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

Data File

High Performance Reversed Phase Chromatography

SOURCE 15RPC is designed for reversed phase chromatography (RPC) of peptides, proteins and oligonucleotides. It is part of the SOURCE media range developed for rapid, high resolution preparative separations. The range is characterized by a monosized, polymeric matrix with a controlled and reproducible pore size distribution. SOURCE 15RPC is an interesting alternative to silica-based RPC matrices as it has a different selectivity, high capacity and wide pH stability range.

SOURCE 15RPC ST 4.6/100, and RESOURCE RPC 1 ml and 3 ml columns are prepacked with SOURCE 15RPC media. 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 media are 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

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.

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

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	Polystyrene/divinyl benzene
Bead form	Rigid, spherical, porous, monodisperse,
Particle size	15 µm
Pore volume	1.9 ml/g
Dynamic capacity*	≈ 10 mg BSA /ml medium at 300 cm/h ≈ 30 mg bacitracin/ml medium at 300 cm/h ≈ 50 mg insulin/ml medium at 300 cm/h
pH stability	
working range	1–12
cleaning range (CIP/SIP)	1–14
Max. linear flow rate	1800 cm/h
Typical flow rate range	200–900 cm/h
Operating temp.	4–40 °C
Autoclavable	20 min at 121°C
Delivery conditions	20% ethanol

* To measure the dynamic capacity, 5 mg bacitracin (MW 1 400), 2 mg BSA (MW 67 000) or 5 mg insulin (MW 5 700) per ml 0.1% TFA in water was applied at 300 cm/h. RESOURCE RPC 1 ml and FPLC System were used.

18-1123-50

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

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%.

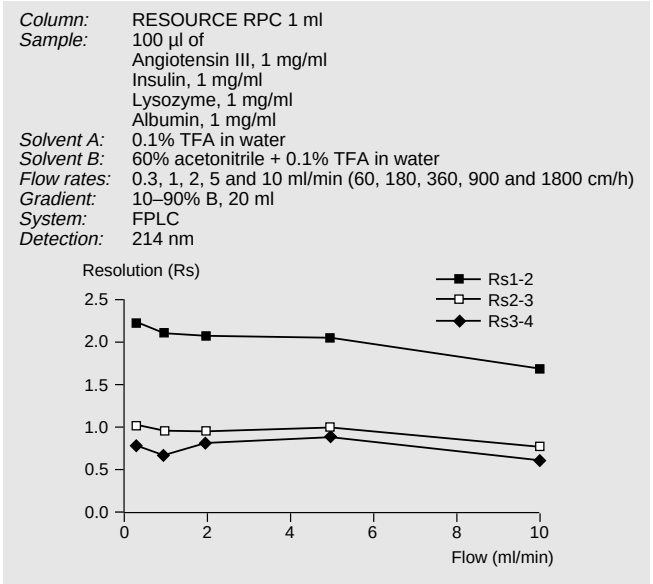


Fig. 3. Resolution versus flow. The resolution is maintained at high flow rates.

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).

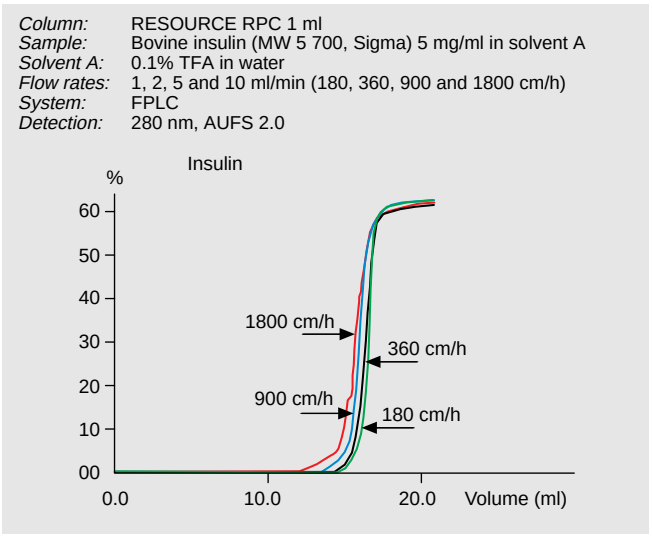


Fig. 4. High binding capacity at high flow rates – breakthrough curve for bovine insulin (MW 5 700, Sigma).

