HiTrap™ Capto™ IEX Selection Kit

HiTrap Capto IEX Selection Kit offers a fast, simple, and convenient way for selecting the best ion exchange chromatography media for a given application. The kit consists of five different high capacity ion exchange and multimodal ion exchange media based on a chemically modified, highly rigid agarose base matrix. The media are prepacked in ready-to-use HiTrap 1 ml columns for small-scale screening and purification (Fig 1). After selecting the best chromatography medium for a target protein, larger prepacked columns and bulk media are available for large-scale preparative work. Separations can be easily performed using a syringe, a pump, an ÄKTA™ system, or other chromatography systems.

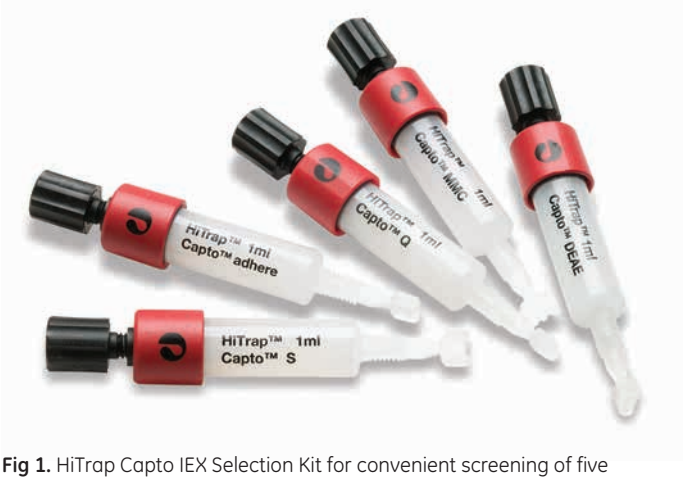


Fig 1. HiTrap Capto IEX Selection Kit for convenient screening of five different Capto media.

HiTrap Capto IEX Selection Kit offers:

- **Convenience:** Fast and easy screening of three traditional ion exchange media and two multimodal ion exchange media in a prepacked format.
- **Speed:** Capto media with high dynamic binding capacity at high flow rates.
- **Simple operation:** Reduces purification time.
- **Easy scale-up:** Larger prepacked columns and bulk chromatography media for preparative purification work are available.

Chromatography media characteristics

The media packed in HiTrap columns are Capto S, Capto Q, Capto DEAE, Capto adhere, and Capto MMC. Capto S and Capto Q are strong ion exchangers that maintain their charge (and thus their function) over a wide pH range. Weak ion exchangers vary with pH regarding the degree of dissociation and hence, ion exchange capacity.

Capto DEAE, although predominantly a weak anion exchanger, cannot be fully discharged by raising the pH due to a minor content of quaternarized amine groups. It is therefore possible to use this specific DEAE chromatography media at higher pH values for the separation of highly charged species such as nucleotides.

Capto MMC and Capto adhere have different selectivity compared to traditional ion exchangers, due to the multimodal ligand that may interact with target molecules in several ways. In addition to the ionic interactions other types of interactions such as hydrogen bonding and hydrophobic interaction are involved. Capto MMC is a multimodal high salt-tolerant cation exchanger allowing the capture of proteins at high conductivity (Fig 2).

Capto adhere is a multimodal strong anion exchanger. It has been designed for the removal of aggregates and host cell proteins after protein A-purification of monoclonal antibodies. The characteristics of Capto S, Capto Q, Capto DEAE, Capto adhere, and Capto MMC are summarized in Table 1.

Capto chromatography media are suitable for scale-up to manufacturing. High throughput in downstream purification requires a matrix with high mechanical strength combined with a pore structure that allows fast mass transfer and high capacity for target molecules. Capto media are based on a highly rigid agarose base matrix that offers outstanding pressure/flow properties with optimized pore structure.

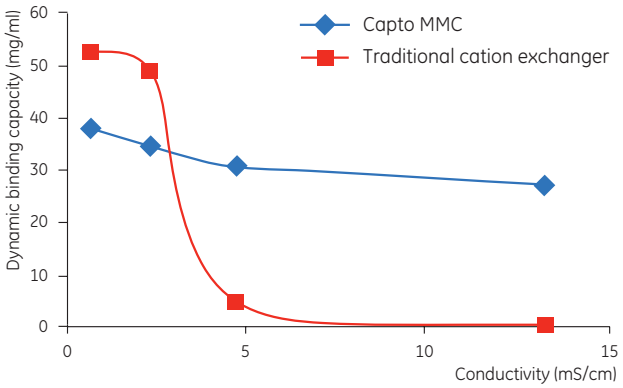
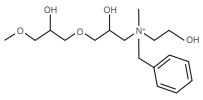
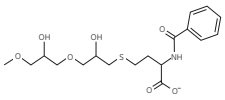


Fig 2. Capto MMC allows a much larger operating range (area below the curves) in terms of conductivity of the starting material than traditional cation exchanges.

