Reversed phase chromatography

SOURCE™ 30RPC

SOURCE 30RPC is an addition to the SOURCE product range, a family of high performance separation resins for fast, preparative purification of biomolecules such as proteins, peptides, and oligonucleotides (Fig 1). SOURCE 30RPC is designed for reversed phase chromatography (RPC) and is an alternative to silica-based RPC matrices. The resin is based on a monodisperse polymeric matrix, offering high capacity and wide pH stability.

SOURCE 30RPC is an excellent chromatography resin for the polishing stage of industrial purification processes where high productivity and stable performance in production are important.

SOURCE 30RPC is characterized by:

- Wide pH operation range
- Batch-to-batch reproducibility
- Excellent pressure/flow characteristics
- Maintained performance at high flow velocities and high sample loads
- Excellent scalability
- High chemical stability

SOURCE 30RPC

SOURCE 30RPC is based on a unique ~ 30 µm, monodisperse, porous, rigid polystyrene/divinyl benzene matrix. A controlled pore size distribution and large specific surface area offer excellent resolution and the capacity for a wide range of molecules, 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 requirements apply. Table 1 summarizes the general characteristics of SOURCE 30RPC.

Fig 1. Butyl Sepharose 4 Fast Flow – for rapid purification.

Wide pH stability

The polystyrene/divinyl benzene matrix provides SOURCE 30RPC with chemical stability over a wide pH range. With an operating range between pH 2 and 12 and a cleaning range between pH 1 and 14, SOURCE 30RPC has broad flexibility in the choice for running conditions and cleaning procedures.

Batch-to-batch reproducibility

The combination of a unique manufacturing process and high quality assurance standards results in reproducible batch-to-batch quality. The process gives consistent pore and bead structure, both within and between batches – an important factor for routine applications and industrial production where regulatory control is strict.

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_{B10}[2]	~ 14 mg BSA/mL resin ~ 23 mg bacitracin/mL resin ~ 72 mg insulin/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 1M HCl, 1M HCl/90% Methanol, 90% HAc, 6M GuHCl, 100 % n-propanol, 100% Ethanol, 100 % Methanol, 100 % Acetone, 0.45M 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]	2 to 12
pH stability, CIP[5]	1 to 14
Recommended operating flow velocity	100 to 1000 cm/h[6,7]
Operating temperature	4°C to 40oC
Delivery conditions	20% ethanol
Storage	25% ethanol, 4°C to 30°C

[1] Monodisperese 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] 1.0 M NaOH should 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] Will depend on the pressure specification of the chromatographic system used, solvent and bed height. A linear flow velocity of 1000 cm/h will give a pressure drop of approximately 10 bar at a bed height of 15 cm using water as eluent.

Excellent pressure/flow characteristics

The SOURCE 30 matrix is composed of uniform ~ 30 µm diameter beads, spherical in shape and free from broken beads, fragments, and fines (see Fig 2). This results in stable, densely packed beds with excellent flow properties. Due to the monosized beads, the back pressure from SOURCE 30RPC is low, although actual values will depend on the solvent used and operating temperature (Fig 3).

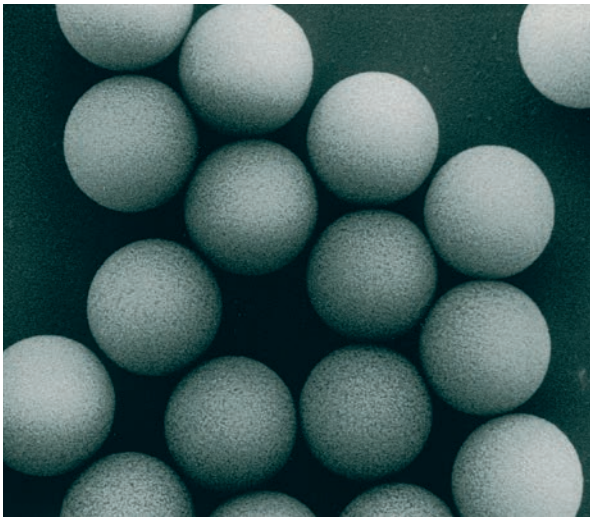
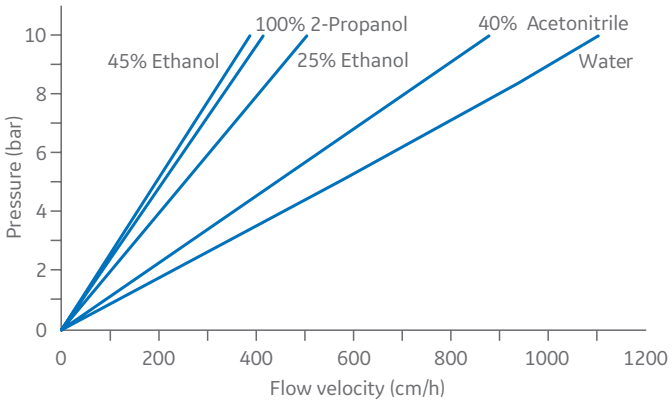


