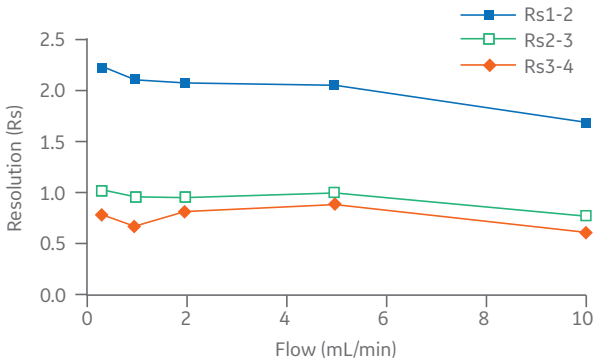


Fig. 3. Resolution versus flow. The resolution is maintained at high flow rates.

Low back pressures

The uniform bead size and spherical shape gives stable packed beds and low back pressures, in contrast to beads with a wide range of particle sizes (see also under 'Operation', Fig. 10).

Column: RESOURCE RPC 1 mL
Sample: Bovine insulin (MW 5 700, Sigma) 5 mg/mL in solvent A
Solvent A: 0.1% TFA in water
Flow rates: 1, 2, 5 and 10 mL/min (180, 360, 900 and 1800 cm/h)
System: FPLC
Detection: 280 nm, AUFS 2.0

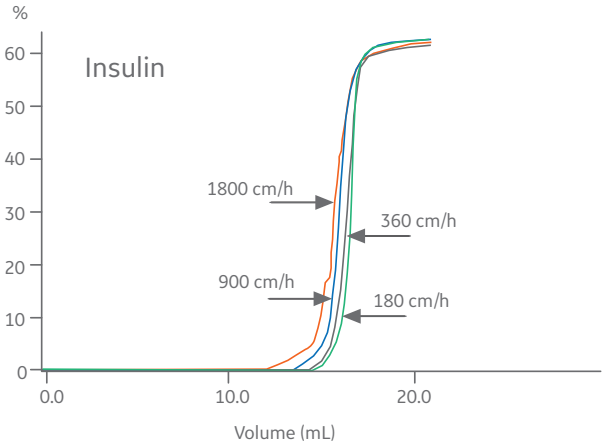


Fig. 4. High binding capacity at high flow rates – breakthrough curve for bovine insulin (MW 5 700, Sigma).

Wide pH stability

The polystyrene/divinyl benzene matrix provides SOURCE 15RPC with chemical stability over a wide pH. With an operating range between pH 2–12 and a cleaning range between pH 1–14, SOURCE 15RPC has unmatched flexibility for running conditions and cleaning procedures.

Reproducible quality

SOURCE 15RPC is manufactured by a patented process that gives a high degree of quality assurance. The procedure results in consistent pore structure, both within and between batches, an important factor for routine applications and industrial production where there are strict regulatory demands (Fig 5).

Column: RESOURCE RPC 1 mL
Sample: 25 µl of
(Ile^7)angiotensin III, 0.5 mg/mL
(Val^4)angiotensin III, 0.5 mg/mL
Angiotensin III, 0.5 mg/mL
Angiotensin I, 0.5 mg/mL
Solvent A: 0.1% TFA in water
Solvent B: 60% acetonitrile + 0.1% TFA in water
Flow rate: 1 mL/min (180 cm/h)
Gradient: 15–65% B in 20 min
System: FPLC
Detection: 214 nm

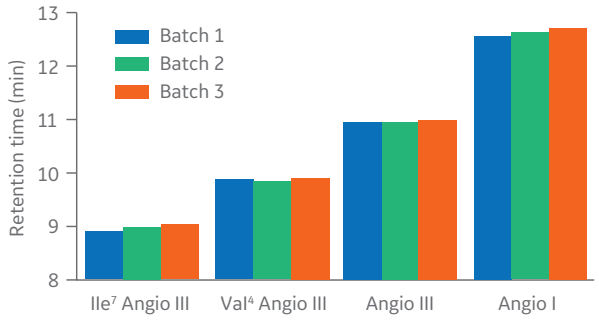


Fig. 5. Reproducibility of three batches of SOURCE 15RPC.