Table 1. Characteristics of Capto chromatography media

	Capto S	Capto Q	Capto DEAE	Capto adhere	Capto MMC
Matrix	Highly cross-linked agarose with dextran surface extender			Highly cross-linked agarose	
Ion exchange type	Strong cation	Strong anion	Weak anion	Strong anion, multimodal	Weak cation, multimodal
Charged group	$-SO_3^-$	$-N^+(CH_3)_3$	$-N^+H(CH_2CH_3)_2$		
Total ionic capacity	0.11 to 0.14 mmol Na^+/ml medium	0.16 to 0.22 mmol Cl^-/ml medium	0.29 to 0.35 mmol Cl^-/ml medium	0.09 to 0.12 mmol Cl^-/ml medium	0.07 to 0.09 mmol H^+/ml medium
Particle size (d_{50v})[1]	90 µm	90 µm	90 µm	75 µm	75 µm
Flow velocity	At least 700 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa, 43.5 psi)			At least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa, 43.5 psi)	
Dynamic binding capacity	> 120 mg lysozyme/ml medium[2]	> 100 mg BSA/ml medium[2]	> 90 mg ovalbumin/ml medium[2]	Not available	> 45 mg BSA/ml medium at 30 mS/cm[3]
pH stability[4]					
Cleaning	3 to 14	2 to 14	2 to 14	2 to 14	2 to 14
Working	4 to 12	2 to 12	2 to 12	3 to 12	2 to 12
Working temperature[5]	4 to 30°C	4 to 30°C	4 to 30°C	4 to 30°C	4 to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH[6], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol				
Storage	20% ethanol, 0.2 M NaAc	20% ethanol	20% ethanol	20% ethanol	20% ethanol
Avoid	Oxidizing agents, cationic detergents	Oxidizing agents, anionic detergents	Oxidizing agents, anionic detergents	Oxidizing agents, anionic detergents	Oxidizing agents, cationic detergents

[1] d_{50v} is the median particle size of the cumulative volume distribution.

[2] Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 min, 600 cm/h in a Tricorn™ 5/100 column with 10 cm bed height in 50 mM Tris-HCl buffer, pH 8.0.

[3] Dynamic binding capacity at 10% breakthrough as measured at a residence time of 2 min, 300 cm/h in a Tricorn 5/100 column with 10 cm bed height in 50 mM sodium acetate, 250 mM NaCl, pH 4.75.

[4] *Cleaning pH*: pH interval that the medium can be subjected to, for cleaning- or sanitization-in-place (accumulated 90 to 300 h at room temperature) without significant change in function. *Working pH*: pH interval where the medium can be operated without significant change in function.

[5] Low temperatures can decrease the capacity of Capto S, Capto DEAE, Capto adhere, and Capto MMC.

[6] No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

Column characteristics

HiTrap columns are made of polypropylene, which is compatible 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. The characteristics of a HiTrap column are listed in Table 2.

Note: The column cannot be opened and refilled.

Table 2. Characteristics of HiTrap 1 ml columns

Column volumes	1 ml
Column dimensions	0.7 × 2.5 cm
Maximum flow rates	4 ml/min
Recommended flow rates	1 ml/min
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)

Operation

It is important to screen for an optimal ion exchange ligand to get the best results and cost efficiency. This can easily be performed using HiTrap Capto IEX Selection Kit. Complete and easy-to-follow instructions are included for fast startup, chromatography media screening, and method optimization. Charged molecules bind to the ion exchange and multimodal ion exchange media 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. Operation is performed using a chromatography system such as ÄKTAdesign (it is also possible to use a peristaltic pump or a syringe and the provided Luer adapter).

Applications

The charged groups of the S, Q, and DEAE ligands used in Capto media are identical to the charged groups used in other ion exchange media from GE Healthcare. However, minor differences in selectivity can occur due to differences in the base matrix, ligand density, and surface extenders. This is illustrated in Figures 3 and 4 where an *E. coli* homogenate was applied to the columns and the target protein was eluted with a linear salt gradient. The separations were monitored by measuring the absorbance at 280 nm and in one case, also at 490 nm. All purifications were performed using ÄKTAexplorer™.

Columns: HiTrap Capto Q, HiTrap Q XL, HiTrap Q FF
Column volume: 1 ml
Sample: Green Fluorescent Protein (GFP) in *E. coli* homogenate
Start buffer: 50 mM Tris-HCl, pH 8.2
Elution buffer: 50 mM Tris-HCl, 1 M NaCl, pH 8.2
Flow: 1 ml/min (156 cm/h)
Gradient: 0 to 100 % elution buffer, 15 column volumes (CV)
System: ÄKTAexplorer 100