Fig 2. Electron micrograph of SOURCE 30RPC beads. Note the uniform size and absence of fines, fragments, and broken beads.

(A) 15 cm bed height



(B) 30 cm bed height

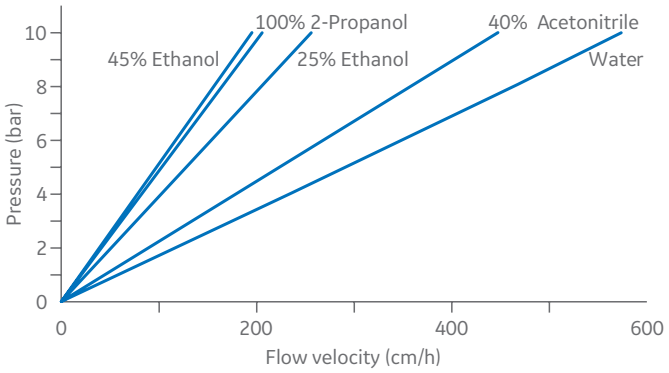


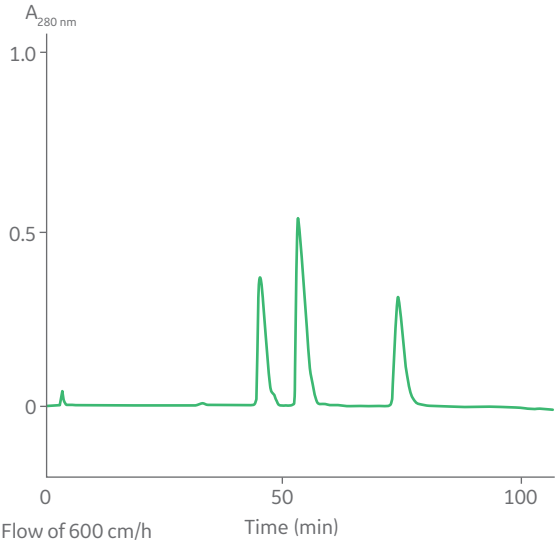
Fig 3. Pressure/flow characteristics of SOURCE 30RPC in various organic solvents and water at room teperature. The pressure/flow velocity data were determined in a FineLINE™ Pilot 35 column with (A) 15 cm and (B) 30 cm bed height.

Maintained performance at high flow velocities and high sample loads

SOURCE performance is well maintained at high flow velocities and high sample loads. Figures 4 and 5 show separations of model proteins at different sample loads and flow velocities.

Column: SOURCE 30RPC, 10 mm i.d. × 100 mm column (8 mL)
Sample: Mixture of Ribonuclease A, Insulin and Albumin
Sample load: 1 mg/mL resin, total load
Solution A: 0.1% TFA
Solution B: 0.1% TFA/60% Acetonitrile
Flow velocity: 150 and 600 cm/h
Gradient: 20%–80% B, 20 column volumes

