HiTrap™ IEX Selection Kit

HiTrap IEX Selection Kit offers a fast, simple, and convenient way to decide which ion exchanger or ion exchange ligand is best for a given application. The kit consists of seven different ion exchange media based on Sepharose™ Fast Flow and Sepharose XL. The media are prepacked in ready-to-use HiTrap 1 ml columns (Fig 1). After choosing the optimal ion exchange medium, prepacked columns and bulk media are available for larger scale preparative work.

Separations are easily performed by simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems.

The columns included in the kit are also available as individual HiTrap 1 ml and 5 ml columns.

HiTrap IEX Selection Kit offers:

- Fast and easy screening with seven different ion exchange media
- Convenient use
- Simple operation
- Easy scale-up

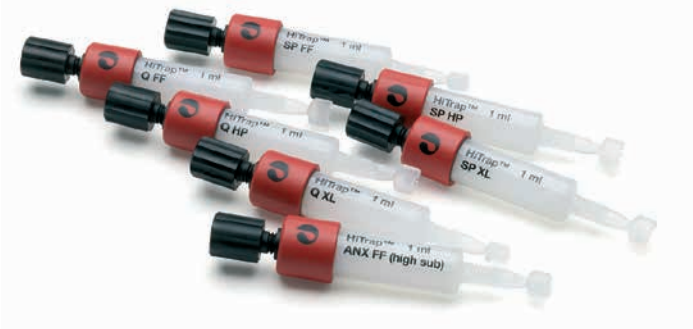

Fig 1. HiTrap IEX Selection Kit.

Media characteristics

The media packed in HiTrap columns are 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.

SP Sepharose Fast Flow, Q Sepharose Fast Flow, DEAE Sepharose Fast Flow, and CM Sepharose Fast Flow are based on a robust, 6% highly cross-linked beaded agarose matrix with good flow properties and high loading capacities.

ANX Sepharose 4 Fast Flow (high sub) is based on a 4% highly cross-linked beaded agarose matrix. This results in a medium with higher porosity, which is particularly useful for the purification of high molecular mass proteins.

SP Sepharose XL and Q Sepharose XL media have long chains of dextran coupled to a robust, 6% highly cross-linked agarose matrix. The dextran chains increase the exposure of the SP or Q charged groups, which results in higher loading capacity in some applications. The functional groups are coupled to the matrices via chemically stable ether linkages. The charged groups are shown in Table 1. The media and the columns display high chemical stability – see Tables 1 and 2.

The flow characteristics of Sepharose Fast Flow and Sepharose XL make these ion exchangers a good choice for separation early in purification schemes. Purification can be scaled up using prepacked columns containing the same media. Larger pack sizes for scale-up to production scale are also available. Full technical and regulatory support for production-scale applications is available for all the ion exchangers described.

Column characteristics

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. The top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Characteristics of HiTrap columns are listed in Table 2.

Table 1. Characteristics of Sepharose Fast Flow and Sepharose XL ion exchangers

Cation exchangers

Property	SP Sepharose Fast Flow	SP Sepharose XL	CM Sepharose Fast Flow
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose with bound dextran	6% highly cross-linked agarose
Mean particle size	45 to 165 µm	45 to 165 µm	45 to 165 µm
Type of medium	Strong cation	Strong cation	Weak cation
Charged group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2CH_2CH_2SO_3^-$	$-O-CH_2COO^-$
Total ionic capacity	0.18 to 0.25 mmol H^+/ml medium	0.18 to 0.25 mmol H^+/ml medium	0.09 to 0.13 mmol H^+/ml medium
Dynamic binding capacity[1]	70 mg ribonuclease A/ml medium	> 160 mg lysozyme/ml medium	50 mg ribonuclease A/ml medium
pH stability:			
Cleaning[2]	3 to 14	3 to 14	2 to 14
Working[3]	4 to 13	3 to 13	4 to 13
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage buffer	supplied in 0.2 M sodium acetate in 20% ethanol	supplied in 0.2 M sodium acetate in 20% ethanol	supplied in 20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		
Avoid	Oxidizing agents, cationic detergents, and buffers		

