SOURCE™ 30RPC

SOURCE 30RPC is a polymer-based BioProcess™ resin for high performance reversed phase chromatography (RPC). 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.

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

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Read these instructions carefully before using the product.

Safety

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

1 Characteristics

SOURCE 30RPC is based on a unique 30 µm, monodisperse, porous, rigid polystyrene/divinyl benzene matrix. A controlled pore size distribution and a large specific surface area offer excellent resolution and capacity for a wide range of biomolecules, from small peptides and oligonucleotides up to large proteins. 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 summarizes the characteristics of SOURCE 30RPC. Fig 1 shows typical pressure flow characteristics for different solvents and bed heights.

Chemical stability

The pronounced chemical stability allows a pH operational range of 2 to 12 and a pH cleaning-in-place (CIP) range of 1 to 14.

SOURCE 30RPC can be used with aqueous and organic solvents commonly used in reversed phase chromatography, see Table 1. RPC is not recommended for protein purifications if recovery of activity and return to a correct tertiary structure are required. Many proteins are denatured in the presence of organic solvents.

Table 1. Characteristics of SOURCE 30RPC

Matrix	Spherical and monodisperse, porous, rigid polystyrene/divinyl benzene particles
Mean particle diameter[1]	~ 30 µm
Dynamic binding capacity, Q_{810}[2]	~ 14 mg BSA/mL resin ~ 23 mg bacitracin/mL resin ~ 72 mg insulin/mL resin
Chemical stability	Stable to commonly used aqueous buffers: 1 M HCl 1 M HCl/90% methanol 90% HAc 6 M GuHCl 100% n-propanol 100% ethanol 100% methanol 100% acetone 0.45 M NaOH/40% 2-propanol 1.0 M NaOH[3] 0.1% TFA in water 0.1% TFA in acetonitrile 100% isopropanol 100% tetrahydrofuran
pH stability, operational[4] **pH stability, CIP**[5]	2 to 12 1 to 14
Recommended operating flow velocity	100 to 1000 cm/h[6, 7]
Operating temperature	4°C to 40°C
Delivery conditions	20% ethanol
Storage	20% ethanol, 4°C to 30°C

[1] Monodisperse size distribution
[2] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in an HR 10/10 cm bed height (2 min residence time) for BSA/bacitracin/insulin in 0.1% TFA in water
[3] 1.0 NaOH must only be used for cleaning purposes.
[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] 3.5 cm diameter, 15 cm bed height, at room temperature using buffers with the same viscosity as water
[7] Depends on the pressure specification of the chromatographic system used, solvent, and bed height. A linear flow velocity of 1000 cm/h gives a pressure drop of approximately 10 bar at a bed height of 15 cm using water as eluent

2 Packing columns

Treat SOURCE carefully and especially avoid “digging” or stirring directly in the sedimented resin or packed beds, since this can generate fines. Resuspend SOURCE 30RPC stored in its container by gentle shaking.

SOURCE 30RPC must be packed in at least 25% ethanol but is delivered in 20%, therefore remember to add ethanol to increase ethanol concentration before starting packing. A packed bed in a column is best removed by dismantling the upper end-piece and pumping liquid in from the opposite end.

Recommended columns and systems

To fully exploit the resolving power of SOURCE 30RPC it is essential to use columns with a well designed flow distribution system at the column inlet and outlet such as in the Tricorn™ and FineLINE™ ranges. To minimize zone spreading, the flow distribution must be effective without resulting in large mixing volumes.

For scouting different separation conditions at small scale or pilot scale, the Tricorn series of columns and FineLINE Pilot 35 column connected to an ÄKTA™ design system are recommended.

For production scale we recommend the FineLINE series of columns. This column family, with an hydraulically controlled adapter, allows for fast and efficient packing which is completed in about 10 minutes. Performance and reproducibility are excellent.

Lab-scale columns

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

Production-scale columns

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

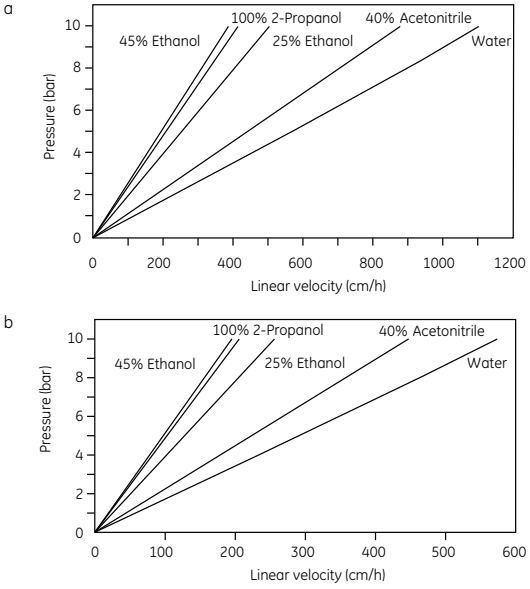


Fig 1. Pressure flow characteristics of SOURCE 30RPC in various organic solvents and water. The pressure flow velocity data were determined in a FineLINE Pilot 35 column with a) 15 cm and b) 30 cm bed height

