Q **Sepharose** High Performance
SP **Sepharose** High Performance

ION EXCHANGE CHROMATOGRAPHY

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Q Sepharose™ High Performance and SP Sepharose High Performance enjoy well-deserved reputations as highly successful anion and cation ion exchange resins 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 cleaning in place (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 Cytiva's 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).



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

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.

Charged molecules bind to the separation resin 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, and so on.

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.

Resin characteristics

Q Sepharose High Performance and SP Sepharose High Performance are high-resolution anion and cation exchange resins based on 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 resin 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 resins remain charged and have high loading capacities over broad pH ranges.

Table 1 lists the main resin characteristics.

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

Matrix	Cross-linked agarose, spherical
Particle size, d_{50V}[1]	~ 34 µm
Functional groups	$-CH_2N^+(CH_3)_3$, quaternary ammonium (Q) $-CH_2CH_2CH_2SO_3^-$, sulphopropyl (SP)
Ionic capacity	0.14 to 0.20 mmol (Cl-)/mL medium (Q) 0.15 to 0.20 mmol (H+)/mL medium (SP)
Dynamic binding capacity	~ 70 mg BSA/mL resin (Q) ~ 55 ribonuclease A/mL resin (SP)
Exclusion limit [Mr] Globular proteins[2]	~ 1 x 10^7
Recommended maximum operating flow velocity (large scale)[3]	90 cm/h
Chemical stability	Stable to commonly used aqueous buffers; 1.0 M NaOH[4], 1.0 M acetic acid[4], M urea, 6 M guanidine hydrochloride, 30% isopropanol, 70% ethanol
pH stability, operational[5]	2 to 12 (Q) 4 to 13 (SP)
pH stability, CIP[6]	2 to 14 (Q) 3 to 14 (SP)
pH ligand fully charged[7]	Entire pH range
Storage	20% ethanol, 4° to 30°C (Q) 20% ethanol with 0.2 M sodium acetate, 4° to 30°C (SP)

[1] Median particle size of the cumulative volume distribution
[2] Tested on the base matrix
[3] In a 10L ReadyToProcess column with 25 cm diameter and 20 cm bed height using buffer with the same viscosity as water at 20°C
[4] Should only be used for cleaning purposes
[5] pH range where resin can be operated without significant change in function
[6] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function
[7] pH range where ligand is fully charged; although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges

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™ systems include 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 resins 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 systems. 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 18112763 AD page 2, table 2 for more info.
* Water at room temperature

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HiScreen Q and SP HP columns

Q and SP Sepharose High Performance resins 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 resin 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 resin 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)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)
Storage	20% ethanol, 4° to 30°C (Q) 20% ethanol, 0.2 M sodium acetate, 4° to 30°C (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. 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 or FPLC system, operation is straightforward.

Related resins available in HiTrap IEX Selection Kit

Although it does not include Q or SP Sepharose High Performance resins, 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 resins 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, see data file HiTrap IEX Selection Kit, 18114048.

(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.

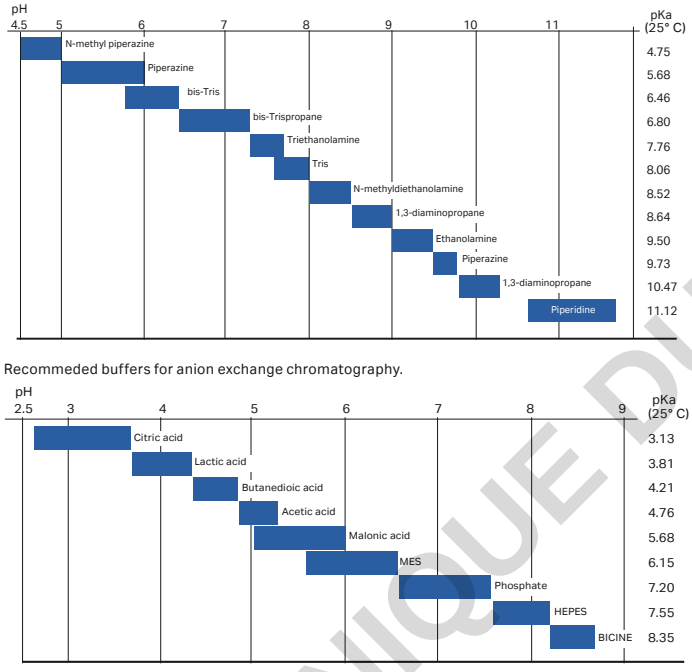
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Chemical stability

Good chemical stability allows the use of effective 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 resins 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/CO_2
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 resins a natural choice for preparative separations during the intermediate and final steps of a purification scheme. In addition, ÄKTA systems 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 size exclusion chromatography 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 size exclusion chromatography columns, for example, prepacked HiLoad™ 16/600 or 26/600 Superdex™ 30 prep grade, 75 prep grade, or 200 prep grade.