Anion exchangers

Property	Q Sepharose Fast Flow	Q Sepharose XL	DEAE Sepharose Fast Flow	ANX Sepharose 4 Fast Flow (high sub)
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose with bound dextran	6% highly cross-linked agarose	4% highly cross-linked agarose
Mean particle size	45 to 165 µm	45 to 165 µm	45 to 165 µm	45 to 165 µm
Type of medium	Strong anion	Strong anion	Weak anion	Weak anion
Charged group	$-N^+(CH_3)_3$	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$*	$-N^+(C_2H_5)_2H$*
Total ionic capacity	0.18 to 0.25 mmol Cl^-/ml medium	0.18 to 0.26 mmol Cl^-/ml medium	0.11 to 0.16 mmol Cl^-/ml medium	0.13 to 0.17 mmol Cl^-/ml medium
Dynamic binding capacity[1]	120 mg HSA/ml medium	> 130 mg BSA/ml medium	110 mg HSA/ml medium	43 mg BSA/ml medium
pH stability:				
Cleaning[2]	1 to 14	2 to 14	1 to 14	2 to 14
Working[3]	2 to 12	3 to 13	2 to 12	3 to 13
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage buffer	supplied in 20% ethanol	supplied in 20% ethanol	supplied in 20% ethanol	supplied in 20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol			
Avoid	Oxidizing agents, anionic detergents, and buffers			

[1] Determination of dynamic binding capacity:

DEAE Sepharose Fast Flow, Q Sepharose Fast Flow, SP Sepharose Fast Flow and CM Sepharose Fast Flow: Samples were applied at 75 cm/h until 50% breakthrough. Column: 0.5 × 5 cm. Buffers: 0.05 M Tris, (2 M NaCl in the elution buffer), pH 7.5 (Q and DEAE) or 0.1 M acetate, (2 M NaCl in the elution buffer), pH 5.0 (SP and CM).
Q Sepharose XL and SP Sepharose XL: Samples were applied at 300 cm/h until 10% breakthrough. Column: 0.75 × 10 cm. Buffers: 0.05 M Tris, (0.5 M NaCl in the elution buffer), pH 7.5 (Q) or 0.05 M glycine, (0.5 M NaCl in the elution buffer), pH 9.0 (SP).
ANX Sepharose 4 Fast Flow (high sub): Sample was applied at 300 cm/h until 10% breakthrough. Column: 1.6 × 13 cm. Buffer: 0.05 M Tris, (1 M NaCl in the elution buffer), pH 7.5.

[2] Refers to the pH interval for regeneration and cleaning.

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

* **Note:** The active end of the charged group is the same for DEAE Sepharose Fast Flow and ANX Sepharose 4 Fast Flow (high sub). The difference is the length of the carbon chain of the charged group. DEAE Sepharose Fast Flow has a diethylaminoethyl-group bound to the agarose whilst ANX Sepharose 4 Fast Flow (high sub) has a diethylaminopropyl-group attached.

Table 2. Characteristics of HiTrap columns

Property	
Column volumes	1 ml or 5 ml
Column dimensions	HiTrap 1 ml: 0.7 × 2.5 cm HiTrap 5 ml: 1.6 × 2.5 cm
Maximum flow rates	HiTrap 1 ml: 4 ml/min HiTrap 5 ml: 20 ml/min
Recommended flow rates	HiTrap 1 ml: 1 ml/min HiTrap 5 ml: 5 ml/min
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Chemical stability	All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol

(A)

(B)

(C)

Fig 2. Using a HiTrap column with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. (B) Load the sample and begin collecting fractions. (C) Wash, elute, and continue collecting fractions.

Operation

Screening for the optimal ion exchange ligand is an important factor when optimizing a separation method. This can easily be performed using the prepacked Sepharose Fast Flow and Sepharose XL media in HiTrap IEX Selection Kit.

Complete, easy-to-follow instructions are included for fast start-up and method optimization. Operation is easy, use a syringe and the provided Luer adapter (Fig 2), a peristaltic pump, or a chromatography system such as an ÄKTA system.

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

Applications

The separations on the next page illustrate the difference in chromatographic performance for the ion exchange media included in HiTrap IEX Selection Kit. Model proteins were used to show the different binding properties of the ion exchange ligands under identical conditions (Figs 3 and 4).