Flow of 150 cm/h



Flow of 600 cm/h

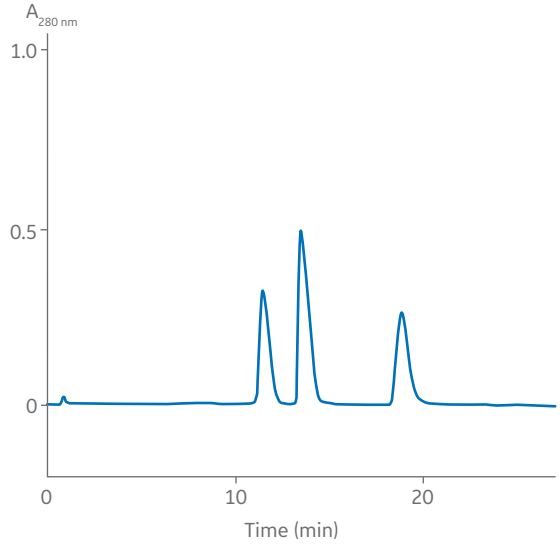
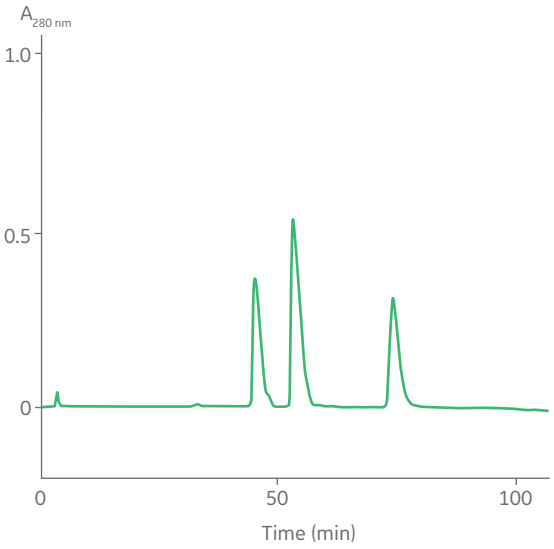


Fig 4. The influence of increasing flow velocity on resolution.

Column: SOURCE 30RPC, 10 mm i.d. × 100 mm column (8 mL)
Sample: Mixture of Ribonuclease A, Insulin and Albumin
Sample load: 1 and 10 mg/mL resin, total load
Solution A: 0.1% TFA
Solution B: 0.1% TFA/60% Acetonitrile
Flow velocity: 150 cm/h
Gradient: 20%–80% B, 20 column volumes

Sample load of 1 mg



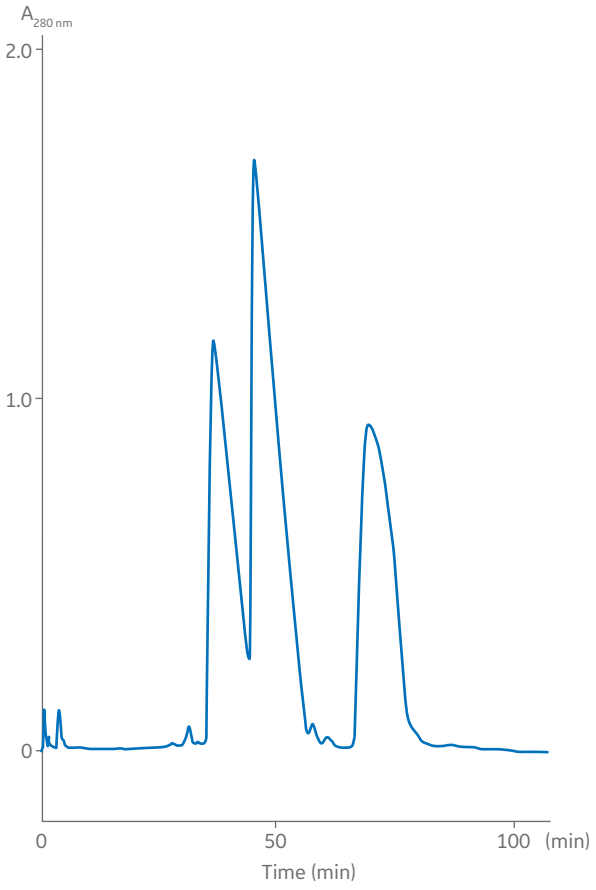


Fig 5. The influence of increasing sample load on resolution.

Excellent scalability

SOURCE 30RPC is easy to pack in both laboratory and large scale columns and maintains its performance during scale up. Furthermore, scale up is very predictable – simply keep flow velocity, sample load per column volume, and bed height constant.

Figure 6 shows a scale-up of a model protein separation on SOURCE 30RPC going from a 24 mL HR 10/30 column to a ten liter FineLINE 200L column in one step. The scale-up factor is 400.

Operation

SOURCE 30RPC can be used with standard methods for RPC. However, if the method used has been developed for silica-based RPC resins, it may require re-optimizing due to different characteristics and selectivities.

