Q Sepharose™ High Performance
SP Sepharose High Performance

Q Sepharose High Performance and SP Sepharose High Performance enjoy well-deserved reputations as highly successful anion and cation ion exchange media for purifying a wide range of biomolecules. Both share an impressive list of operational characteristics that includes:

- High-resolution, high-capacity separations with high recovery
- Reliable and reproducible
- High chemical stability for effective CIP/sanitization
- Available in convenient HiPrep™, HiScreen™, and HiTrap™ prepacked columns plus laboratory packs
- Easy to scale up

With these attributes, Q and SP Sepharose High Performance occupy a central position in the broad spectrum of GE Healthcare ion exchange products. Their high resolution generates distinct, high purity separations and their high capacity and ease of use encourages preparative use and scale-up, primarily in intermediate and final purification. Availability in different prepacked HiPrep and HiTrap column formats plus laboratory packs gives users the freedom to enjoy the convenience and simplicity of ready-to-use columns or the flexibility of choosing column type and size (Fig 1).

Ion exchange chromatography

Ion exchange chromatography is probably the most frequently used and versatile method for fractionating biological substances, even proteins and peptides with small differences in charge can be separated. Furthermore, binding and elution conditions are easy to optimize, resulting in fast, high-resolution separations that are reproducible and cost-effective to scale up.

Fig 1. Q and SP Sepharose High Performance, available in lab packs and prepacked HiPrep and HiTrap columns.

Charged molecules bind to the separation medium at low ionic strength and are then eluted with a salt or pH gradient. Whereas continuous gradient elution is most frequently used in high resolution ion exchange chromatography, simple stepwise gradient elution is recommended for sample preparation, concentration, etc.

These factors have all contributed to making ion exchange chromatography a leading technique in biomolecule separation today. Thanks to the highly efficient, high-resolution separations that Q and SP Sepharose High Performance deliver, they continue to play a key role in the purification of biomolecules.

Media characteristics

Q Sepharose High Performance and SP Sepharose High Performance are high-resolution anion and cation exchange media based on highly cross-linked 34 µm agarose beads. This matrix provides excellent chemical and physical stability. The rigidity and small size of the particles allows fast adsorption and desorption, even at high sample loadings and flow rates.

Q Sepharose High Performance is a strong anion exchange medium and SP Sepharose High Performance a strong cation exchanger. The Q functional group is a quaternary amino group and the SP sulphopropyl. Both are coupled to the matrix via chemically stable ether linkages. The two media remain charged and have high loading capacities over broad pH ranges.

Table 1 lists the main media characteristics.

Table 1. Main media characteristics of Q and SP Sepharose High Performance

Matrix	6% spherical, cross-linked agarose
Functional groups	$-CH_2N^+(CH_3)_3$, quaternary ammonium (Q) $-CH_2CH_2CH_2SO_3^-$, sulphopropyl (SP)
Total ionic capacity	0.14 to 0.20 mmol (Cl^-)/mL medium (Q) 0.15 to 0.20 mmol (H^+)//mL medium (SP)
Binding capacity	70 mg BSA/mL medium (Q) 55 mg ribonuclease A/mL medium (SP)
Average particle size	34 µm
Exclusion limit	4×10^6 daltons (globular proteins)
Rec. linear flow rate	Up to 150 cm/h
Chemical stability	1 M sodium hydroxide 1 M acetic acid 8 M urea 6 M guanidine hydrochloride 30% acetonitrile 30% isopropanol 70% ethanol 2% SDS
pH stability	
short term*	1 to 14 (Q), 3 to 14 (SP)
long term and working†	2 to 12 (Q), 4 to 13 (SP)
Storage	20% ethanol (Q) 20% ethanol, 0.2 M sodium acetate (SP)

* Refers to the pH interval for regeneration

† Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

Packing in laboratory columns

Q and SP Sepharose High Performance are supplied in laboratory packs of 75 mL, which is ideal for users who prefer the flexibility of packing columns of their choice. Straightforward and well-proven recommendations for packing, operation and maintenance are included in the instructions.

Empty high-resolution columns from the Tricorn™, XK, and HiScale ranges are available in a variety of sizes and recommended for users who want to pack their own columns.