Wide pH stability

The polystyrene/divinyl benzene matrix provides SOURCE 15RPC with chemical stability over a wide pH. With an operating range between pH 1–12 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).

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 medium. 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.

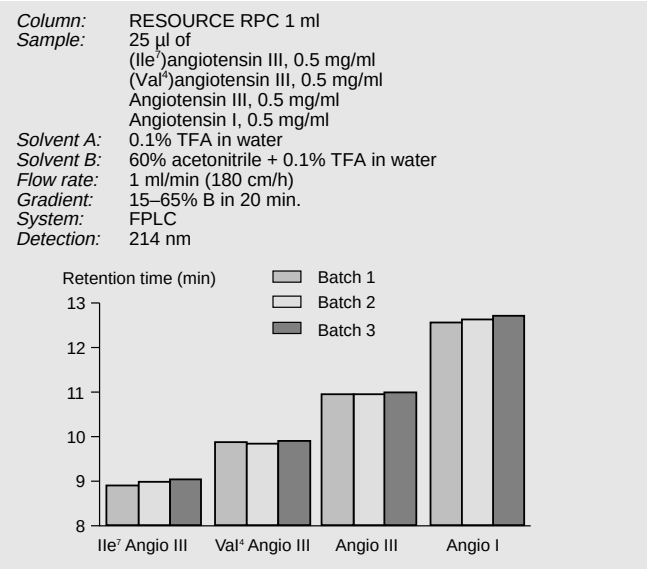


Fig. 5. Reproducibility of three batches of SOURCE 15RPC.

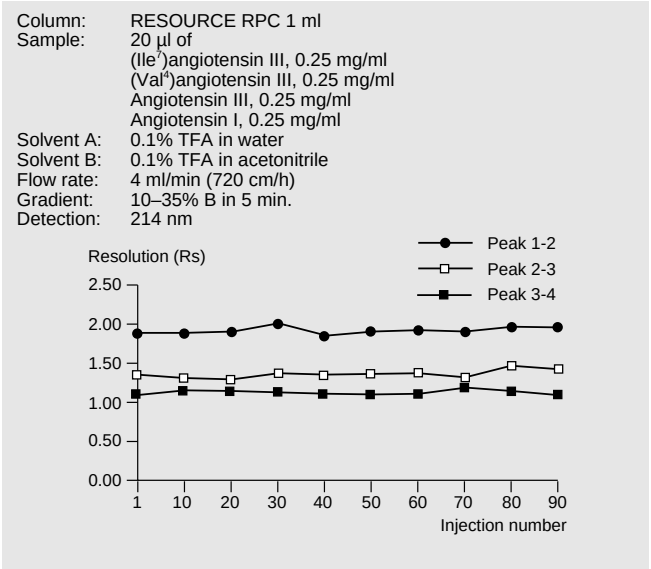


Fig. 6. Reproducibility on a RESOURCE RPC column.

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.

Operation

SOURCE 15RPC and pre-packed SOURCE columns can be used with standard methods for RPC. However, if using a method developed for silica-based RPC media, it must be optimized for SOURCE 15RPC.

Chemical stability

SOURCE 15RPC and pre-packed SOURCE columns are resistant to the solvents commonly used in RPC. These are typically 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) when the sample is eluted in an acetonitrile gradient. SOURCE 15RPC is resistant to other organic solvents such as isopropanol, methanol, ethanol, acetic acid, tetrahydrofuran. It is also resistant to 6 M guanidine-hydrochloride, 0.1% SDS and pH from 1 to 14.

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 (working range pH 1–12, cleaning range pH 1–14).

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

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.

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

	RESOURCE RPC		
	1 ml	**3 ml**	**SOURCE 15RPC ST 4.6/100**
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 flow rate (ml/min)	1–5	1–5	0.5–2.5
Max. recommended flow rate (ml/min)	10	10	5.0

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.