SOURCE 15RPC ST column

SOURCE 15RPC ST 4.6/100 is ideal for separations where high resolution is most important. This column is an excellent choice for preparative reversed phase purifications at laboratory scale because of the high capacity of the resin. It can also be used to advantage during optimization before scaling up.

The ST column is made of stainless steel. Table 2 lists the main chromatographic properties of SOURCE 15RPC ST 4.6/100.

Table 2. Main chromatographic properties of pre-packed columns with SOURCE 15RPC

	RESOURCE RPC		SOURCE 15RPC ST 4.6/100
	1 mL	3 mL	
Col. dimensions, i.d. × bed height (mm)	6.4 × 30	6.4 × 100	4.6 × 100
Bed volume (mL)	1	3	Approx. 1.7
Recommended operating flow rate (mL/min)	1–5	1–5	0.5–2.5
Maximum operating flow rate (mL/min)	10	10	5.0

RESOURCE RPC

RESOURCE RPC 1 mL and 3 mL columns give fast and convenient separations on ÄKTA™design, FPLC and HPLC systems. The columns are available with a choice of two volumes, 1 mL and 3 mL. RESOURCE RPC 1 mL is ideal for rapid screening experiments whereas RESOURCE RPC 3 mL is better suited to applications in which high resolution is critical. Both columns are made of PEEK (polyetheretherketone), which has a high pressure tolerance and high chemical resistance. Reproducibility of separations is high (Fig. 6). Table 2 lists the main chromatographic properties of RESOURCE RPC columns.

Column: RESOURCE RPC 1 mL
Sample: 20 µl of
(Ile^7)angiotensin III, 0.25 mg/mL
(Val^4)angiotensin III, 0.25 mg/mL
Angiotensin III, 0.25 mg/mL
Angiotensin I, 0.25 mg/mL
Solvent A: 0.1% TFA in water
Solvent B: 0.1% TFA in acetonitrile
Flow rate: 4 mL/min (720 cm/h)
Gradient: 10–35% B in 5 min.
Detection: 214 nm

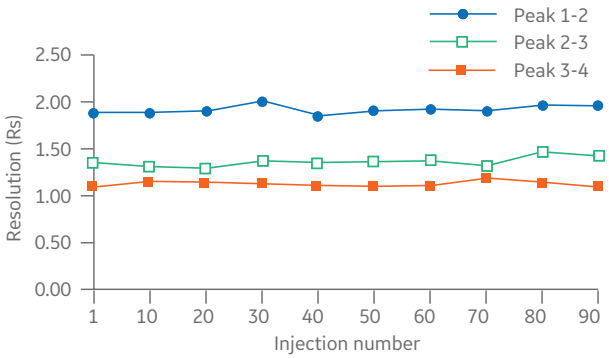


Fig. 6. Reproducibility on a RESOURCE RPC column.

Operation

SOURCE 15RPC and pre-packed SOURCE columns can be used with standard methods for RPC. RPC is not recommended for protein purifications if recovery of activity and return to a correct tertiary structure are required, since many proteins are denatured in the presence of organic solvents.

Separations at high pH

Solubility of peptides is often pH dependent and successful separations of some peptides require operation at a high pH. Compared to silica-based matrices, SOURCE 15RPC has high pH stability (operational range pH 2–12, cleaning range pH 1–14).

In the example illustrated in Figure 7A and B, Angiotensin II and Angiotensin III peptides were successfully separated on SOURCE 15RPC at pH 12 but not at pH 2.