Prepacked Q and SP Sepharose High Performance columns

By providing added speed, convenience and reproducibility, prepacked columns extend the usefulness of Q and SP Sepharose High Performance. ÄKTA™ design system includes preset method templates based on these prepacked columns, which further improves convenience and results, particularly their reproducibility, plus the speed at which they are achieved.

Both media are supplied in two types of column, each available in two sizes; HiPrep Q and SP Sepharose High Performance, and HiTrap Q HP and SP HP.

HiPrep Q HP 16/10 and HiPrep SP HP 16/10

HiPrep columns are made of polypropylene, which is biocompatible with biomolecules. The columns are easily connected to a variety of chromatographic systems, including simple pump-based configurations and ÄKTA design. The columns are not designed to be opened or repacked.

HiPrep Q HP and HiPrep SP HP are available in 16 mm diameter, with a bed height of 10 cm giving a column volume of 20 mL.

Table 2. Characteristics of prepacked HiPrep Q HP 16/10 and HiPrep SP HP 16/10 columns

Column dimension	1.6 × 10.0 cm
Bed volume	20 mL
Recommended flow rate*	2 to 5 mL/min (60 to 150 cm/h)
Maximum flow rate*	5 mL/min (150 cm/h)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)
Storage	20% ethanol (Q) 20% ethanol, 0.2 M sodium acetate (SP)

See 18-1127-63 AD page 2, table 2 for more info.

*Water at room temperature

HiScreen Q and SP HP columns

Q and SP Sepharose High Performance media are also available prepacked in HiScreen columns. These columns are made of biocompatible polypropylene that does not interact with biomolecules. They can be run on peristaltic pumps or on chromatography systems such as ÄKTA. The columns are delivered with a stopper at the inlet and at the outlet. Table 3 lists the characteristics of HiScreen columns. Note that HiScreen columns cannot be opened or repacked.

Table 3. Characteristics of HiScreen columns

Column dimensions	0.77 × 10 cm
Column volume	4.7 mL
Column hardware pressure limit[3]	8 bar (0.8 MPa, 117 psi)

[3] The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

HiTrap Q HP 1 mL and 5 mL
HiTrap SP HP 1 mL and 5 mL

HiTrap Q HP and HiTrap SP HP are small, prepacked columns made of biocompatible polypropylene. The column is delivered with a stopper on the inlet and a snap-off end on the outlet. All necessary connectors are included for connection to different systems as well as to a laboratory pump and a simple syringe. Note that HiTrap columns cannot be opened or repacked. Two sizes are available, 1 mL and 5 mL (Fig 1).

The 1-mL column is often used for method screening to quickly establish optimal binding and elution conditions for specific applications. Its fast and simple operation is well-suited to this role, as well as to small-scale purifications. The larger 5-mL column is an excellent choice for group separations and sample concentration, and when the purification method has been established and larger amounts of protein need to be purified. Two or three columns can be connected in series. Further scale-up can be done on HiPrep Q HP 16/10 or HiPrep SP HP 16/10 columns (see Applications).

Table 4. Characteristics of HiTrap Q HP and HiTrap SP HP. See Table 1 for media characteristics

Column dimensions	0.7 × 2.5 cm (1 mL), 1.6 × 2.5 cm (5 mL)
Column volumes	1 mL and 5 mL
Rec. flow rate	1.0 mL/min (1 mL), 5.0 mL/min (5 mL)
Max. flow rate*	4.0 mL/min (1 mL), 20.0 mL/min (5 mL)
Max. back pressure	0.3 MPa, 3 bar, 42 psi
Storage	20% ethanol (Q)
	20% ethanol, 0.2 M sodium acetate (SP)

* Room temperature, aqueous buffers

Operating HiTrap Q HP and HiTrap SP HP

Using HiTrap Q HP and HiTrap SP HP prepacked columns is simple. Complete, easy-to-follow instructions are included for fast start-up and method optimization. Whether you use a syringe and the provided luer adaptor (Fig 2), a peristaltic pump, or a chromatography system such as ÄKTA design or FPLC™ System, operation is straightforward.

