Q Sepharose High Performance
SP Sepharose High Performance

Prepacked HiLoad and HiTrap columns

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 HiLoad™ 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 HiLoad 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.

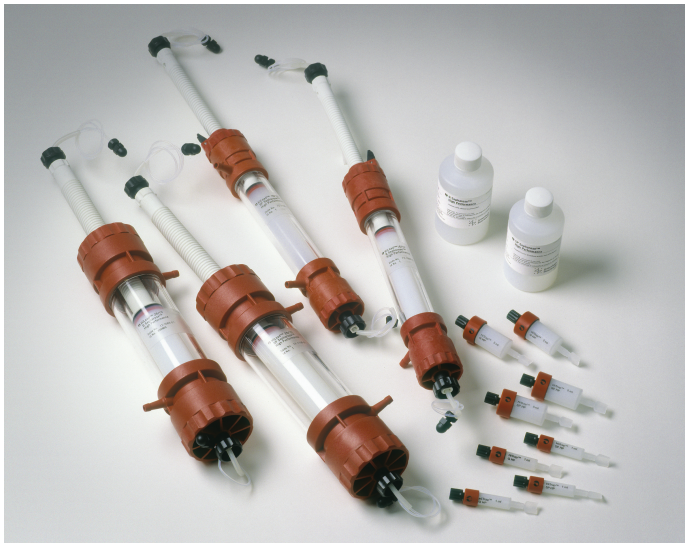


Fig 1. Q and SP Sepharose High Performance, available in lab packs and prepacked HiLoad and HiTrap columns, are a dominant force in the high-resolution, high-capacity purification of biomolecules.

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]	1 to 14 (Q), 3 to 14 (SP)
long term and working[2]	2 to 12 (Q), 4 to 13 (SP)
Storage	20% ethanol (Q) 20% ethanol, 0.2 M sodium acetate (SP)

[1] Refers to the pH interval for regeneration

[2] 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™ and XK 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. ÄKTAdesign™ 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; HiLoad Q and SP Sepharose High Performance, and HiTrap Q HP and SP HP.

HiLoad 16/10 and 26/10 Q Sepharose High Performance

HiLoad 16/10 and 26/10 SP Sepharose High Performance

HiLoad prepacked columns are XK laboratory columns prepacked with Q or SP Sepharose High Performance.

XK columns are the perfect partners for preparative separations with Sepharose High Performance. The columns have an advanced design and are constructed from precision bore borosilicate glass. Dead volumes are less than 0.1% of the total column volume. A thermostatic jacket is fitted as standard. HiLoad Q and SP Sepharose High Performance are available in two sizes, 16 or 26 mm diameter, both with a bed height of 10 cm (Fig 1) and giving column volumes of 20 ml and 53 ml, respectively.

In addition to the built-in benefits of high-resolution, high-capacity preparative separations, HiLoad columns offer great reproducibility and convenience. Every HiLoad column is packed under stringent control and tested individually for bed volume, efficiency (theoretical plates/m), asymmetry factor, and flow characteristics. They can be used in a variety of systems, such as ÄKTAdesign. Flanged tubing and M6 connectors for connection to FPLC™ System are supplied as standard, as are connectors for ÄKTAdesign systems.

Table 2 lists the key characteristics of HiLoad 16/10 and 26/10 Q and SP Sepharose High Performance columns.

Table 2. HiLoad Q and SP Sepharose High Performance column characteristics. See Table 1 for media characteristics.

Bed diameter	16 or 26 mm
Bed height	10 cm
Bed volume	20 ml (16/10) 53 ml (26/10)
Theoretical plates	>12 000 /m
Rec. flow rate	up to 150 cm/h (5 ml/min for 16/10, 13 ml/min for 26/10)
Max. back pressure over the packed bed during operation	0.3 MPa, 3 bar, 42 psi
HiLoad column hardware pressure limit	0.5 MPa, 5 bar, 73 psi
Storage	20% ethanol (Q) 20% ethanol, 0.2 M sodium acetate (SP)

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 HiLoad Q or SP Sepharose High Performance columns (see Applications).

Table 3. Characteristics of HiTrap Q HP and HiTrap SP HP. See Table 1 for media characteristics.

Column dimensions	0.7 x 2.5 cm (1 ml), 1.6 x 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.

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.