Column: RESOURCE RPC 3 mL
Sample: 150 µl of
Angiotensin II, 0.25 mg/mL
Angiotensin III, 0.25 mg/mL
Solvent A: (A) 0.1% TFA (pH 2) or (B) 10 mM NaOH (pH 12) in water
Solvent B: 60% Acetonitrile in 0.1% TFA (pH 2) or 10 mM NaOH (pH 12)
Flow rate: 2 mL/min (360 cm/h)
Gradient: 10–65% B in 10 min

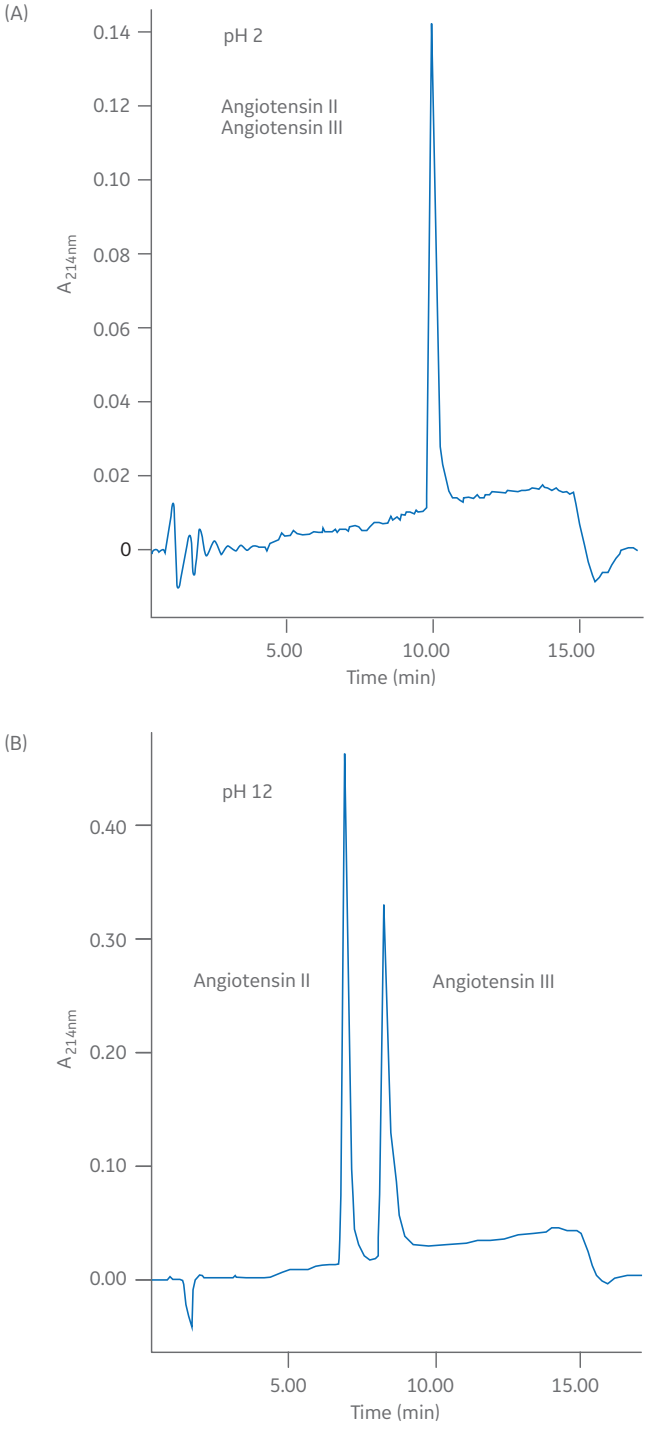


Fig. 7. Separation of Angiotensin II and Angiotensin III at (A) pH 2 and (B) pH 12. The peptides were not separated at low pH.

During the purification of Beta–lipotropin (Fig. 8), contaminants eluted together with Beta–lipotropin at pH 2, but were separated at pH 12. The earlier elution position of the peptide at pH 12 also meant that less organic solvent was required, which can be an important consideration for scale up.

