Ion exchange chromatography

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 resins based on Sepharose™ Fast Flow and Sepharose XL. The resins are prepacked in ready-to-use HiTrap 1 mL columns (Fig 1). After choosing the optimal ion exchange resin, prepacked columns and bulk resins 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 resins
- Convenient use
- Simple operation
- Easy scale-up

Resin characteristics

The resins 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 resin with higher porosity, which is particularly useful for the purification of high molecular mass proteins.

Fig 1. HiTrap IEX Selection Kit.

SP Sepharose XL and Q Sepharose XL resins 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 resins and the columns display high chemical stability (Table 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 resin. 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	Cross-linked agarose, 6%, spherical	cross-linked agarose, with dextran surface extender, spherical	Cross-linked agarose, 6%, spherical
Particle size, d_{50V}[1]	~ 90 µm	~ 90 µm	~ 90 µm
Type of resin	Strong cation	Strong cation	Weak cation
Charged group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2CH_2CH_2SO_3^-$	$-O-CH_2COO^-$
Ionic capacity	0.18 to 0.25 mmol H^+/mL resin	0.18 to 0.25 mmol H^+/mL resin	0.09 to 0.13 mmol H^+/mL resin
Dynamic binding capacity	~ 70 mg ribonuclease A/ mL resin[2]	≥ 160 mg Lysozyme/ mL resin[3]	~ 50 mg ribonuclease A/ mL resin[2]
pH stability, operational[4]	4 to 13	4 to 13	4 to 13
pH stability, CIP[5]	3 to 14	3 to 14	2 to 14
pH ligand fully charged[6]	Entire pH range	Entire pH range	Above 6
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		
Avoid	Oxidizing agents, cationic detergents and buffers		
Storage	0.2 M sodium acetate in 20% ethanol, 4 to 30˚C	0.2 M sodium acetate in 20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C

Anion exchangers

Property	Q Sepharose Fast Flow	Q Sepharose XL	DEAE Sepharose Fast Flow	ANX Sepharose Fast Flow (high sub)
Matrix	Cross-linked agarose, 6%, spherical	cross-linked agarose, with dextran surface extender, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 4%, spherical
Particle size, d_{50V}[1]	~ 90 µm	~ 90 µm	~ 90 µm	~ 90 µm
Type of resin	Strong anion	Strong anion	Weak anion	Weak anion
Charged group	$-N^+(CH_3)_3$	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$*	$-N^+(C_2H_5)_2H$*
Ionic capacity	0.18 to 0.24 mmol Cl^-/ mL resin	0.18 to 0.26 mmol Cl^-/ mL resin	0.11 to 0.16 mmol Cl^-/ mL resin	0.13 to 0.18 mmol Cl^-/ mL resin
Dynamic binding capacity	~ 42 mg BSA/mL resin[7]	≥ 160 mg BSA/mL resin[8]	~ 110 mg HAS/mL resin[2]	~ 43 mg BSA /mL resin[9]
pH stability, operational4	2 to 12	2 to 12	2 to 12	3 to 13
pH stability, CIP5	2 to 14	2 to 14	2 to 14	2 to 14
pH ligand fully charged[6]	Entire pH range	Entire pH range	Below 9	Below 9
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH7, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol			
Avoid	Oxidizing agents, anionic detergents and buffers			
Storage	20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C

[1] Median particle size of the cumulative volume distribution.

[2] Determination of dynamic binding capacity: *DEAE 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 (DEAE) or 0.1 M acetate, (2 M NaCl in the elution buffer), pH 5.0 (SP and CM).

[3] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for Lysozyme in 50 mM Glycine-NaOH, pH 9.

[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] pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

[7] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a Tricorn 5/100 at 10 cm bed height (2 min residence time) for BSA in 50mM Tris, pH 8.0.

[8] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for BSA in 50 mM Tris-HCl, pH 7.5.

[9] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a XK 16/20 column at 13 cm bed height (2.6 min residence time) for BSA in 0.05 M Tris, pH 7.5.

* 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	Stable to commonly used aqueous buffers, 1.0 M NaOH[1], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol

[1] 1.0 M NaOH should only be used for cleaning purposes

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 resins 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 resin 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 resins included in HiTrap IEX Selection Kit. Model proteins were used to show the different binding properties of the ion exchange ligands under identical conditions (Fig 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.

(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.

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 gelifesciences.com/proteinpurification.