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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

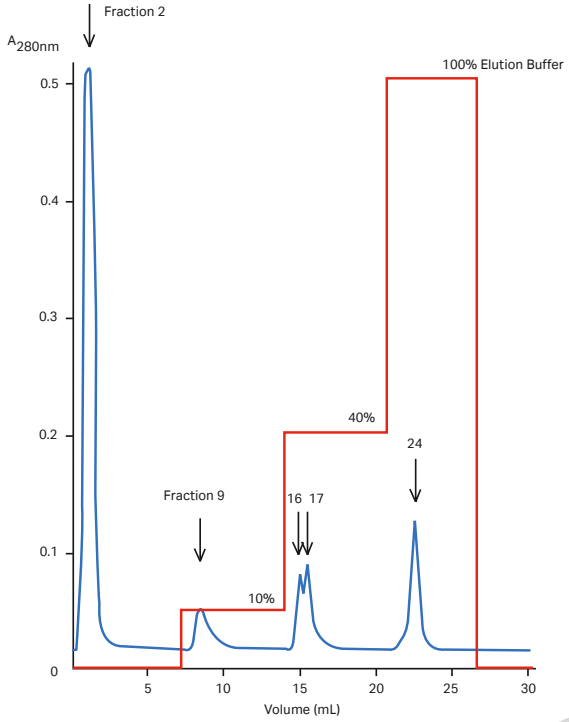
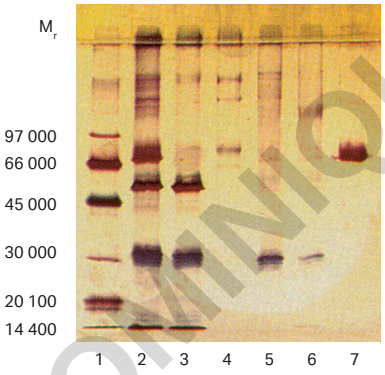


Fig 4. Group separation of proteins from casein-precipitated human milk on HiTrap SP HP 1 mL.



Lanes

1. LMW markers
2. Start material, casein-precipitated human milk, diluted 5x
3. Fraction 2, diluted 2x
4. Fraction 9
5. Fraction 16
6. Fraction 17
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 resins 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 Cytiva.

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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, α-chymotrypsinogen A, lysozyme, 4 mg protein/mL (3:3:1:1) in start buffer
Sample load:	1 mg protein/mL resin
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 resin
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, α-chymotrypsinogen A, lysozyme, 4 mg protein/mL (3:3:1:1) in start buffer
Sample load:	1 mg protein/mL resin
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)

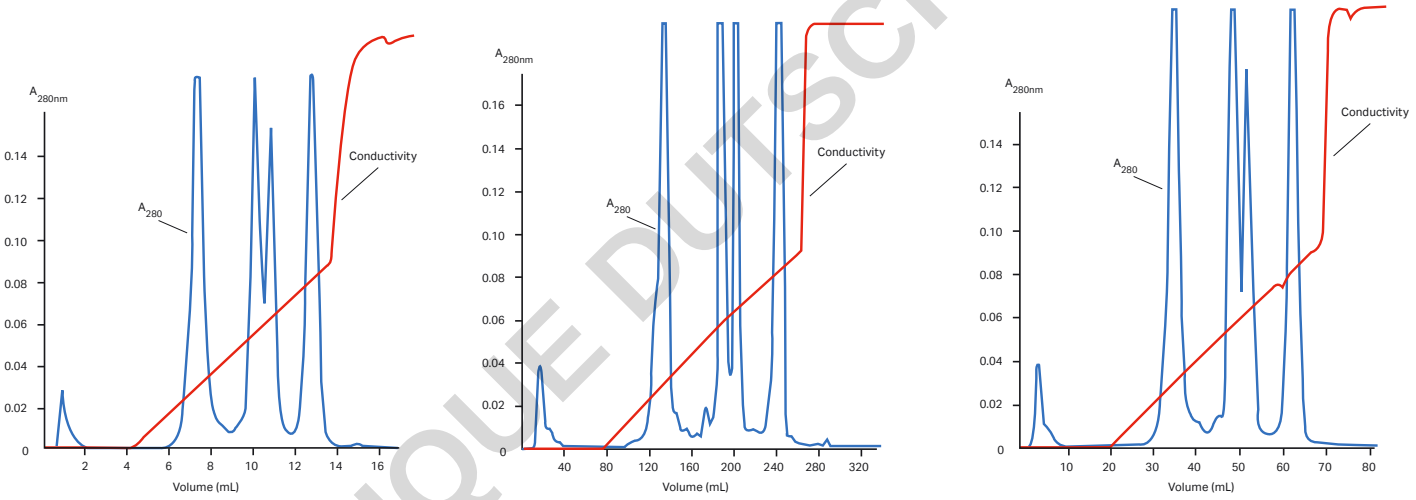


Fig 6. Comparing separations on HiTrap SP HP 1 mL and 5 mL and HiPrep SP HP 16/10 columns confirms the predictable nature of the results and the ease with which separations can be scaled up.

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Ordering information

Products	Quantity	Code number
Q Sepharose High Performance	75 mL	17101401
	1 L	17101403
	5 L	17101404
	10 L	17101405
SP Sepharose High Performance	75 mL	17108701
	1 L	17108703
	5 L	17108704
	10 L	17108705
	60 L	17108708
Prepacked columns		
HiPrep Q HP 16/10	1 (20 mL)	29018182
HiPrep SP HP 16/10	1 (20 mL)	29018183
HiScreen Q HP	1 × 4.7 mL	28950511
HiScreen SP HP	1 × 4.7 mL	28950515
HiTrap Q HP	1 × 1 mL	29051325
	5 × 1 mL	17115301
	5 × 5 mL	17115401
HiTrap SP HP	1 × 1 mL	29051324
	5 × 1 mL	17115101
	5 × 5 mL	17115201
HiTrap IEX Selection Kit	7 × 1 mL	17600233
Related products		
Ion exchange chromatography Handbook, Principles and methods		11000421
Ion exchange columns and media, Product Profile		18112731
HiTrap IEX Selection Kit		18114048
Accessories		
1/16" male/luer female*	2	18111251
Tubing connector flangeless/ M6 female	2	18100368
Tubing connector flangeless/ M6 male	2	18101798
Union 1/16" female/M6 male	6	18111257
Union M6 female /1/16" male	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

* One connector included in each HiTrap package
† Two, five, or seven stop plugs female included in HiTrap packages depending on products
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery

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