Column: RESOURCE RPC 3 mL
Sample: Beta-lipotropin (fragment 1–10), 0.5 mg in 300 µl water
Solvent A: (A) 0.1% TFA (pH 2) or (B) 10 mM NaOH (pH 12) in water
Solvent B: 60% Acetonitrile in 0.1% TFA (pH 2) or 10 mM NaOH (pH 12)
Flow rate: 2 mL/min (360 cm/h)
Gradient: 0–30% B in 10 min

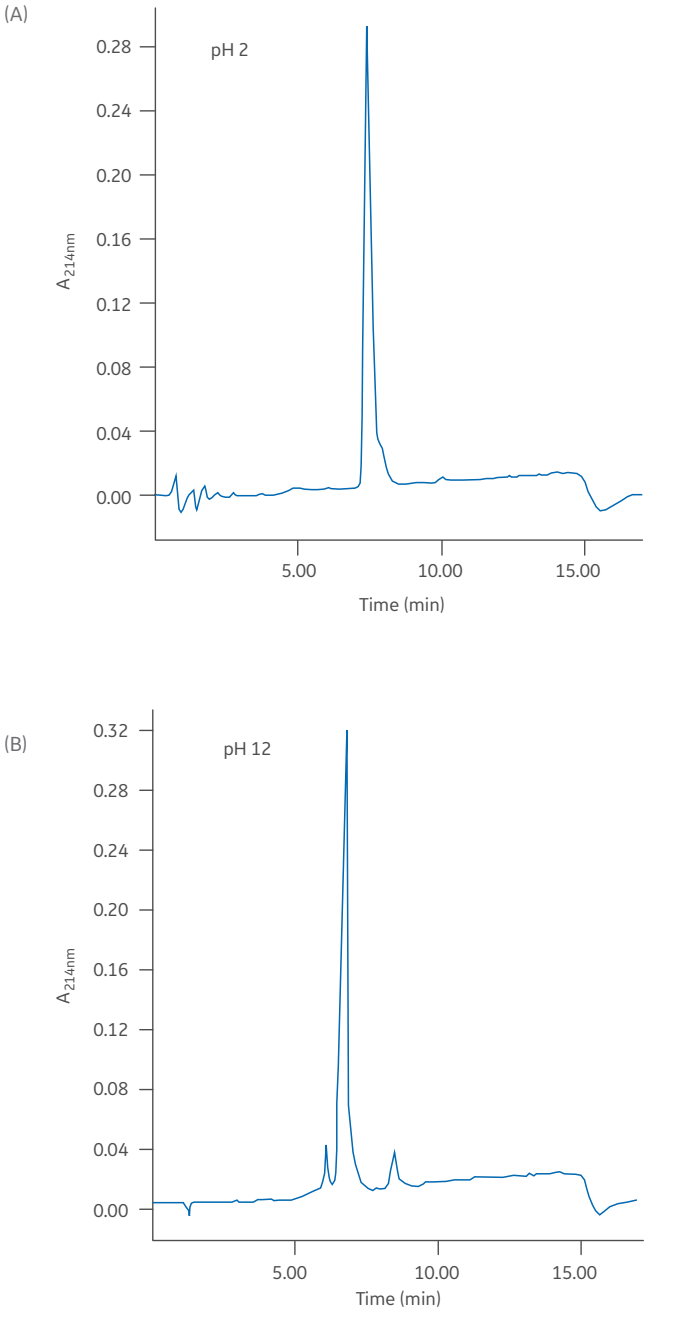


Fig. 8. Purification of Beta-lipotropin fragment 1–10 (MW 950, Sigma) at a) pH 2 and b) pH 12. At pH 2 the contaminants are eluted in the Beta-lipotropin peak, at pH 12 they are separated.

In a third example (Fig. 9), a novel growth factor which was found to be unstable at low pH could be purified at pH 8.3 with good recovery of biological activity.