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 HiPrepTM 16/10 columns are available. The different ion exchange resins 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 a-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

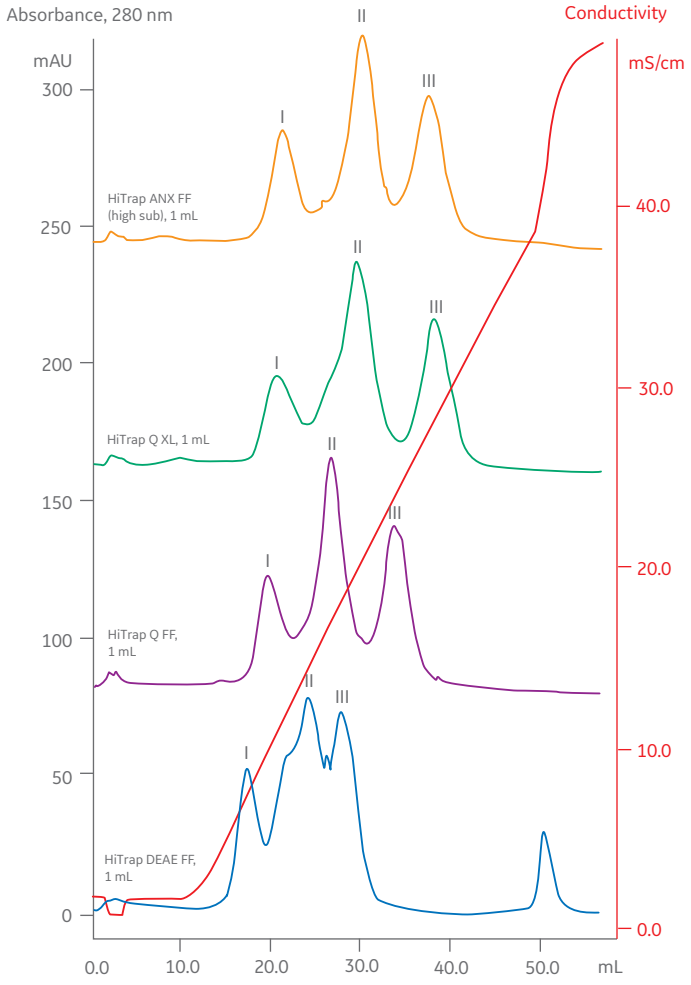

Fig 3. Separation of conalbumin (I), α-lactoglobulin (II) and soya bean trypsin inhibitor (III) on HiTrap DEAE FF 1 mL, HiTrap Q FF 1 mL, HiTrap Q XL 1 mL, and HiTrap ANX FF (high sub) 1 mL.

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

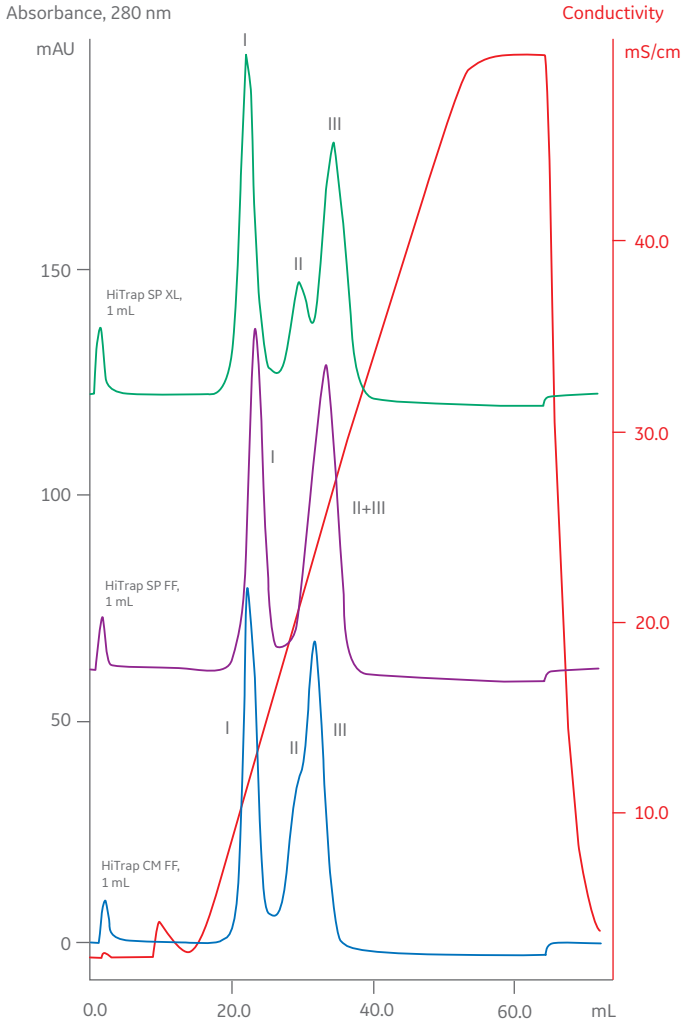

Fig 4. Separation of ribonuclease A (I), cytochrome C (II) and lysozyme (III) on HiTrap CM FF 1 mL, HiTrap SP FF 1 mL, and HiTrap SP XL 1 mL.