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

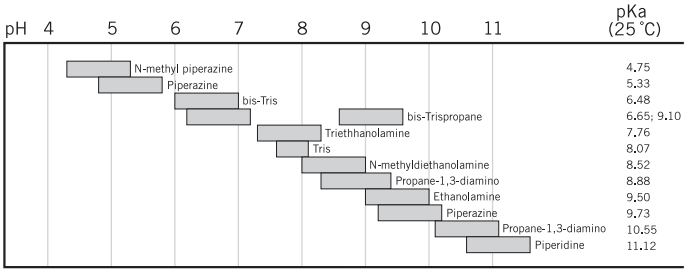
The seven columns included in the kit are also available as individual HiTrap 1-ml and 5-ml columns. For more information about the kit, request Data File HiTrap IEX Selection Kit, Code no. 18-1140-48.

Chemical stability

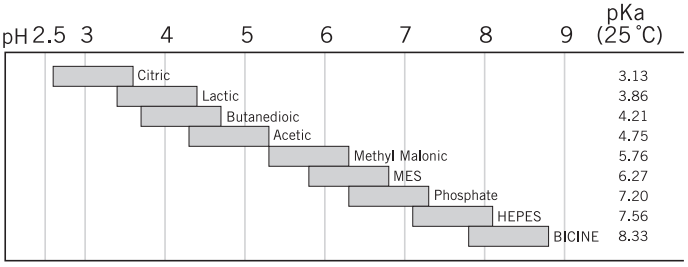
Good chemical stability allows the use of effective cleaning-in-place (CIP) schemes that result in high recoveries over many purification cycles. Likewise, it allows regular sanitization to hinder microbial growth and maintain a high level of hygiene. Both CIP and sanitization thus promote good economy 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 solvents 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 4. 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 extreme 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. The excellent availability of suitable equipment also contributes to widespread use, especially as systems in the ÄKTAdesign platform have ready-programmed method templates for both HiLoad and HiTrap columns.

Figure 6 shows the separation of standard proteins when scaling-up on HiLoad 16/10 and HiLoad 26/10 SP Sepharose High Performance. Figure 4 shows a DNA application, the purification of supercoiled plasmid DNA on HiLoad 16/10 Q Sepharose High Performance resulting in 220 µg of biologically active material.

Column:	HiLoad 16/10 Q Sepharose High Performance
Sample:	2.0 ml of a bacterial alkaline extract of HB 101 (pBR322) corresponding to 400 ml amplified bacterial culture
Start buffer:	10 mM Tris-HCl, 1 mM EDTA, 0.7 M NaCl, pH 8.0
Elution buffer:	10 mM Tris-HCl, 1 mM EDTA, 0.85 M NaCl, pH 8.0
Flow rate:	3 ml/min (90 cm/h)
Result:	220 µg biologically active supercoiled DNA

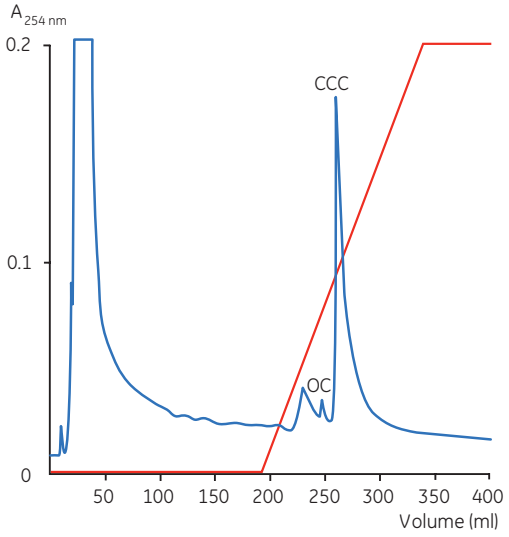


Fig 4. Purification of supercoiled plasmid DNA on HiLoad 16/10 Q Sepharose High Performance.

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 5A and B 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 5 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 16/60 or 26/60 Superdex™ 30 prep grade, 75 prep grade, or 200 prep grade.

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 HiLoad columns.

Figure 6 shows the scale-up of a standard protein mixture on different sizes of HiTrap (1 ml and 5 ml) and HiLoad (20 ml and 53 ml) columns. Flow rate (ml/min) was scaled according to the ratio between the cross-sectional areas of the columns, and gradient length and sample loading according to the ratio between the column volumes.

The reproducibility of the separation result confirms how easy this scale-up strategy is to apply in practice.

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.