In summary, the pH stability of SOURCE 15RPC gives flexibility for the control of selectivity and recovery, in addition to allowing aggressive cleaning conditions – especially important when working with biological extracts.

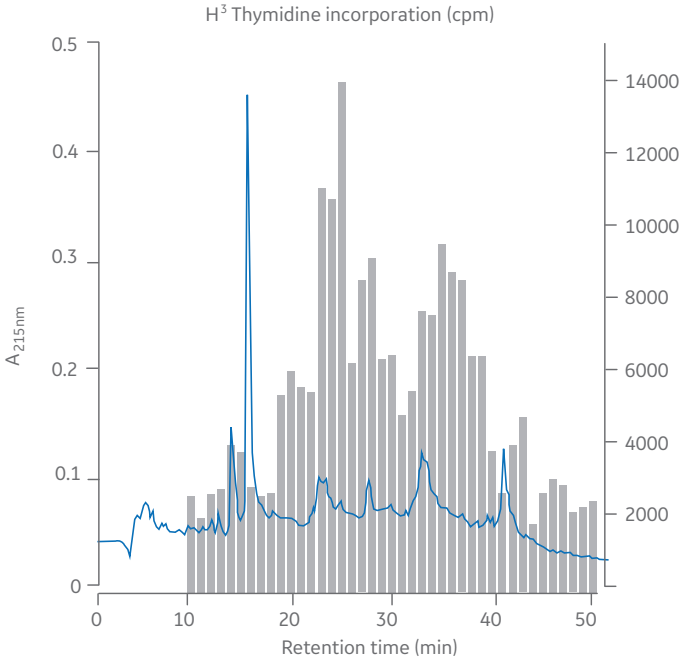


Fig. 9. Purification of a novel growth factor not stable at low pH. Work by Dr Ed Nice, Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Victoria, 3050, Australia.

High flow rate

SOURCE 15RPC resin has a pressure/flow characteristic of 400 cm/h at < 1 MPa in a column with 10 cm diameter and a 10 cm bed height (see Table1).

Chromatography systems

The pre-packed SOURCE columns can be used with ÄKTA chromatography systems, FPLC and HPLC systems which tolerate organic solvents and the required operating pressures. Figure 10a, b shows pressure/flow graphs for several solvents with RESOURCE RPC columns.

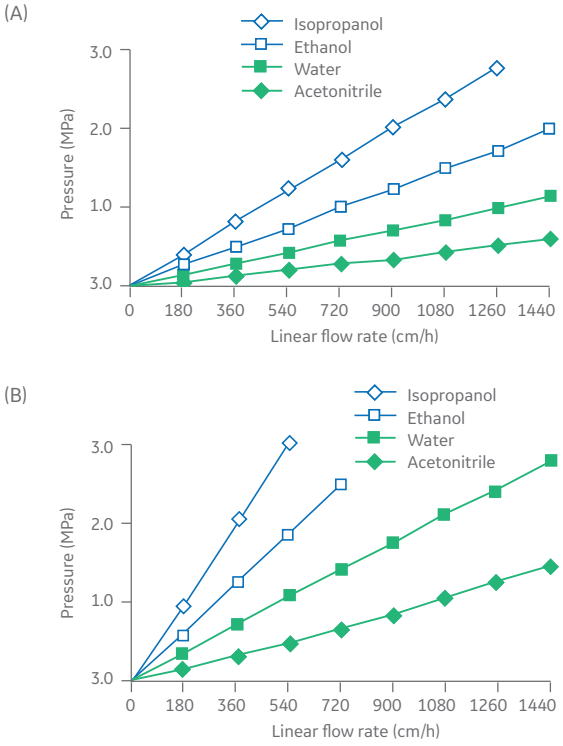


Fig. 10. Pressure flow curves of (A) RESOURCE 1 mL, and (B) RESOURCE 3 mL, in various organic solvents and water.