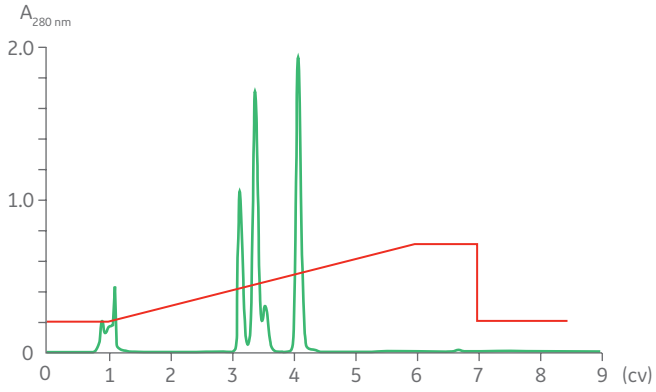
High chemical stability

SOURCE 30RPC is based on polystyrene/divinylbenzene, which gives high chemical stability. With an operating range from pH 2 to 12 and a cleaning range from pH 1 to 14, SOURCE 30RPC offers flexibility in the choice of running conditions, and cleaning and sanitization methods. This high chemical stability is illustrated in Figure 7, which shows the separation of angiotensins on SOURCE 30RPC before and after treatment with typical cleaning agents. Following incubation of SOURCE 30RPC for one week at 40°C in either 1.0 M NaOH or 1.0 M HCl, its chromatographic performance was verified. As can been seen from the chromatograms in Figure 7, the separation patterns were practically unaffected by the treatment.

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.

Column:	SOURCE 30RPC, 10 mm i.d. × 300 mm column (24 mL) 200 mm i.d. × 300 mm column (10 l)
Sample:	Mixture of Angiotensin II, Ribonuclease A and Insulin
Sample load:	0.064 mg/mL resin, total load
Solution A:	0.1% TFA/0.05 M NaCl
Solution B:	0.1% TFA/60% n-Propanol
Flow velocity:	150 cm/h
Gradient:	20%–70% B, 5 column volumes (cv)

HR 10/30 column



FineLINE 200L column

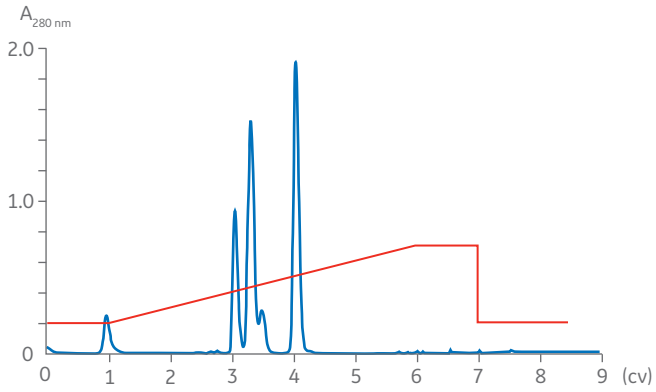


Fig 6. A separation on 24 mL laboratory scale HR column (upper figure) scaled up to a 10 liter production scale FineLINE 200L column (lower figure). The scale-up factor is 400.

Column: SOURCE 30RPC, 5 mm i.d. × 50 mm column (1 mL)
Sample: Mixture of (Ile^{7}) Angiotensin III, (Val4) Angiotensin III, Angiotensin III, and Angiotensin II
Sample load: 0.13 mg/mL resin of each peptide
Solution A: 0.1% TFA
Solution B: 0.1% TFA/60% Acetonitrile
Flow velocity: 300 cm/h
Gradient: 15% to 65% B, 20 column volumes