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

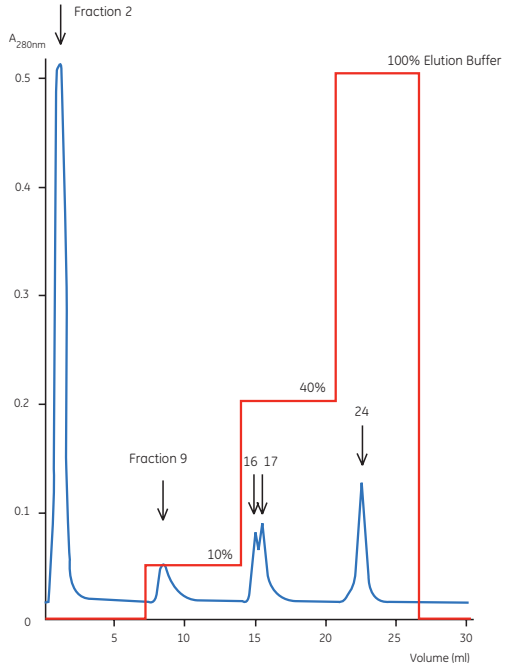
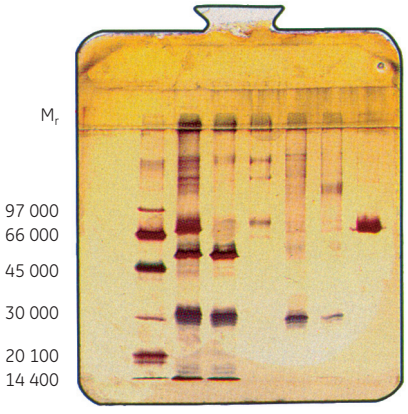


Fig 5A. Group separation of proteins from casein-precipitated human milk on HiTrap SP HP 1 ml.



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

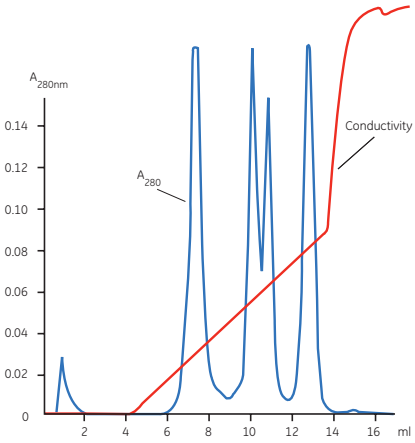
Fig 5B. SDS electrophoresis (PhastSystem™, PhastGel™ 10–15, silver staining) on fractions from the group separation of casein-precipitated human milk on HiTrap SP HP 1 ml shown in Fig. 5A.

Table 5. 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

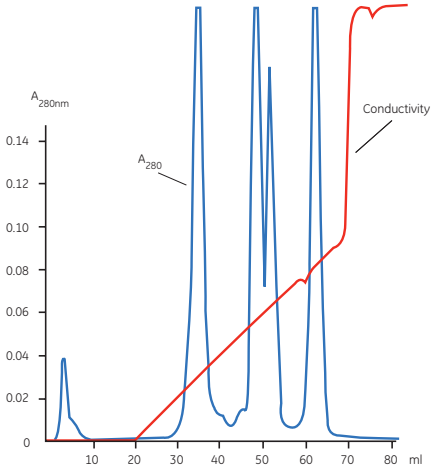
HiTrap SP HP, 1 ml

Sample: Concanavalin A, ribonuclease A, α-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: –43% elution buffer over 10 ml (10 column volumes)



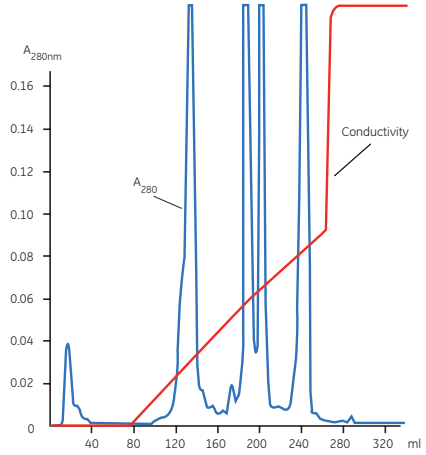
HiTrap SP HP, 5 ml

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: 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: 0–43% elution buffer over 50 ml (10 column volumes)



HiLoad 16/10 SP Sepharose High Performance

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: 0–43% elution buffer over 200 ml (10 column volumes)