Related media available in HiTrap IEX Selection Kit

Although it does not include Q or SP Sepharose High Performance media, the HiTrap IEX Selection Kit will be of interest to many potential users of these two ion exchangers. The kit consists of seven different ion exchange media prepacked in HiTrap 1 mL columns; SP Sepharose Fast Flow, Q Sepharose Fast Flow, DEAE Sepharose Fast Flow, CM Sepharose Fast Flow, ANX Sepharose 4 Fast Flow (high sub), SP Sepharose XL and Q Sepharose XL.

The HiTrap IEX Selection Kit offers a fast, simple and convenient way to decide which ion exchange matrix and ligand is best suited for a given application. In other words, it is a useful tool in helping to speed up the development of an optimized ion exchange separation.

The seven columns included in the kit are also available as individual HiTrap 1-mL and 5-mL columns. For more information about the kit, request Data File HiTrap IEX Selection Kit, Code no. 18-1140-48.

A)

B)

C)

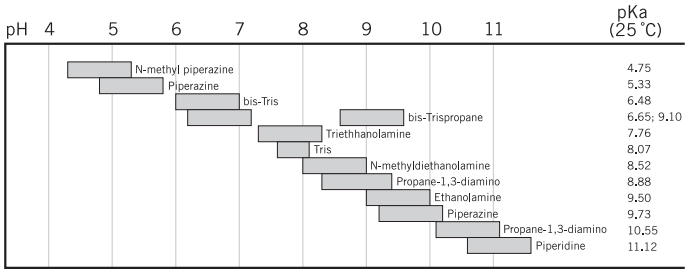
Fig 2. Using a HiTrap Q HP or HiTrap SP HP 1 mL column with a syringe. A) Prepare buffers and sample. Remove stop plug from top of the column and snap off the end. Equilibrate. B) Load the sample and begin collecting fractions. C) Wash, elute and continue collecting fractions.

Chemical stability

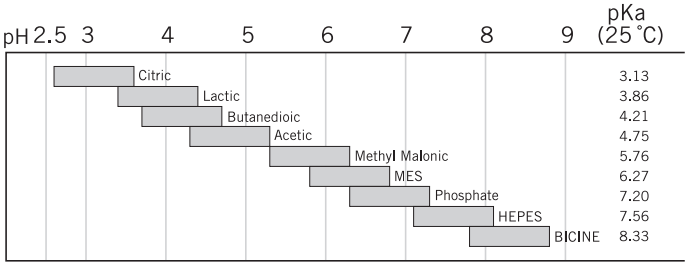
Good chemical stability allows the use of effective cleaning-in-place (CIP) schemes that result in high recoveries over many purification cycles. Likewise, it allows regular sanitization to prevent microbial growth and maintain a high level of hygiene. Both CIP and sanitization thus promote good process economy and are therefore key factors to consider when selecting ion exchange media and prepacked columns for preparative applications.

For CIP, regular washing with 0.5 to 1.0 M sodium hydroxide should be sufficient to remove most contaminating material, although very hydrophobic molecules may bind so tightly that they must be eluted with organic agents like 70% ethanol or 30% isopropanol, or with strong detergents.

CIP and sanitization protocols for Q and SP Sepharose High Performance are included in the packages. Note that specific protocols should be developed according to the nature and condition of the starting material.



Recommeded buffers for anion exchange chromatography.



Recommeded buffers for cation exchange chromatography.

Fig 3. Recommeded buffers for ion exchange chromatography.

Table 5. Recommended volatile buffers for ion exchange chromatography

pH	Volatile buffer systems
2.3–3.5	Pyridine/formic acid
3.0–5.0	Trimethylamine/formic acid
4.0–6.0	Trimethylamine/acetic acid
6.8–8.8	Trimethylamine/HCl
7.0–8.5	Ammonia/formic acid
8.5–10.0	Ammonia/acetic acid
7.0–12.0	Trimethylamine/CO2
8.0–9.5	Ammonium carbonate/ammonia
8.5–10.5	Ethanolamine/HCl

Applications

Protein and nucleic acid purifications

The applications of Q and SP Sepharose High Performance are many. The versatility of ion exchange plus the performance benefit of high resolution with high capacity make both media a natural choice for preparative separations during the intermediate and final steps of a purification scheme. In addition, systems in the ÄKTA design platform have ready-programmed method templates for both HiPrep and HiTrap columns, contributing to the widespread use of Q and SP Sepharose High Performance columns.