Clarified lysate from a strain of wild-type *E. coli*, grown at 37°C, was used as a source for capturing alkaline phosphatase in a natural environment. This sample was used to compare the different anion exchange ligands and illustrates the small, but significant difference in retention time for the alkaline phosphatase (Figs 5 to 8).

All purifications were done using an ÄKTAexplorer 100 and after sample application the columns were washed and eluted with a linear gradient.

The separations were monitored by measuring the conductivity and absorbance at 280 nm. The phosphatase activity in the fractions from the *E. coli* lysate were also assayed by a spectrophotometric method at 405 nm.

Further optimization

HiTrap IEX Selection Kit is also an excellent aid in more detailed optimization studies. The effects of buffer composition, pH, flow rate, sample loading, and elution scheme can be studied with small quantities of sample before proceeding to the working scale.

More information regarding optimization can be found in the handbook, Ion Exchange Chromatography and Chromatofocusing: Principles and Methods; see ordering information or visit www.gelifesciences.com/protein-purification.

Scale-up

For quick scale-up of purification, two or three HiTrap ion exchange columns can easily be connected in series.[1] All HiTrap columns in HiTrap IEX Selection Kit are available as individual 1 ml and 5 ml columns.

For further scale-up, prepacked HiPrep™ 16/10 columns are available. The different ion exchange media are also available in lab packs and process-scale quantities; see ordering information.

[1] Connecting columns in series increases backpressure.

Storage

HiTrap Q FF, HiTrap DEAE FF, HiTrap ANX FF (high sub) and HiTrap Q XL are delivered in 20% ethanol. HiTrap SP FF and HiTrap SP XL are delivered in 20% ethanol, 0.2 M sodium acetate.

Columns:	HiTrap DEAE FF, 1 ml HiTrap Q FF, 1 ml HiTrap Q XL, 1 ml HiTrap ANX FF (high sub), 1 ml
Sample:	0.4 mg conalbumin (pI = 6.3), 0.8 mg α-lactoglobulin (pI = 5.8), 1.2 mg soya bean trypsin inhibitor (pI = 4.5) dissolved in 2 ml start buffer
Start buffer:	20 mM Tris-HCl, pH 7.4
Elution buffer:	20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
Flow rate:	1 ml/min (150 cm/h)
Running parameters:	Equilibration: 20 ml start buffer Sample application: 2 ml Wash: 5 ml start buffer Elution 40 ml, linear gradient, 0% to 80% elution buffer
System:	ÄKTAexplorer 100