Scaling up

SOURCE 15RPC allows separations achieved with SOURCE 15RPC ST and RESOURCE RPC 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 (Fig. 11A and B).

Applications

SOURCE 15RPC and pre-packed SOURCE columns are for high resolution, preparative chromatography of peptides, proteins and oligonucleotides.

Figures 8 and 9 illustrate separations of synthetic peptides while Fig. 12 shows separation of a synthetic oligonucleotide. Figure 11 shows a high resolution preparative separation of recombinant human epidermal growth factor (EGF) expressed in yeast. Most impurities have been removed by other chromatographic techniques, in this case by an initial hydrophobic interaction chromatography step on Phenyl Sepharose 6 Fast Flow (high sub) followed by ion exchange on Q Sepharose high performance. Fig 11 shows the final step on SOURCE 15RPC at lab-scale and after scale-up to a pilot-scale column.

GE 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 minutes with excellent performance and reproducibility. See also Table 3 with a list of recommended columns for lab-scale applications.

Recommended columns

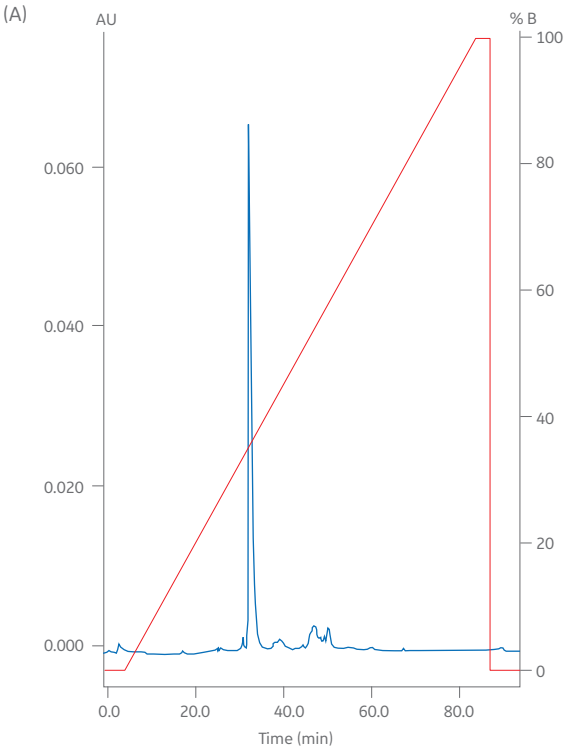
Table 3. Recommended lab–scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
FineLINE Pilot 35	35	29–140	30–150
TricornTM 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

Table 4. Recommended production–scale columns

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)
FineLINE 70	70	580	30–150
FineLINE 70L	70	1200	50–300
FineLINE 100P	100	1200	30–150
FineLINE 100PL	100	240	50–300
FineLINE 200P	200	470	30–150
FineLINE 200PL	200	940	50–300
FineLINE 350P, PFR, 2µm	350	14 400	30–150
FineLINE 350PL, EPDM, 10µm	350	28 800	50–300

Column: RESOURCE RPC 3 mL
Sample: 2.14 mL EGF pool after Q Sepharose High Performance
Eluent A: 0.05% TFA +5% acetonitrile in water
Eluent B: 0.05% TFA +80% acetonitrile in water
Flow rate: 1.6 mL/min (300 cm/h)
Gradient: 0–100% B in 40 column volumes
System: FPLC with FPLC director



Column: SOURCE 15RPC 35 × 100 mm
Sample: 62.5 mL EGF pool after Q Sepharose High Performance
Load: 0.1 mg/mL resin. 10 mg total load
Buffer A: 0.05% TFA +5% acetonitrile in water
Buffer B: 0.05% TFA +80% acetonitrile in water
Flow rates: 50 mL/min (300 cm/h)
Gradient: 0–100% B in 40 column volumes
System: BioPilot System with UNICORN™ control software