Group separation and sample concentration

The 1-mL and 5-mL HiTrap columns complement the other Q and SP Sepharose High Performance products by providing rapid and reliable method scouting as well as group separation and sample concentration. Figures 4 and 5 illustrate an example of group separation on HiTrap SP HP 1 mL and SDS-PAGE analysis of the fractions respectively.

Sample concentration is frequently required to improve subsequent purification steps. For example, concentration is necessary prior to gel filtration to reduce sample volume, which is one of the more important factors affecting resolution with this technique.

Table 6 shows the efficiency of HiTrap Q HP 1 mL and HiTrap SP HP 5 mL for concentrating standard proteins. Samples were dissolved in start buffer and applied to the columns at flow rates of 0.5 mL/min (HiTrap Q HP) and 2.5 mL/min (HiTrap SP HP) with a peristaltic pump. Columns were then washed with start buffer and eluted with elution buffer using a syringe. Fractions were collected for analysis and yield was determined by measuring absorbance at 280 nm.

As can be seen, yields are high, even for very dilute samples. After concentration, the sample is eluted in volumes suitable for direct loading onto gel filtration columns, e.g. prepacked HiLoad™ 16/600 or 26/600 Superdex™ 30 prep grade, 75 prep grade, or 200 prep grade.

Column	HiTrap SP HP, 1 mL
Sample	Casein-precipitated human milk, filtered (0.45 µm filter) and buffer exchanged to start buffer on a PD-10 Desalting column
Sample volume	0.5 mL
Flow rate	1.0 mL/min (150 cm/h)
Start buffer	50 mM sodium acetate, pH 6.0
Elution buffer	50 mM sodium acetate, 1.0 M NaCl, pH 6.0

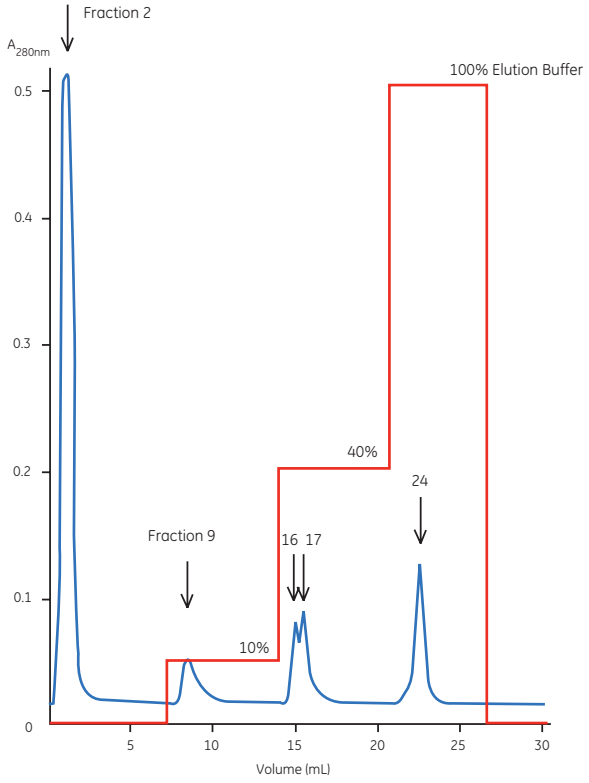
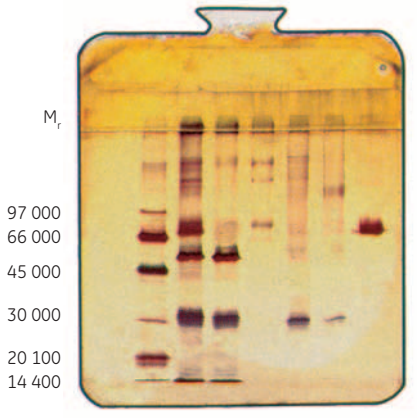


Fig 4. Group separation of proteins from casein-precipitated human milk on HiTrap SP HP 1 mL.