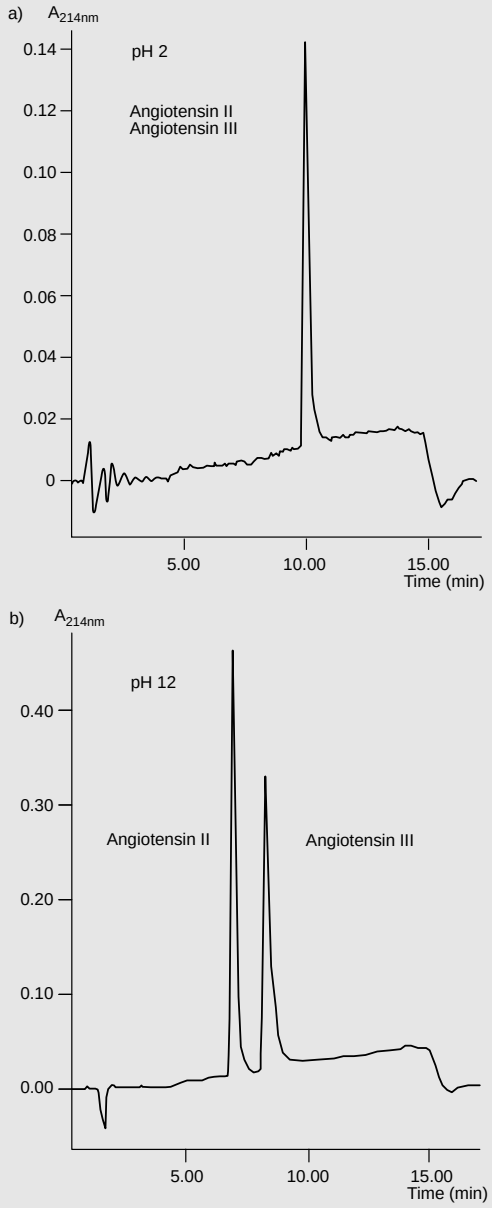


Fig. 7. Separation of Angiotensin II and Angiotensin III at a) pH 2 and b) pH 12. The peptides were not separated at low pH.

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.

High flow rate

The maximum linear flow rate that can be used is 1800 cm/h. Flow rates during a separation, however, are more typically 200–900 cm/h. Separation times at these flow rates range from a couple of minutes to 30 minutes.

Chromatography systems

The pre-packed SOURCE columns can be used with ÄKTAdesign, 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.

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, 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.

Amersham Biosciences offers a range of high quality RPC media, which includes silica based media with different selectivities. For more information about Sephasil® Protein C4, Sephasil Peptide C8, Sephasil Peptide C18, µRPC C2/C18, PepRPC® and ProRPC® pre-packed columns, please ask for Data File 18-1119-45. Sephasil Protein C4, Sephasil Peptide C8, and Sephasil Peptide C18 are also available in bulk quantities on request, please contact Amersham Biosciences.

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

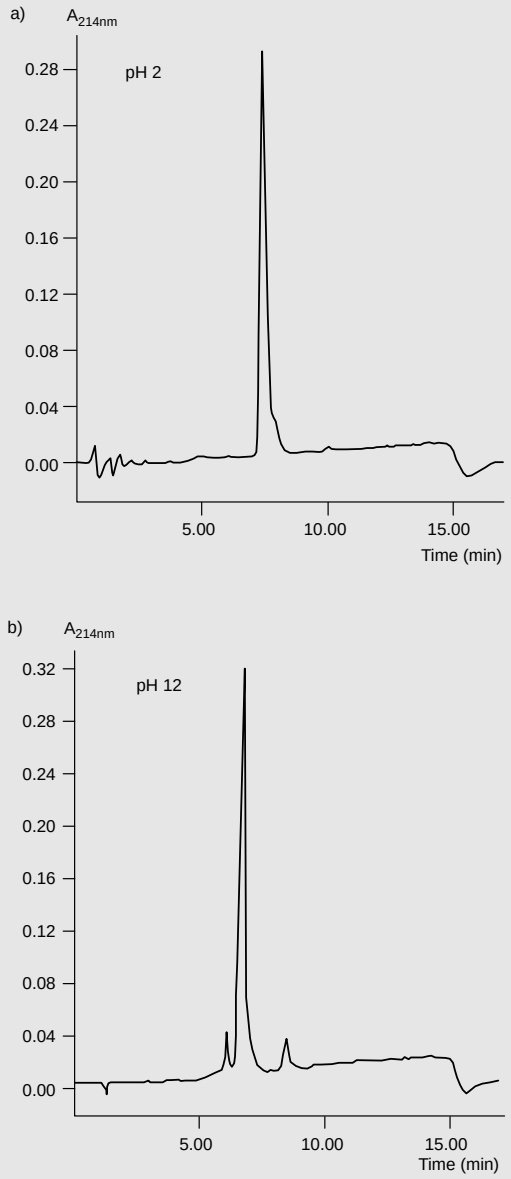


Fig. 8. Purification of Beta-lipotropin fragment 1–10 (MW 950, Sigma) at a) pH 2 and b) pH 12. At pH 2 the contaminants are eluted in the Beta-lipotropin peak, at pH 12 they are separated.