Packing recommendations

Columns are packed according to the resin and equipment used. Always read and follow the relevant column instructions carefully.

Recommended packing solvents are 25% to 100% ethanol or 100% methanol. Methanol gives a lower viscosity which allows for higher flow velocities during packing.

The best slurry concentration is between 25% and 50%. Slurry concentrations higher than 50% often lead to more asymmetric peaks.

Packing Tricorn columns

Below is a general description of how to pack Tricorn columns, with specific conditions for the different columns.

Table 2.

Column	Slurry conc.	Pack flow rate	Back pressure at given flow rate	Packing equipment (product code)
Tricorn 10/100	25%	18 mL/min for 10 min	12 bar	Packing connector: 10-10 (18115323) Packing tube: Tricorn glass tube 10/300 (18115318)
Tricorn 10/300	25%	13 mL/min for 10 min	20 bar	Packing connector: 10-10 (18115323) Packing tube: Tricorn glass tube 10/600 (18115319)

1. Assemble the column and packing equipment.
2. Eliminate air from the column dead space by flushing the end piece and adapter with 25% ethanol. Close the column outlet leaving the bottom filter covered with 25% ethanol.
3. Resuspend SOURCE 30RPC resin stored in its container by shaking (avoid stirring of sedimented resin). Use a slurry concentration of 25% in 25% ethanol.

   Note: *Add ethanol to increase concentration from 20% to 25%.*

4 Pour the slurry into the packing equipment and column in one continuous motion. Pouring the slurry into the packing glass tube in a slow motion will minimize the introduction of air bubbles.

5 Attach the adapter or the end-piece to the packing tube and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

6 Open the bottom outlet of the column and let SOURCE 30RPC sediment by pumping in 25% ethanol through the column at a flow rate given in the table above.

7 When the bed has stabilized, close the bottom valve and stop the pump.

8 Disconnect the packing tube and connector and carefully place the top filter on top of the bed before fitting the adapter.

9 With the adapter inlet disconnected, screw down the adapter approximately 1 mm into the bed, allowing the packing solution to flush the adapter inlet.

10 Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will form between the bed surface and the adapter. Mark the bed height on the column.

11 Disconnect the column inlet and lower the adapter approximately 1 mm under the mark into the bed surface.

12 Check once more that there is no space between the adapter and bed surface. If there is space, repeat steps 10–12. If not, the column is ready for use.

Packing FineLINE 100 and FineLINE 200 columns

The FineLINE columns allow an extremely simple, rapid, and reproducible packing procedure. With this procedure, SOURCE is packed at the same time as the adapter is adjusted into position at the correct pressure on top of the bed.

Material needed

- An adjustable pressure relief valve
- A 4-port (2-way) valve attached to the lower column outlet
- Columns with 6 mm ID tubing and 25 mm OD clamp connectors, gaskets, and blind plates (stop plugs)
- A pump that can provide a linear flow rate of at least 20 cm/min through the column at a pressure of 0.6 MPa (6 bar, 85 psi) for FineLINE 100, and at 0.4 MPa (4.0 bar, 57 psi) for FineLINE 200

1. Assemble the column.
2. Add 25% ethanol to the bottom of the column and drain the column outlet be opening the bottom valve. Close the bottom valve.
3. Connect a tube to the suction side of a pump. Place the tube on top of the net and start the pump to extract any remaining air bubbles trapped under the net. Leave the net covered with 25% ethanol.
4. Resuspend SOURCE resin stored in its container by shaking (avoid stirring sedimented resin). Adjust the slurry concentration to 40% to 50%.
5. Pour the slurry fully into the column. Top up with 25% ethanol to approximately 1.0 and 2.5 cm below the rim for FineLINE 100 and FineLINE 200 respectively.
6. Attach the adapter and the column lid. Avoid trapping air bubbles under the adapter by slightly tilting the adapter while attaching it. Block the tubing at the top of the adapter with a blind plate.
7. Connect the pump to the hydraulic chamber inlet with the pressure relief valve in line. Remove air from the chamber by pumping liquid through it.
8. Switch off the pump and block the hydraulic chamber outlet with a blind plate.