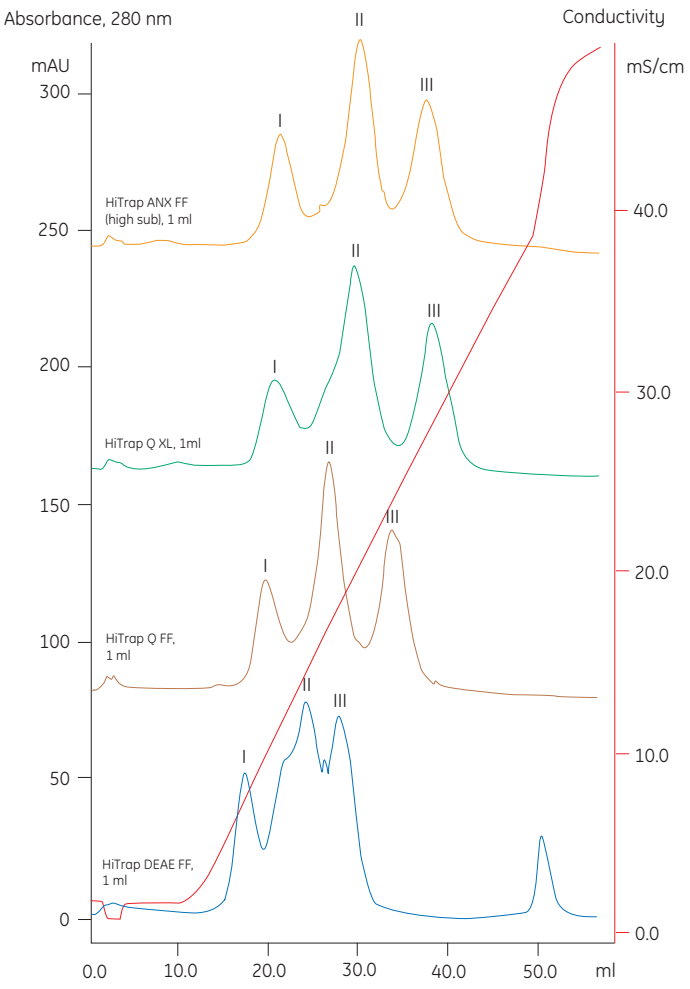

Fig 3. Separation of conalbumin (I), α-lactoglobulin (II) and soya bean trypsin inhibitor (III) on HiTrap DEAE FF 1 ml, HiTrap Q FF 1 ml, HiTrap Q XL 1 ml, and HiTrap ANX FF (high sub) 1 ml.

Columns:	HiTrap CM FF, 1 ml HiTrap SP FF, 1 ml HiTrap SP XL, 1 ml
Sample:	3 mg ribonuclease A (pI = 9.3), 0.8 mg cytochrome C (pI = 10.3), 0.8 mg lysozyme (pI > 11) dissolved in 2 ml start buffer
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, pH 6.8
Flow:	1 ml/min (150 cm/h)
Running parameters:	Equilibration: 20 ml start buffer Sample application: 2 ml Wash: 5 ml start buffer Elution: 40 ml, linear gradient, 0% to 100% elution buffer
System:	ÄKTAexplorer 100

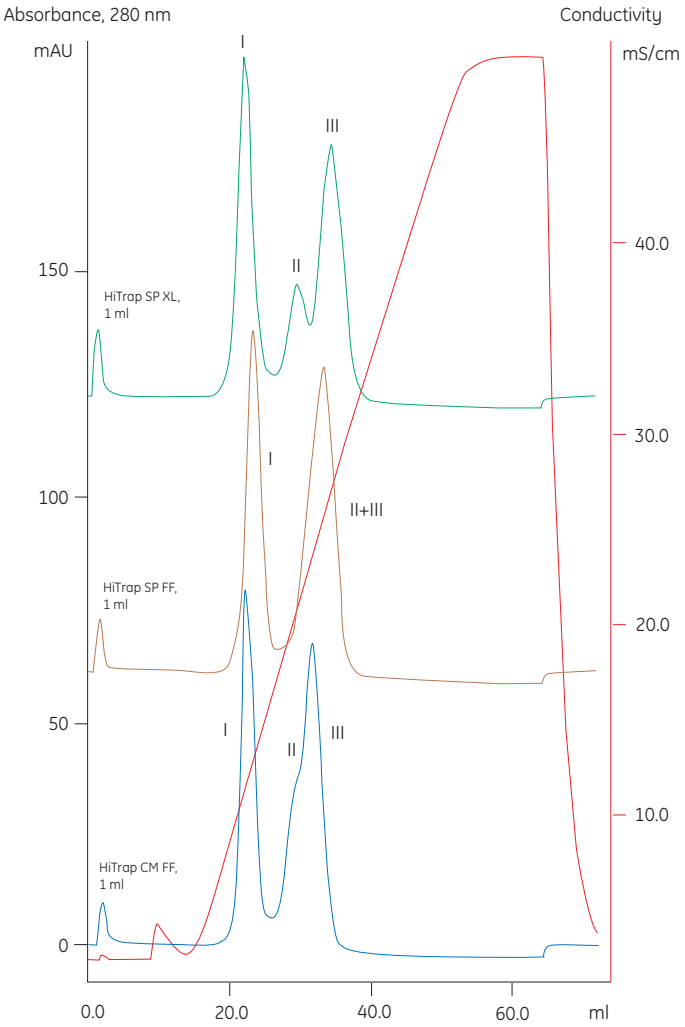

Fig 4. Separation of ribonuclease A (I), cytochrome C (II) and lysozyme (III) on HiTrap CM FF 1 ml, HiTrap SP FF 1 ml, and HiTrap SP XL 1 ml.