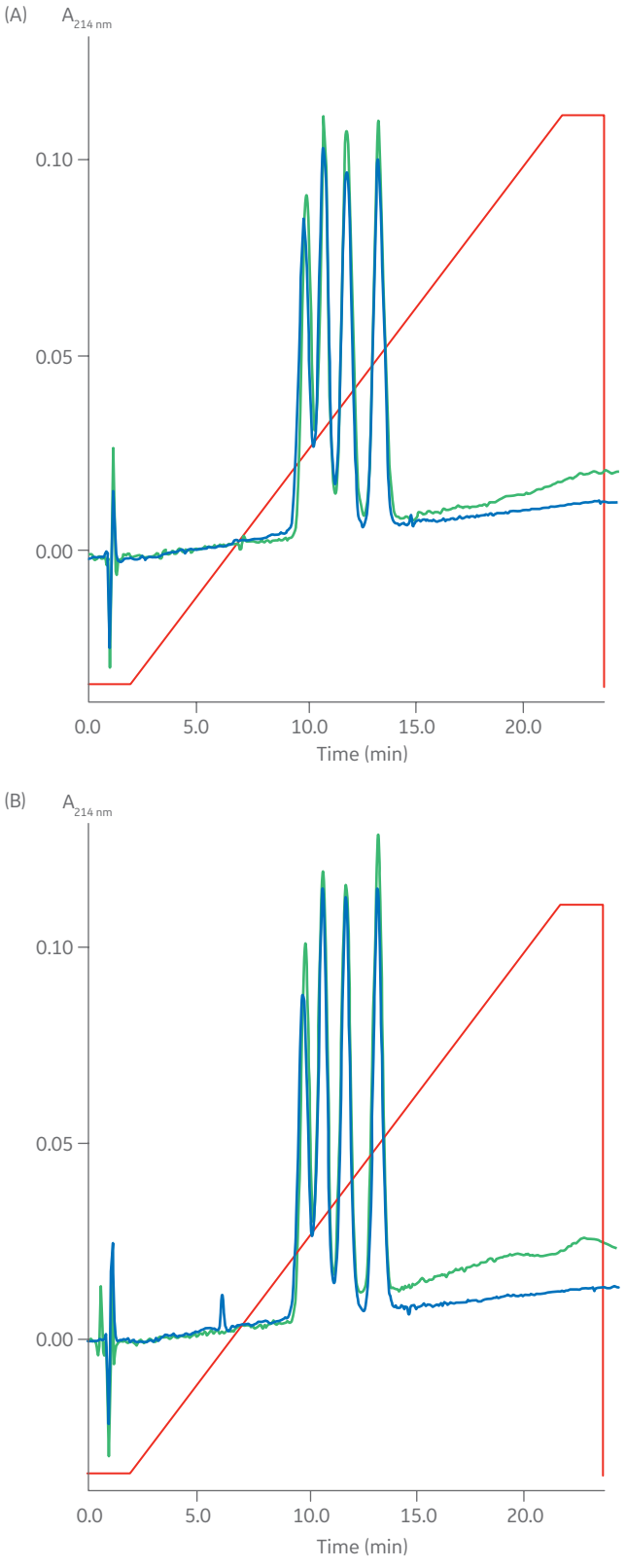


Fig 7. Separation of model protein mixture on SOURCE 30RPC before and after incubation of the resin for one week at 40°C in (A) 1.0 M HCl and in (B) 1.0 M NaOH. Yellow curves are before incubation, and blue curves are after incubation.

Application

Separation of Angiotensin II and III at high pH

Solubility of peptides is often pH-dependent and successful separation of some peptides requires operation at high pH. In contrast to silica-based matrices, SOURCE 30RPC is stable at high pH. In the example illustrated in Figure 7, Angiotensin II and Angiotensin III were successfully separated at pH 12, but not at pH 2.

Equipment

Optimal conditions for SOURCE 30RPC are achieved using a suitable column, such as the FineLINE series. FineLINE columns are specially designed for SOURCE resins, enabling column packing to be completed in 10 min, while producing stable, reproducible beds.

For scouting different separation conditions at small scale, and for lab-scale preparative applications, FineLINE Pilot 35 connected to an ÄKTA™ chromatography system and Tricorn™ columns are recommended (see Table 2). For large-scale applications, the FineLINE series of columns are recommended (see Table 2).

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 volumetric flow rates. Pressure/flow characteristics with different solvents and bed heights are shown in Figures 3A and 3B.

Typically, flow velocities in the range 100 to 1000 cm/h will provide the desired resolution, productivity, and product yield with convenient process times of a few minutes to one hour.

Further recommendations for method design and optimization, column packing, cleaning, and sanitization of SOURCE 30RPC are found in the instructions enclosed with each package.

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	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, 2 µm	350	14 400	30–150
FineLINE 350PL, EPDM, 10 µm	350	28 800	50–300

Ordering information

Resin

Product	Pack size	Product Code
SOURCE 30RPC	10 mL	17512020
SOURCE 30RPC	200 mL	17512002
SOURCE 30RPC	500 mL	17512003
SOURCE 30RPC	1 L	17512004
SOURCE 30RPC	5 L	17512005

Column

Lab-scale 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
Large-scale Column	
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

Product	Product Code
Hydrophobic Interaction and Reversed Phase Chromatography: Principles and Methods, Handbook	11001269
FineLINE Pilot 35, Data file	18110495
FineLINE 100/100L and 200/200L, Data file	18113000

gelifesciences.com/bioprocess

GE, the GE Monogram, ÄKTA, FineLINE, SOURCE, and Tricorn are trademarks of General Electric Company.
© 2000-2018 General Electric Company.
All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.
GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
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

KA1372220818DF 18112973 AE