Lane 1:	LMW markers
Lane 2:	Start material, casein-precipitated human milk, diluted 5x
Lane 3:	Fraction 2, diluted 2x
Lane 4:	Fraction 9
Lane 5:	Fraction 16
Lane 6:	Fraction 17
Lane 7:	Fraction 24

Fig 5. SDS electrophoresis (PhastSystem™, PhastGel™ 10–15, silver staining) on fractions from the group separation of casein-precipitated human milk on HiTrap SP HP 1 mL shown in Figure 4.

Initial scale-up

HiTrap columns also offer a quick start to scaled-up purifications, either by two or three HiTrap columns being connected in series, or by progressing to larger HiPrep columns.

Process development and scale-up to production

The excellent performance of Q and SP Sepharose High Performance for laboratory scale preparative applications naturally lends itself to the process development and scale-up of ion exchange separations. The media are well supported for this task.

As members of the BioProcess™ family, both are backed up with special services and documentation to facilitate the development, scale-up and routine operation of production applications. Validated manufacture, secure supply and regulatory support comprise just part of this package. For more information, please contact GE Healthcare.

Table 6. HiTrap Q HP 1 mL and HiTrap SP HP 5 mL used for concentrating standard proteins. Note the high yields, even for very dilute samples

Column	Sample	Sample conc. µg/mL	Sample volume mL	Eluted conc. µg/mL	Volume eluted mL	Conc. factor (volume)	Yield %
HiTrap Q HP, 1 mL	Human IgG	23	450	3180	3.0	150	92
		10	100	4700	2.0	50	93
		1010	10	3370	3.0	3	100
HiTrap SP HP, 5 mL	Lysozyme	333	150	3170	16.0	9	100
		33	1500	3720	13.2	114	98

HiTrap SP HP, 1 mL

Sample	Concanavalin A, ribonuclease A, αα-chymotrypsinogen A, lysozyme, 4 mg protein/mL (3:3:1:1) in start buffer
Sample load	1 mg protein/mL medium
Sample volume	0.25 mL, 25% of column volume
Column volume	1 mL
Flow rate	0.5 mL/min (75 cm/h)
Start buffer	50 mM MES, pH 6.0
Elution buffer	50 mM MES, 1.0 M NaCl, pH 6.0
Gradient	0% to 43% elution buffer over 10 mL (10 column volumes)

HiPrep SP HP 16/10, 20 mL

Sample	Concanavalin A, ribonuclease A, α-chymotrypsinogen A, lysozyme, 4 mg protein/mL (3:3:1:1) in start buffer
Sample load	1 mg protein/mL medium
Sample volume	5.0 mL, 25% of column volume
Column volume	20 mL
Flow rate	2.5 mL/min (75 cm/h)
Start buffer	50 mM MES, pH 6.0
Elution buffer	50 mM MES, 1.0 M NaCl, pH 6.0
Gradient	0% to 43% elution buffer over 200 mL (10 column volumes)

HiTrap SP HP, 5 mL

Sample	Concanavalin A, ribonuclease A, αα-chymotrypsinogen A, lysozyme, 4 mg protein/mL (3:3:1:1) in start buffer
Sample load	1 mg protein/mL medium
Sample volume	1.25 mL, 25% of column volume
Column volume	5 mL
Flow rate	2.5 mL/min (75 cm/h)
Start buffer	50 mM MES, pH 6.0
Elution buffer	50 mM MES, 1.0 M NaCl, pH 6.0
Gradient	0% to 43% elution buffer over 50 mL (10 column volumes)