9 While running the pump against the closed hydraulic chamber inlet, adjust the pressure relief valve to 0.6 MPa (6 bar, 85 psi) for FineLINE 100, and to 0.4 MPa (4.0 bar, 57 psi for FineLINE 200.

Note: *Make sure that the pressure relief valve is correctly adjusted! If not, the weakest seal in the system will leak when its limit is exceeded.*

10 Open the lower column outlet and the hydraulic chamber inlet. The adapter now moves down as the bed is packed at a constant hydraulic pressure, continuously regulated by the pressure relief valve.

11 When the adapter has reached its lowest position, let the pump run for one more minute. In the meantime, secure the adapter with the locking bar. (Do not use tools to fasten the screws.)

12 Close the column outlet and the hydraulic chamber inlet. Stop the pump.

13 Run the column with an upward flow to remove any residual air trapped in the adapter.

Evaluation of packing

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

Calculate the number of theoretical plates per metre (N/m) as follows:

$N/m = 5.54 (V_e/W_h)^2 \times 1000/L$

Calculate the peak asymmetry factor as follows:

$As = b/a$

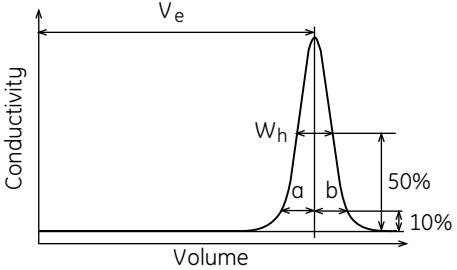


Fig 2. Example showing results obtained from the column evaluation method described above

V_e = Volume eluted from start of sample application to peak maximum

W_h = Peak width at half of peak height

L = Bed height in mm

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

Suggested protocol

Eluent:	0.5 M NaCl
Sample:	0.8 M NaCl
Sample volume:	1% of bed volume
Flow velocity:	60 cm/h
Detection:	Conductivity

Typical efficiency values for a well packed column are greater than 11 000 theoretical plates per meter, N/m, when measuring the peak width at 50% of the peak height. Typical values for the asymmetry factor are 0.8–1.8 when measuring half-peak widths at 10% of the peak height.

Note that system contribution must be minimized, reducing dead volumes as much as possible to achieve the required efficiency.

3 Maintenance

All samples and buffers must be filtered and free of particulate matter. Fouling can be prevented and resin lifetime prolonged if regeneration procedures are developed for each application. The high chemical stability of SOURCE 30RPC allows harsh cleaning-in-place (CIP) procedures to be used.

Cleaning-in-place

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denaturated substances generated in previous purification cycles. For a successful CIP procedure contact time, organic solvent, and pH are important; and depending on the nature of the contamination, different protocols maybe have to be developed and used in combination. To achieve an efficient CIP procedure two requirements must be fulfilled:

1 The substance must be soluble in the CIP solution used.
2 The substance must have higher affinity for the CIP solution than the hydrophobic surface of the RPC matrix.

A combination of solutions with high and low pH and a solvent gradient is very efficient. However, isocratic CIP procedures at constant pH are probably sufficient in many cases. Flow rates/CIP time are different for each application, but as a general guideline, use a flow rate that is significantly lower than during the chromatographic run.

Three different CIP alternatives are suggested.

CIP solutions: Solution A: Ultra pure water.
Solution B: 5 mM H_3PO_4/60% n-Propanol
Solution C: 0.3 M NaOH/ 60% n-Propanol

Alternative 1

- (Removal of acid soluble substances)
  Gradient 1: 0–100% of solution B, 3 column volumes
- (Removal of solution B from the column)
  Isocratic: 1 column volume of solution A
- (Removal of basic soluble substances)
  Gradient 2: 0–100% of solution C, 3 column volumes
- Wash with 1 column volume of solution A

Alternative 2

- (Removal of acid soluble substances only) Replace both gradients in Alternative 1 with an isocratic elution for 60 minutes with solution B

Alternative 3

- (Removal of base soluble substances only) Replace both gradients in Alternative 1 with an isocratic elution for 60 minutes with solution C

Storage

Store SOURCE 30RPC in 20% ethanol to prevent microbial growth.

4 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.

It is recommended to perform optimization at laboratory scale.

