SOURCE™ 15RPC

SOURCE 15RPC is a polymer-based, BioProcess™ resin for high-performance reversed phase chromatography (RPC).

These instructions contain information about resin characteristics, operation (including column packing), method optimization, scale-up, maintenance, and equipment.

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Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Introduction

BioProcess chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Characteristics

SOURCE 15RPC is based on spherical and monodisperse, porous, rigid, polystyrene/divinyl benzene, ~15µm particles. The resin is without functional groups, instead the inherent hydrophobicity of the particles is utilized, which results in a unique selectivity for RPC. These characteristics, together with the controlled pore size distribution, give reproducible and scalable results and make SOURCE 15RPC even suitable for difficult separations such as those encountered in the final stages of an industrial purification process.

The following table summarizes the characteristics of SOURCE 15RPC. Figure 1 shows typical pressure flow-rate characteristics.

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] pH stability, CIP [4]	2 to 12 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	4°C to 30°C, 20% ethanol
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 for operation without significant change in function.

4 pH range for cleaning or sanitization without significant change in function.

5 1.0 M NaOH should only be used for cleaning purposes.

6 The pressure/flow characteristics describes the relationship between pressure and the flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

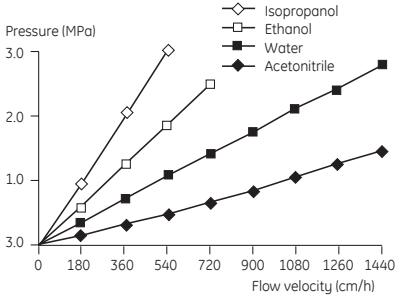


Fig 1. Pressure flow-rate characteristics of SOURCE 15RPC in a 6.4 × 100 mm prepacked column (RESOURCE™ RPC, 3 mL).

3 Stability

SOURCE 15RPC can be used with aqueous and organic solvents commonly used in reversed phase chromatography. The chemical stability is excellent, with an operational pH range of 2–12 and a pH cleaning range of 1–14.

Swelling/shrinking in water/organic solvents is less than 3%.

RPC is not recommended for protein purification when recovery of activity and return to a correct tertiary structure are required, since many proteins are denatured in the presence of organic solvents.

4 Packing columns

SOURCE 15RPC is supplied in 20% ethanol. Decant the ethanol solution and replace with packing solvent before use.

Recommended columns

SOURCE 15RPC can be used with columns and equipment commonly used for preparative RPC.

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 100	100	1200	3-15
FineLINE 10PL	100	240	5-30
FineLINE 200	200	470	3-15
FineLINE 200PL	200	940	5-30
FineLINE 350P, PFR, 2µm	350	14 400	3-15
FineLINE 350PL, EPDM, 10µm	350	28 800	5-30

Packing recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully.

Recommended packing solvent is either 25% to 100% ethanol or 100% methanol.

The best slurry concentration is between 2% and 10% resin but up to 35% is acceptable. Higher slurry concentrations often lead to more assymetric peaks.

Apply a flow rate that gives a back pressure between 25 to 30 bar. Do not exceed the maximum operating pressure of the column and other equipment in use.

Evaluation of packing

Column performance can be evaluated using conventional efficiency and asymmetry factor testing.

Suggested protocol:

Eluent:	100% methanol
Sample:	3% acetone
Sample volume:	1% of bed volume
Flow velocity:	2 cm/min
UV detection:	280 nm

Typical efficiency values are greater than 15 000 plates/m when measuring the peak width at 50% of the peak height. Typical values for the asymmetry factor are 0.8 to 1.8 when measuring half-peak widths at 10% of the peak height.

Note:

Reduce dead volume in the system should be reduced as much as possible to achieve the required efficiency.

5 Maintenance

All samples should be filtered and free of particulate matter. Use a filter with a porosity less than 2 µm.

Fouling can be prevented and the resin lifetime lengthened if regeneration procedures are developed for each application. The high chemical stability of SOURCE 15RPC allows harsh cleaning and sanitization procedures to be used. The following cleaning agents, alone or in combination, are generally efficient for regeneration.

- Up to 90% acetonitrile or iso-propanol
- 0.5 to 1.0 M NaOH
- 90% acetic acid
- 1.0 M HCl or 3% TFA

6 Storage

Store SOURCE 15RPC at 4°C to 40°C in 20% ethanol to prevent microbial growth.

7 Method design and optimization

The main purpose of optimizing a preparative chromatographic step is to reach the predefined purity level with the highest possible recovery and productivity by choosing the most suitable combination of the critical chromatographic parameters.

To save both time and material, it is recommended that the procedure is optimized at laboratory-scale.

8 Selectivity, loading capacity and recovery

The following parameters are important for selectivity, loading capacity and recovery:

- Type and concentration of organic solvent

  Acetonitrile is usually considered to give the best resolution. It also has low viscosity and good UV transparency. However, because of the toxicity of acetonitrile, low alcohols are often preferred for process-scale applications. They are cheaper but more viscous, giving much higher back pressures than with acetonitrile (see pressure/flow data in Fig. 1).