Amersham Biosciences 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 minutes with excellent performance and reproducibility.

Column: RESOURCE RPC 1 ml
Sample: Tissue extract purified on Mono Q, 1.4 ml pool of active fractions
Solvent A: 0.1% NH_4HCO_3, pH 8.3
Solvent B: 0.1% NH_4HCO_3 +60% Acetonitrile in water
Flow rate: 1 ml/min (180 cm/h)
Gradient: 0–100% B in 60 min

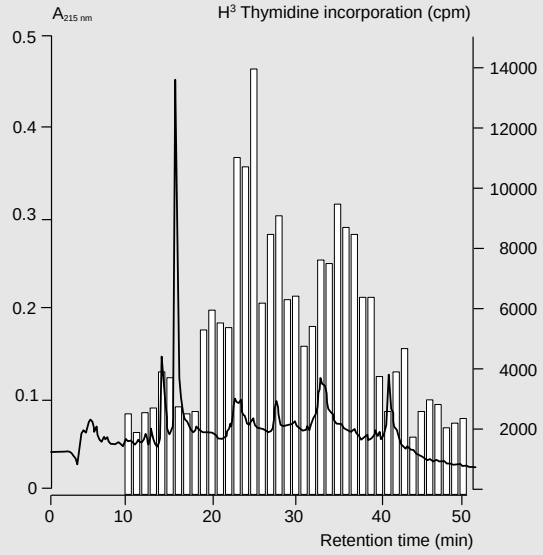


Fig. 9. Purification of a novel growth factor not stable at low pH. Work by Dr Ed Nice, Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Victoria, 3050, Australia.

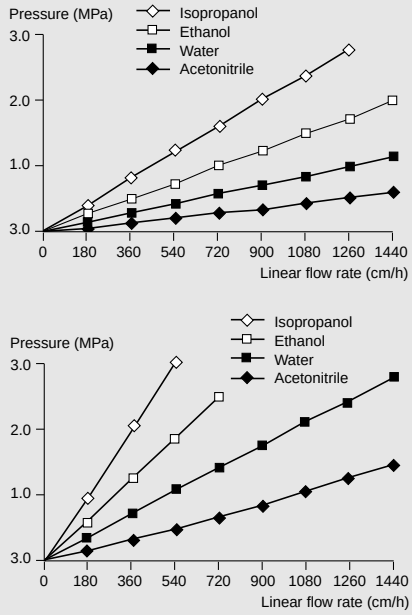


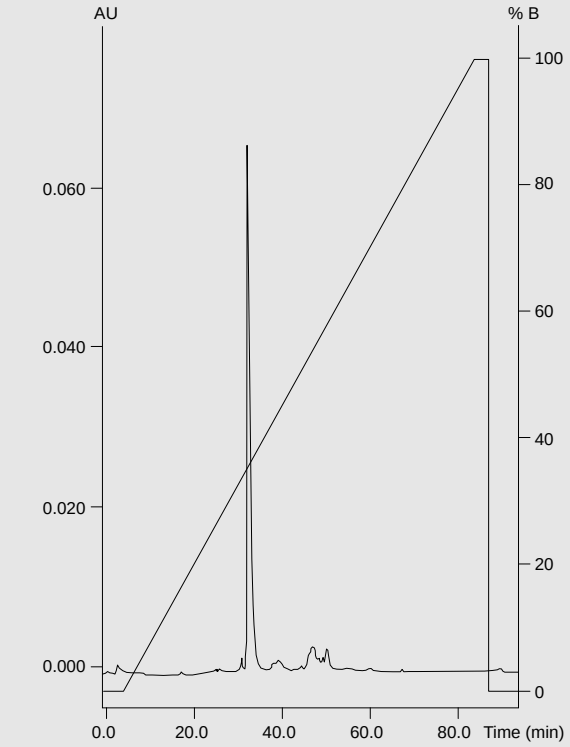
Fig. 10. Pressure flow curves of a) RESOURCE 1 ml, and b) RESOURCE 3 ml, in various organic solvents and water.