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. Alcohols are in general more viscous, giving higher back pressures than 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. It is recommended to begin gradients with at least 5% of organic solvent and 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 must also be considered.

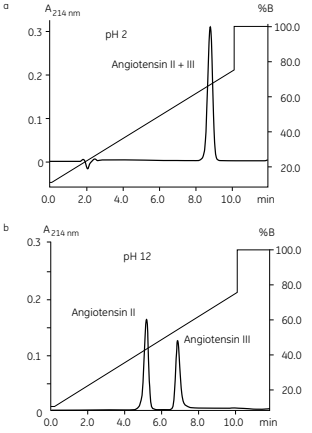
- Type and concentration of ion-pairing agents and buffer components

Trifluoroacetic acid (TFA) is widely used as an ion-pairing agent. It gives high resolution of peptide and protein separations, 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 excellent pH stability of SOURCE 30RPC makes it possible to use a wide pH range to improve selectivity. However, the stability of the protein/peptide at extreme pH must be considered. Figure 3 illustrates the difference in selectivity obtained by changing pH from 2 to 12.



Column:	SOURCE 30RPC, 10 mm i.d. × 100 mm column (8 mL)
Sample:	Angiotensin II and Angiotensin III
Sample load:	0.25 mg/mL resin of each peptide
Solution A1: Solution B1:	0.1% TFA pH = 2 0.1% TFA/50% Acetonitrile pH = 2
Solution A2: Solution B2:	0.1% TFA pH = 12 0.1% TFA/50% Acetonitrile pH = 12
Flow velocity: Gradient	300 cm/h 10–75% B, 10 minutes

Fig 3. a, b Separation of Angiotensin II and Angiotensin III at pH 2 (a), and pH 12 (b). The peptides were not separated at low pH.

- Temperature

Temperature can effect the performance of an RPC method. In process scale applications, the choice of temperature is often restricted by practical limitations. Develop your method within the same temperature ranges which will be used when running the final separation.

Resolution vs productivity

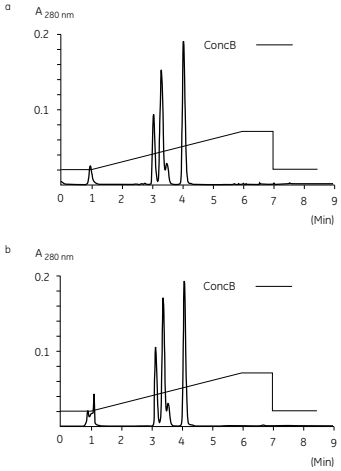
Apart from working on the factors affecting the selectivity, you can often increase resolution further by decreasing the sample load, gradient slope, flow rate, and/or increasing the bed height. These changes will, however, effect throughput. For process-scale

methods, it is important to optimize sample load, gradients slope, flow rate, and bed height to achieve the required resolution at the best possible productivity.

5 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 which remain constant include bed height, flow velocity, sample concentration and volume (in relation to bed volume), and the ratio gradient volume: bed volume. The column diameter and volumetric flow will increase.

The larger equipment needed when scaling up must cause some deviations from the method optimized at small scale. In such cases check the solvent delivery system and monitoring system and try to 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



Column:	SOURCE 30RPC: a) 10 mm i.d. × 300 mm column (24 mL) b) 200 mm i.d. × 300 mm column (10 L)
Sample:	Mixture of Angiotensin, Ribonuclease A and Insulin
Sample load:	0.076 mg/mL resin
Solution A: Solution B:	0.1% TFA/0.05 M NaCl 0.1% TFA/60% n-Propanol
Flow velocity: Gradient:	150 cm/h 20–70% B, 5 column volumes

Fig 4. a, b A 400-fold scale up from (a) 24 mL laboratory scale HR 10/30 column to (b) 10 L production scale FineLINE 200L column.

6 Ordering information

Product	Quantity	Product code
SOURCE 30RPC	10 mL	17512020
SOURCE 30RPC	200 m	17512002
SOURCE 30RPC	500 mL	17512003
SOURCE 30RPC	1 L	17512004
SOURCE 30RPC	5 L	17512005

Related products	Product code
FineLINE Pilot 35	18110202
Tricorn 5/20	28406408
Tricorn 5/20	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
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

Literature	Product code
Hydrophobic Interaction and Reversed Phase Chromatography	11001269

Data Files	Product code
FineLINE Pilot 35	18110495
FineLINE 100/100L & 200/200L	18113000
SOURCE 30RPC	18112973

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