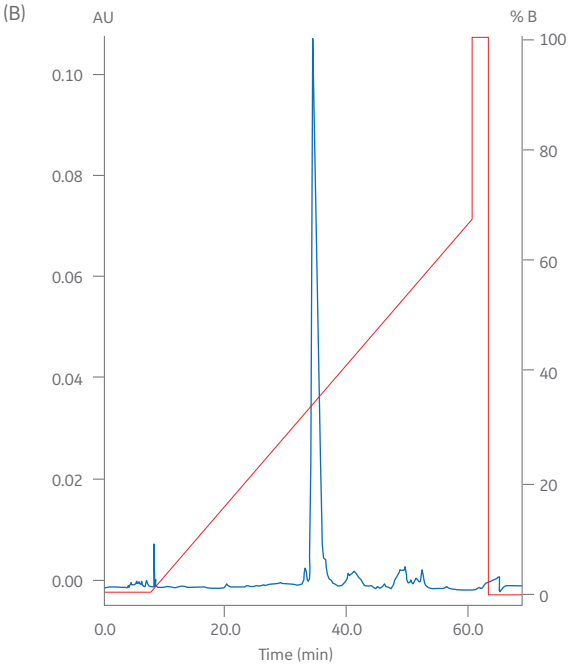


Fig. 11A and B. Final polishing of recombinant epidermal growth factor.

Column: RESOURCE RPC 1 mL
Sample: de-protected 25-mer oligonucleotide in crude reaction mixture
Sample load: 120 µl of 1 µM solution
Flow rate: 0.5 mL/min (90 cm/h)
Buffer A: 100 mM triethylaminoacetate (TEAA), pH 7.0, 5% acetonitrile
Buffer B: 100 mM triethylaminoacetate (TEAA), pH 7.0, 90% acetonitrile

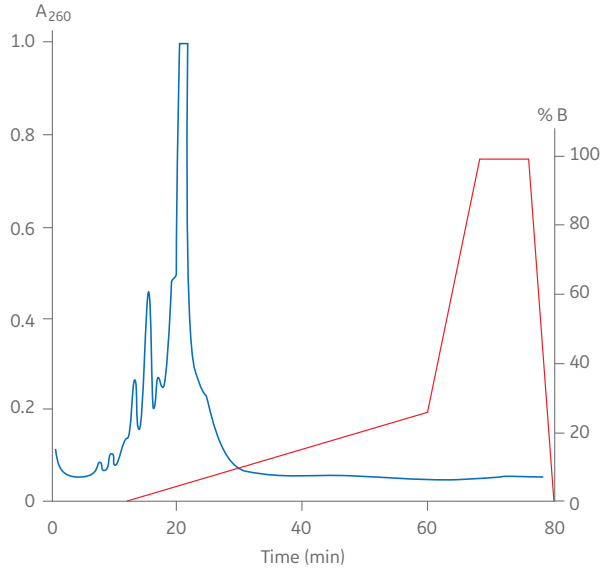


Fig. 12. Purification of a 25 mer DNA oligonucleotide on RESOURCE RPC 1 mL. Work by Dr Chris Fuller, Cambridge Centre for Molecular Recognition, Univ. of Cambridge, England.

Ordering Information

RESOURCE RPC columns

Column	Pack size	Product Code
SOURCE 15RPC ST 4.6/100		17506801
RESOURCE RPC 1 mL		17118101
RESOURCE RPC 3 mL		17118201
Ordering Information		

SOURCE 15RPC matrix

SOURCE 15 RPC	10 mL	17072720
SOURCE 15RPC	200 mL	17072702
SOURCE 15RPC	500 mL	17072703
SOURCE 15RPC	1 L	17072704
SOURCE 15RPC	5 L	17072705

Lab-scale columns

FineLINE Pilot 35	18110202
TricornTM 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

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

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GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

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