  Shallow solvent gradients are often necessary to achieve the required resolution; a 5% increase/hour is not unusual. Begin gradients with at least 5% of organic solvent and do not exceed 95%. Use of 0% and 100% organic solvent will result in very long equilibration times.

  The risk of precipitating sample substances and salts at high solvent concentrations should also be considered.

- Type and concentration of ion-pairing agents and buffer components

  Trifluoroacetic acid (TFA) is widely used as ion-paring agent. It gives high resolution of peptide and protein separations and it is volatile and relatively easy to remove. Useful alternatives that can offer different selectivities are triethylammonium phosphate or acetate (TEAP, TEAA).

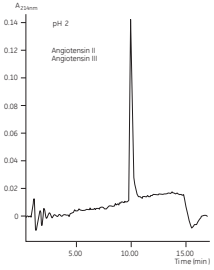
  Other buffer components used are acids, for example phosphoric or acetic acid and neutral salts like ammonium sulphate.

- pH

  The pH stability of SOURCE 15RPC makes possible the use of a wide pH range to improve selectivity. For example, where the separation of two Angiotensin peptides is improved significantly by going from pH 2 to pH 12 (Fig 2).

Column: RESOURCE RPC 3 mL
Sample: Mixture of Angiotensin II and Angiotensin III
Sample load: 25 µg in 150 µL
Eluent A: 0.1% TFA (a), 10 mM NaOH (b)
Eluent B: 0.1% TFA, 60% acetonitrile (a), 10 mM NaOH, 60% acetonitrile (b)
Flow rate: 2 mL/min
Gradient: 10% B in 2 min, 10 to 65% B in 10 min

a



b

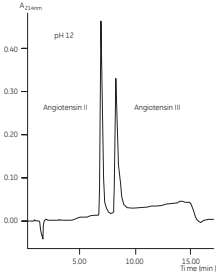


Fig 2. Separation of two Angiotensin peptides at pH 2 (a), and pH 12 (b).

9 Resolution vs productivity

Resolution can be further increased by decreasing the sample load, gradient slope, flow rate and/or increasing the bed height. However, these changes have a negative effect on the throughput. Therefore it is important to optimize sample load, gradient slope, flow rate and bed height to get the required resolution at the best possible productivity for process-scale methods.

10 Scaling up

After the RPC step has been optimized at laboratory-scale, the method can be scaled up. Scale-up is carried out by increasing the diameter of the column. Parameters that remain constant include bed height, linear flow, sample concentration and volume (in relation to bed volume), and the ratio gradient volume/bed volume. The column diameter and volumetric flow rate will increase.

The larger equipment needed when scaling up may cause some deviations from the method optimized at small scale. In such cases check the solvent delivery system and monitoring system and try and minimize the effects of liquid delays and volume changes in the flow path. The increased lengths and diameters of outlet pipes can also cause zone spreading with larger systems.

11 Ordering information

Product	Quantity	Product code
	10 mL	17071720
	200 mL	17072702
SOURCE 15RPC	500 mL	17072703
	1 L	17072704
	5 L	17072705

Recommended lab-scale columns.

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)	Product code
FineLINE Pilot 35	35	29-140	30-150	18110202
Tricorn 5/20	5	0.0-0.5	0-26	28406408
Tricorn 5/50	5	0.2-1.1	8-56	28406409
Tricorn 10/20	10	0.0-2.1	0-26	28406413
Tricorn 10/50	10	0.0-4.4	0-56	28406414
Tricorn 10/100	10	3.6-8.4	46-106	28406415
Tricorn 10/150	10	7.6-12.3	96-156	28406416
Tricorn 10/200	10	11.5-16.2	146-206	28406417
Tricorn 10/300	10	19.4-24.1	246-306	28406418

Recommended production-scale columns.

Column	i.d. (mm)	Approx. bed volume (mL)	Bed height (mm)	Product code
FineLINE 70	70	580	3-15	18115298
FineLINE 70L	70	1200	5-30	18115299
FineLINE 100	100	1200	3-15	11002798
FineLINE 10PL	100	240	5-30	11002799
FineLINE 200	200	470	3-15	11003114
FineLINE 200PL	200	940	5-30	11003115
FineLINE 350P, PFR, 2µm	350	14 400	3-15	11002792
FineLINE 350PL, EPDM, 10µm	350	28 800	5-30	11002785

Prepacked columns with SOURCE 15RPC include RESOURCE columns and a stainless steel SOURCE column.

Product	Quantity	Product code
RESOURCE RPC, 1 mL	1	17118101
RESOURCE RPC, 3 mL	1	17118201
SOURCE 15RPC ST 4.6/100, 1.7 mL	1	17506801

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