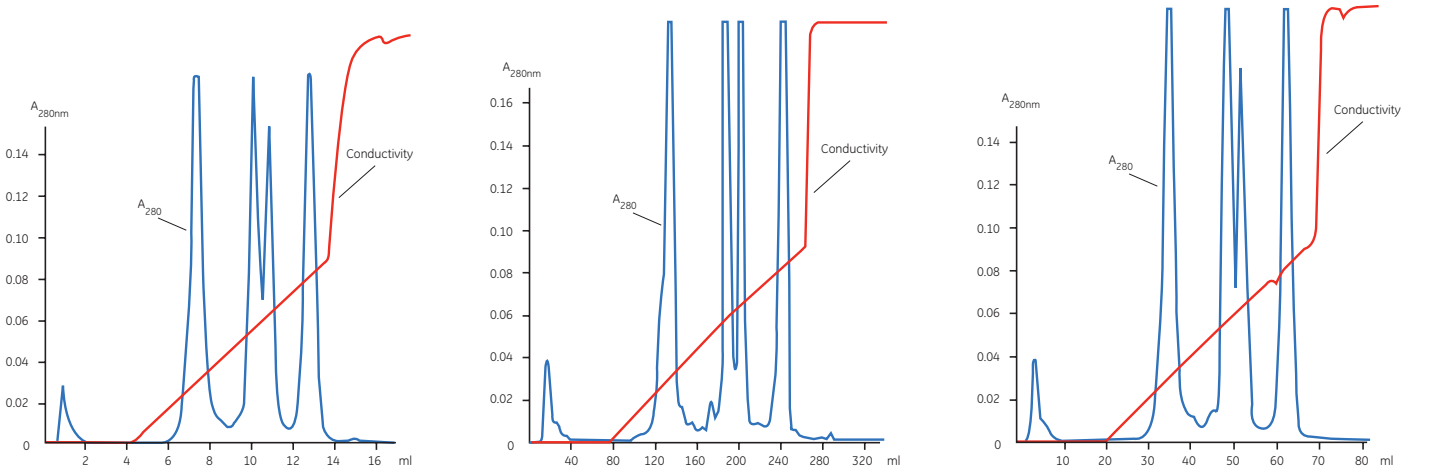


Fig 6. Comparing separations on HiTrap SP HP 1 mL and 5 mL and HiPrep SP HP 16/10 columns confirms the predictable nature of the results and the ease with which separations can be scaled up.

Ordering information

Products	Quantity	Code number
Q Sepharose High Performance	75 mL	17-1014-01
Q Sepharose High Performance	1 L	17-1014-03
Q Sepharose High Performance	5 L	17-1014-04
Q Sepharose High Performance	10 L	17-1014-05
SP Sepharose High Performance	75 mL	17-1087-01
SP Sepharose High Performance	1 L	17-1087-03
SP Sepharose High Performance	5 L	17-1087-04
SP Sepharose High Performance	10 L	17-1087-05

Prepacked columns		
HiPrep Q HP 16/10	1 (20 mL)	29-0181-82
HiPrep SP HP 16/10	1 (20 mL)	29-0181-83
HiScreen Q HP	1 × 4.7 mL	28-9505-11
HiScreen SP HP	1 × 4.7 mL	28-9505-15
HiTrap Q HP	5 × 1 mL	17-1153-01
HiTrap Q HP	5 × 5 mL	17-1154-01
HiTrap SP HP	5 × 1 mL	17-1151-01
HiTrap SP HP	5 × 5 mL	17-1152-01
HiTrap IEX Selection Kit	7 × 1 mL	17-6002-33

Related products	
Ion Exchange Chromatography and Chromatofocusing Handbook, Principles & Methods	11-0004-21
Ion Exchange Columns and Media, Product Profile	18-1127-31
Convenient Protein Purification, HiTrap Column Guide	18-1129-81
Column Packing CD, "The Movie"	18-1165-33

HiTrap accessories	Quantity	Code number
1/16" male/luer female†	2	18-1112-51
Tubing connector flangeless/M6 female†	2	18-1003-68
Tubing connector flangeless/M6 male†	2	18-1017-98
Union 1/16" female/M6 male†	6	18-1112-57
Union M6 female /1/16" male†	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16"‡	5	11-0004-64
Fingertight stop plug, 1/16"§	5	11-0003-55

† One connector included in each HiTrap package

‡ Two, five, or seven stop plugs female included in HiTrap packages depending on the product

§ One fingertight stop plug is connected to the top of each HiTrap column at delivery

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/bioprocess
www.gelifesciences.com/protein-purification

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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