Columns:	HiTrap DEAE FF, 1 ml HiTrap Q FF, 1 ml HiTrap Q XL, 1 ml HiTrap ANX FF (high sub), 1 ml
Sample:	2 ml *E. coli* lysate clarified by centrifugation
Start buffer:	20 mM Tris-HCl, pH 7.4
Elution buffer:	20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
Flow rate:	1 ml/min (150 cm/h)
Running parameters:	Equilibration: 20 ml start buffer Sample application: 2 ml Wash: 10 ml start buffer Elution: 40 ml, linear gradient, 0% to 100% elution buffer
System:	ÄKTAexplorer 100
Analysis:	Alkaline Phosphatase Assay: 75 µl sample + 100 µl substrate, SIGMA*FAST*™ pNPP substrate tablet set N-2770, prepared according to the manufacturer's instructions. Blank: 75 µl water + 100 µl substrate, incubated in dark at room temperature for 2 h before reading the absorbance at 405 nm

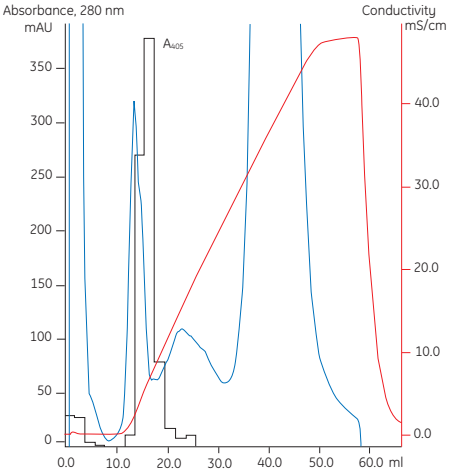

Fig 5. Clarified *E. coli* lysate on HiTrap DEAE FF 1 ml.

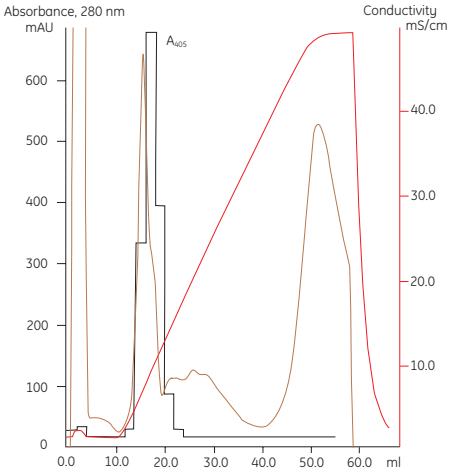

Fig 6. Clarified *E. coli* lysate on HiTrap Q FF 1 ml.

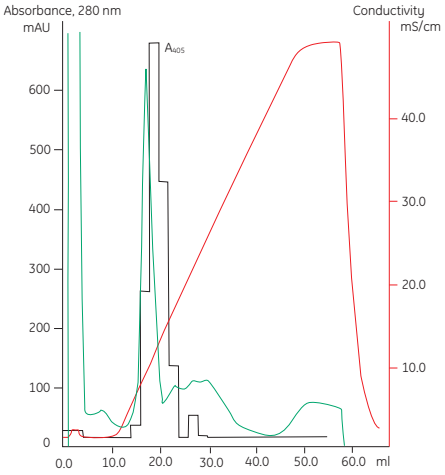

Fig 7. Clarified *E. coli* lysate on HiTrap Q XL 1 ml.

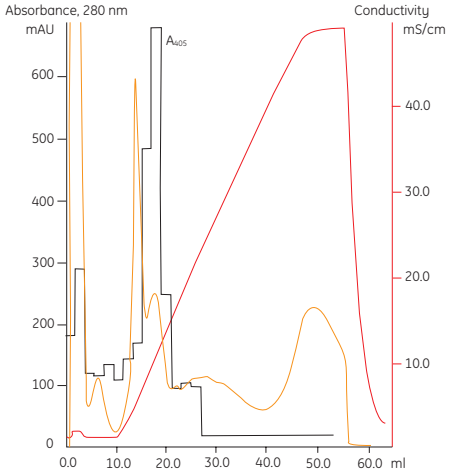

Fig 8. Clarified *E. coli* lysate on HiTrap ANX FF (high sub) 1 ml.