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, SIGMAFAST™ 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

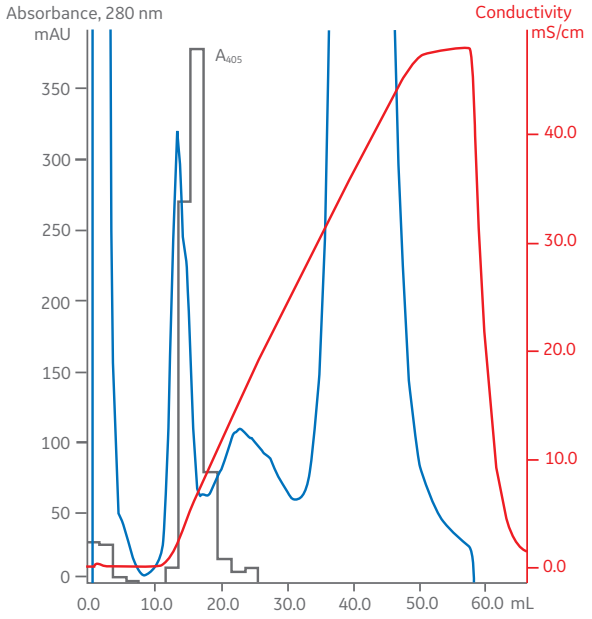

Fig 5. Clarified *E. coli* lysate on HiTrap DEAE FF 1 mL.

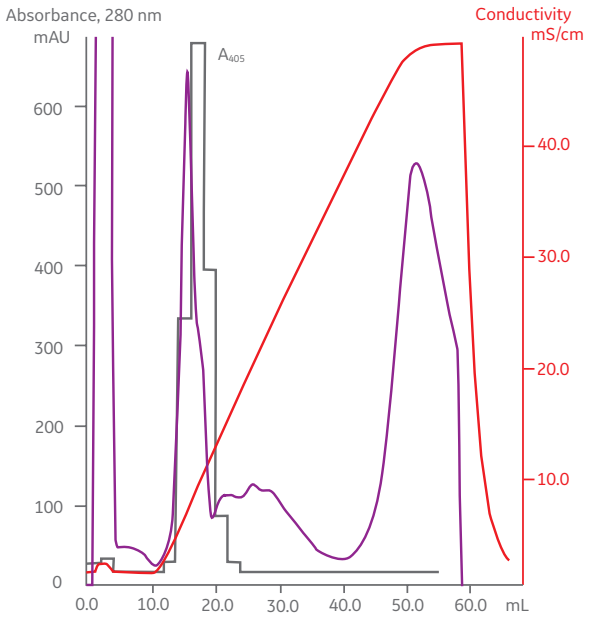

Fig 6. Clarified *E. coli* lysate on HiTrap Q FF 1 mL.

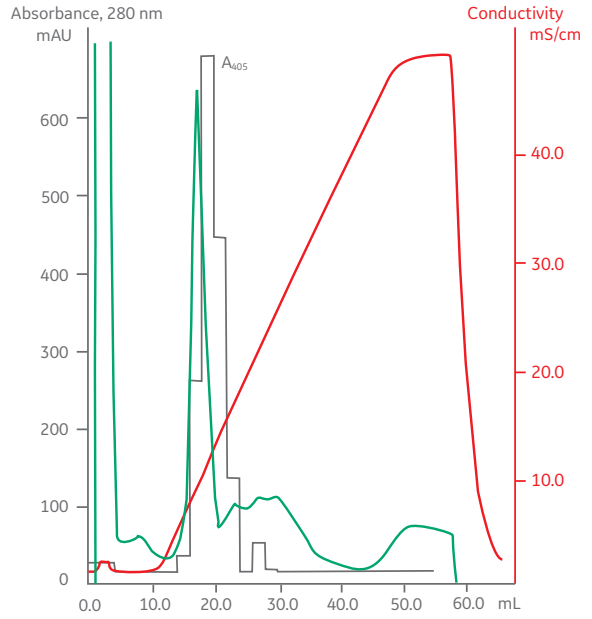

Fig 7. Clarified *E. coli* lysate on HiTrap Q XL 1 mL.