HiLoad 26/10 SP Sepharose High Performance

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: 13.25 ml, 25% of column volume
Column volume: 53 ml
Flow rate: 6.6 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: 0–43% elution buffer over 530 ml (10 column volumes)

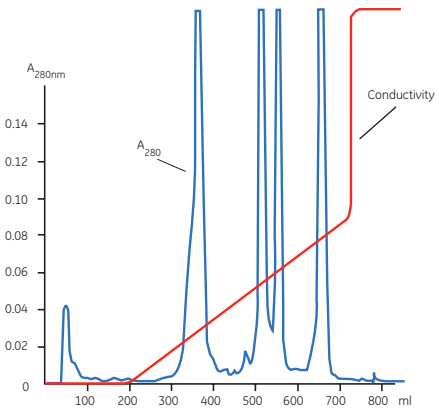


Fig 6. Comparing separations on HiTrap SP HP 1 ml and 5 ml and HiLoad 16/10 and 26/10 SP Sepharose High Performance columns confirms the predictable nature of the results and the ease with which separations can be scaled up.

Ordering information

Products	Quantity	Code no.
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

HiLoad 16/10 Q Sepharose High Performance	1 (20 ml)	17-1064-01
HiLoad 26/10 Q Sepharose High Performance	1 (53 ml)	17-1066-01
HiLoad 16/10 SP Sepharose High Performance	1 (20 ml)	17-1137-01
HiLoad 26/10 SP Sepharose High Performance	1 (53 ml)	17-1138-01
HiTrap Q HP	5 x 1 ml	17-1153-01
HiTrap Q HP	5 x 5 ml	17-1154-01
HiTrap SP HP	5 x 1 ml	17-1151-01
HiTrap SP HP	5 x 5 ml	17-1152-01
HiTrap IEX Selection Kit	7 x 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

HiLoad accessories	No. Supplied	Code no.
To connect HiLoad columns to ÄKTAdesign		
Union M6 female/1/16" male*	5	18-3858-01
Transport syringe	1	18-1017-61
Domed nut	4	18-2450-01

* Two unions are included in the HiLoad package

HiTrap accessories

1/16" male/luer female[1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap package

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

[3] One fingertight stop plug is connected to the top of each HiTrap column at delivery

www.gehealthcare.com/protein-purification
www.gehealthcare.com/hitrap
www.gehealthcare.com

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

GE Healthcare Bio-Sciences AB, a General Electric Company.

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

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

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

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

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

Asia Pacific Tel +65 6275 1830 Fax +65 6275 1829 • Australasia Tel +61 2 9899 0999 Fax +61 2 9899 7511 • Austria Tel 01/57606-1619 Fax 01/57606-1627 • Belgium Tel 0800 73 888 Fax 02 416 82 06 Canada Tel 800 463 5800 Fax 800 567 1008 • Central, East, & South East Europe Tel +43 1 972720 Fax +43 1 97272 2750 • Denmark Tel 45 16 2400 Fax 45 16 2424 • Finland & Baltics Tel +358 (0)9 512 39 40 Fax +358 (0)9 512 39 439 • France Tel 01 69 35 67 00 Fax 01 69 41 96 77 Germany Tel 089 96281 660 Fax 089 96281 620 • Greater China Tel +852 2100 6300 Fax +852 2100 6338 • Italy Tel 02 27322 1 Fax 02 27302 212 • Japan Tel +81 3 5331 9336 Fax +81 3 5331 9370 • Latin America Tel +55 11 3933 7300 Fax +55 11 3933 7304 Middle East & Africa Tel +30 210 9600 687 Fax +30 210 9600 693 • Netherlands Tel 0800 82 82 82 1 Fax 0800 82 82 82 4 • Norway Tel 815 65 555 Fax 815 65 666 • Portugal Tel 21 417 7035 Fax 21 417 3184 • Russia & other C.I.S. & N.I.S Tel +7 (495) 956 5177 Fax +7 (495) 956 5176 • Spain Tel 93 594 49 50 Fax 93 594 49 55 • Sweden Tel 018 612 1900 Fax 018 612 1910 • Switzerland Tel 0848 8028 12 Fax 0848 8028 13 • UK Tel 0800 616928 Fax 0800 616927 • USA Tel 800 526 3593 Fax 877 295 8102

imagination at work

18-1172-88 AB 04/2006