Ordering information

Product	Quantity	Code number
HiTrap IEX Selection Kit	7 × 1 ml	17-6002-33
Related products	**Quantity**	**Code number**
HiTrap Q FF	5 × 1 ml	17-5053-01
HiTrap Q FF	5 × 5 ml	17-5156-01
HiTrap SP FF	5 × 1 ml	17-5054-01
HiTrap SP FF	5 × 5 ml	17-5157-01
HiTrap DEAE FF	5 × 1 ml	17-5055-01
HiTrap DEAE FF	5 × 5 ml	17-5154-01
HiTrap CM FF	5 × 1 ml	17-5056-01
HiTrap CM FF	5 × 5 ml	17-5155-01
HiTrap Q XL[1]	5 × 1 ml	17-5158-01
HiTrap Q XL[1]	5 × 5 ml	17-5159-01
HiTrap SP XL	5 × 1 ml	17-5160-01
HiTrap SP XL	5 × 5 ml	17-5161-01
HiTrap ANX FF (high sub)	5 × 1 ml	17-5162-01
HiTrap ANX FF (high sub)	5 × 5 ml	17-5163-01
HiTrap SP HP	1 × 1 ml	29-0513-24
HiTrap SP HP	5 × 1 ml	17-1151-01
HiTrap SP HP	5 × 5 ml	17-1152-01
HiTrap Q HP	1 × 1 ml	29-0513-25
HiTrap Q HP	5 × 1 ml	17-1153-01
HiTrap Q HP	5 × 5 ml	17-1154-01
Q Sepharose Fast Flow	25 ml	17-0510-10
Q Sepharose Fast Flow[2]	300 ml	17-1510-01
SP Sepharose Fast Flow	25 ml	17-0729-10
SP Sepharose Fast Flow[2]	300 ml	17-0729-01
DEAE Sepharose Fast Flow	25 ml	17-0709-10
DEAE Sepharose Fast Flow[2]	500 ml	17-0709-01
CM Sepharose Fast Flow	25 ml	17-0719-10
CM Sepharose Fast Flow[2]	500 ml	17-0719-01
ANX Sepharose 4 Fast Flow (high sub)	25 ml	17-1287-10
ANX Sepharose 4 Fast Flow (high sub)[2]	500 ml	17-1287-01
Q Sepharose XL[1,2]	300 ml	17-5072-01
SP Sepharose XL[2]	300 ml	17-5073-01
Q Sepharose XL virus licensed	25 ml	17-5437-10
Q Sepharose XL virus licensed	300 ml	17-5437-01

Related products	Quantity	Code number
Q Sepharose High Performance[2]	75 ml	17-1014-01
SP Sepharose High Performance[2]	75 ml	17-1087-01
HiLoad™ 16/10 Q Sepharose HP	1 × 20 ml	17-1064-01
HiLoad 26/10 Q Sepharose HP	1 × 53 ml	17-1066-01
HiLoad 16/10 SP Sepharose HP	1 × 20 ml	17-1137-01
HiLoad 26/10 SP Sepharose HP	1 × 53 ml	17-1138-01
HiPrep 16/10 DEAE FF	1 × 20 ml	17-5090-01
HiPrep 16/10 CM FF	1 × 20 ml	17-5091-01
HiPrep 16/10 Q XL[1]	1 × 20 ml	17-5092-01
HiPrep 16/10 SP XL	1 × 20 ml	17-5093-01
HiTrap Desalting	1 × 5 ml	29-0486-84
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/120 Desalting	4 × 53 ml	17-5087-02

[1] May require a license; see legal information on this page.

[2] Process-scale quantities are available. Please contact your local representative.

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"†	2	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector is 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.

Related literature	Code number
Convenient Protein Purification, HiTrap Column Guide	18-1129-81
Ion Exchange Chromatography and Chromatofocusing Handbook: Principles and Methods	11-0004-21

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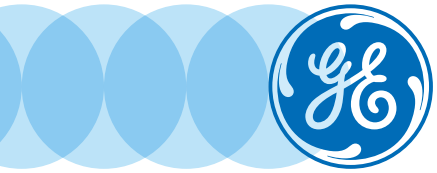

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