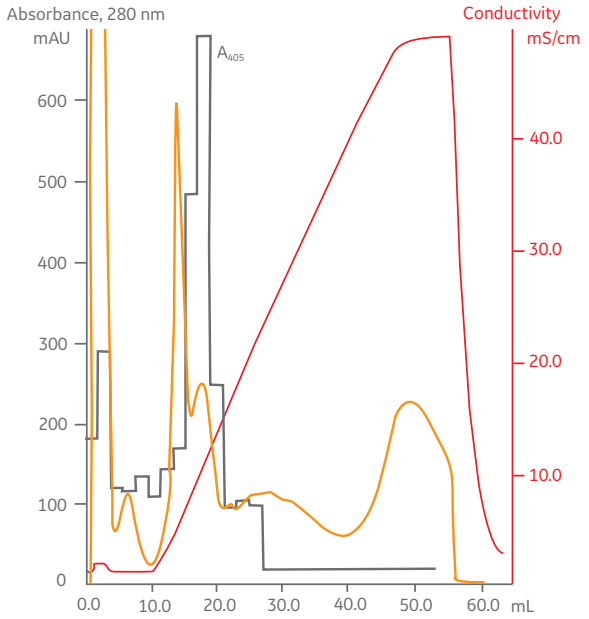

Fig 8. Clarified *E. coli* lysate on HiTrap ANX FF (high sub) 1 mL.

Ordering information

Product	Quantity	Product code
HiTrap IEX Selection Kit	7 × 1 mL	17600233

Related products

HiTrap Q FF	5 × 1 mL	17505301
HiTrap Q FF	5 × 5 mL	17515601
HiTrap SP FF	5 × 1 mL	17505401
HiTrap SP FF	5 × 5 mL	17515701
HiTrap DEAE FF	5 × 1 mL	17505501
HiTrap DEAE FF	5 × 5 mL	17515401
HiTrap CM FF	5 × 1 mL	17505601
HiTrap CM FF	5 × 5 mL	17515501
HiTrap Q XL1	5 × 1 mL	17515801
HiTrap Q XL1	5 × 5 mL	17515901
HiTrap SP XL	5 × 1 mL	17516001
HiTrap SP XL	5 × 5 mL	17516101
HiTrap ANX FF (high sub)	5 × 1 mL	17516201
HiTrap ANX FF (high sub)	5 × 5 mL	17516301
HiTrap SP HP	1 × 1 mL	29051324
HiTrap SP HP	5 × 1 mL	17115101
HiTrap SP HP	5 × 5 mL	17115201
HiTrap Q HP	1 × 1 mL	29051325
HiTrap Q HP	5 × 1 mL	17115301
HiTrap Q HP	5 × 5 mL	17115401
Q Sepharose Fast Flow	25 mL	17051010
Q Sepharose Fast Flow2	300 mL	17151001
SP Sepharose Fast Flow	25 mL	17072910
SP Sepharose Fast Flow2	300 mL	17072901
DEAE Sepharose Fast Flow	25 mL	17070910
DEAE Sepharose Fast Flow2	500 mL	17070901
CM Sepharose Fast Flow	25 mL	17071910
CM Sepharose Fast Flow2	500 mL	17071901
ANX Sepharose 4 Fast Flow (high sub)	25 mL	17128710
ANX Sepharose 4 Fast Flow (high sub)	2 500 mL	17128701
Q Sepharose XL1,2	300 mL	17507201
SP Sepharose XL2	300 mL	17507301
Q Sepharose XL virus licensed	25 mL	17543710
Q Sepharose XL virus licensed	300 mL	17543701

Related products	Quantity	Product code
Q Sepharose High Performance[2]	75 mL	17101401
SP Sepharose High Performance[2]	75 mL	17108701
HiLoad™ 16/10 Q Sepharose HP	1 × 20 mL	17106401
HiLoad 26/10 Q Sepharose HP	1 × 53 mL	17106601
HiLoad 16/10 SP Sepharose HP	1 × 20 mL	17113701
HiLoad 26/10 SP Sepharose HP	1 × 53 mL	17113801
HiPrep 16/10 DEAE FF	1 × 20 mL	17509001
HiPrep 16/10 CM FF	1 × 20 mL	17509101
HiPrep 16/10 Q XL1	1 × 20 mL	17509201
HiPrep 16/10 SP XL	1 × 20 mL	17509301
HiTrap Desalting	1 × 5 mL	29048684
HiTrap Desalting	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/120 Desalting	4 × 53 mL	17508702

[1] May require a license; see legal information on this page.
[2] Process-scale quantities are available. Please contact your local representative.

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”†	2	11000464
Fingertight stop plug, 1/16”‡	5	11000355

* 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

Convenient Protein Purification, HiTrap Column Guide	18112981
Ion Exchange Chromatography Handbook: Principles and Methods	11000421

gelifesciences.com/protein-purification

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
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., 100 Results Way, Marlborough, MA 01752 USA
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073 Japan
For local office contact information, visit gelifesciences.com/contact

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