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	35	3–15	50– 145
FineLINE 100	100	3–15	235–1 180
FineLINE 100L	100	5–30	390–2 350
FineLINE 200	200	3–15	940–4 710
FineLINE 200L	200	5–30	1 570–9 420

a)

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™



b)

Column:	SOURCE 15RPC 35 × 100 mm
Sample:	62.5 ml EGF pool after Q Sepharose High Performance
Load:	0.1 mg/ml media. 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

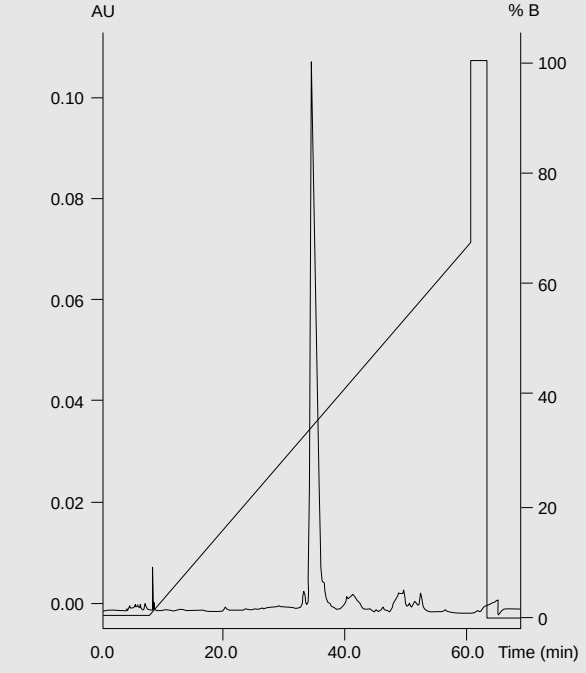


Fig. 11a,b. Final polishing of recombinant epidermal growth factor.

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

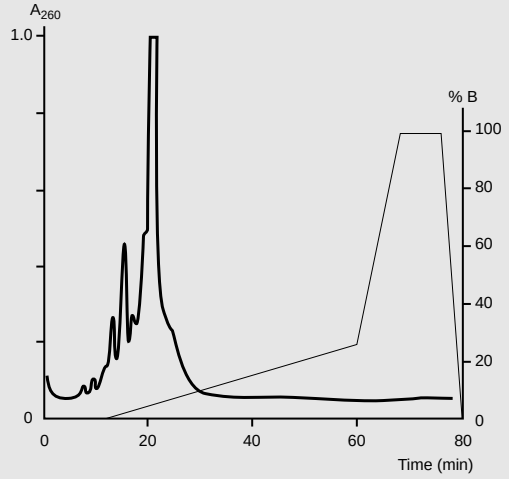


Fig. 12. Purification of a 25 mer DNA oligonucleotide on RESOURCE RPC 1 ml. Work by Dr Chris Fuller, Cambridge Centre for Molecular Recognition, Univ. of Cambridge, England.

Ordering Information

RESOURCE RPC columns	Code No.
SOURCE 15RPC ST 4.6/100	17-5068-01
RESOURCE RPC 1 ml	17-1181-01
RESOURCE RPC 3 ml	17-1182-01

SOURCE 15RPC matrix	Pack size	Code No.
SOURCE 15 RPC	10 ml	17-0727-20
SOURCE 15RPC	200 ml	17-0727-02
SOURCE 15RPC	500 ml	17-0727-03
SOURCE 15RPC	1 litre	17-0727-04
SOURCE 15RPC	5 litre	17-0727-05

Column	Code No.
FineLINE Pilot 35	18-1102-02
Pressure relief valve to FineLINE Pilot 35	18-1110-90
FineLINE 100	18-1105-35
FineLINE 100L	18-1119-56
Stand 100	18-1031-10
FineLINE 200	18-1105-77
FineLINE 200L	18-1119-57
Stand 200	18-1031-20
Pressure relief valve to FineLINE 100 and 200	18-1105-36

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