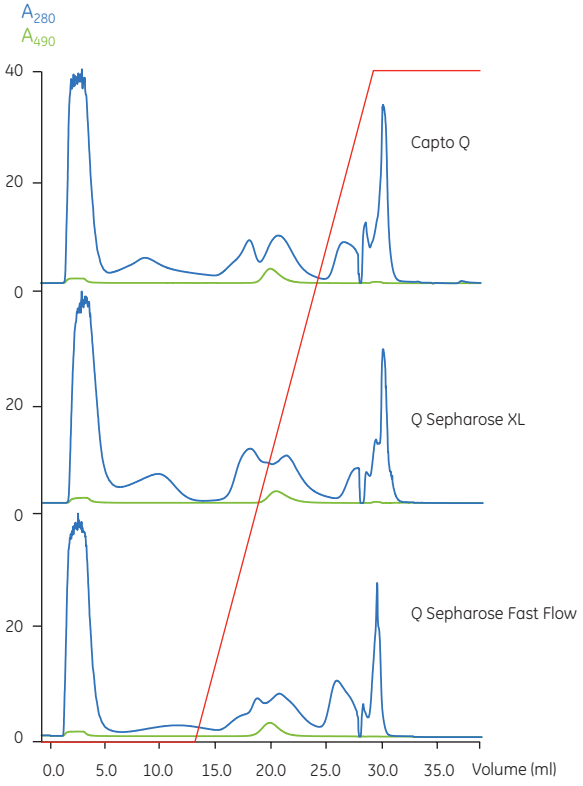


Fig 3. The separation ability of Capto Q for GFP compared to Q Sepharose™ Fast Flow and Q Sepharose XL media in 1 ml prepacked HiTrap columns. Red line = conductivity.

Column: HiTrap Capto S, HiTrap SP XL, HiTrap SP FF
Column volume: 1 ml
Sample: α-chymotrypsin in *E. coli* homogenate
Start buffer: 50 mM sodium acetate, pH 4.8
Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 4.8
Flow: 1 ml/min (156 cm/h)
Gradient: 0 to 100 % elution buffer, 10 CV
System: ÄKTAexplorer 100

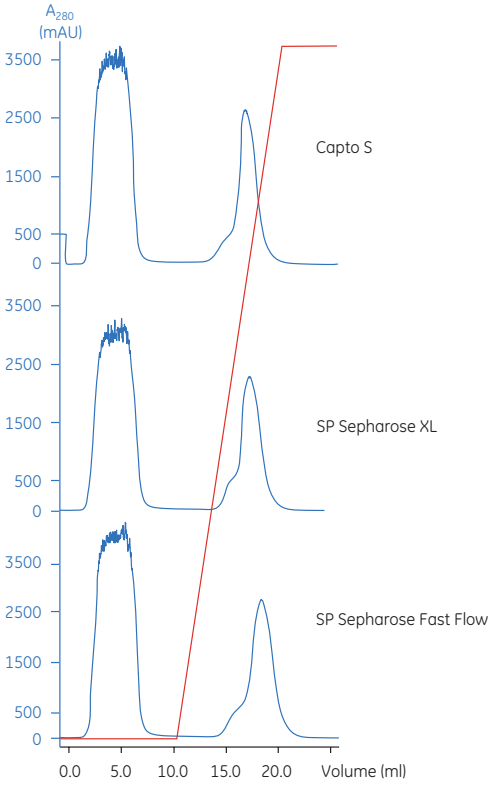


Fig 4. The separation ability of Capto S for α-chymotrypsin compared to SP Sepharose Fast Flow and SP Sepharose XL media in 1 ml prepacked HiTrap columns. Red line = conductivity.

Capto MMC is immobilized with a multimodal ligand that interacts with target molecules in several ways, but its main interaction is that of a weak cation exchanger. The difference in selectivity is illustrated in Figure 5 where human blood plasma was purified using Capto MMC or SP Sepharose Fast Flow under identical conditions. In this case, a Tricorn 5/100 GL column was used and the separations were monitored by measuring the absorbance at 280 nm. The elution curves reflect the different properties of the two media. In addition to multimodal selectivity, Capto MMC also enables the binding of proteins at high salt concentrations. This minimizes the need for prior dilution in order to reduce the conductivity of the starting material.

Capto adhere is a strong anion exchange media with multimodal properties. In addition to ionic interactions, Capto adhere provides hydrogen bonding and hydrophobic interactions with biomolecules. The advantages of the multimodal properties of Capto adhere were demonstrated in a separation process in which a supernatant containing monoclonal IgG_1 (Polymun Scientific, Austria) was first purified on MabSelect SuRe™ and then further polished on Capto adhere. Aggregates and other contaminants were bound to the Capto adhere medium while the monomeric monoclonal IgG passed through the column. Analysis by gel filtration shows the high purity of IgG in the flowthrough and a number of contaminants in the elution pool (Fig 6). The yield of IgG for the Capto adhere step was 92%.

Column: Tricorn 5/100 GL
Media: A) Capto MMC B) SP Sepharose Fast Flow
Sample: Human blood plasma diluted 5 times, 10 CV
Start buffer: 100 mM acetic acid, 50 mM sodium phosphate, 20 mM sodium succinate, pH 5.0
Elution buffer: 100 mM acetic acid, 50 mM sodium phosphate, 20 mM sodium succinate, 1 M NH_4Cl, pH 8.0
Flow: 0.96 ml/min (150 cm/h)
Gradient: 0 to 100 % elution buffer, 10 CV
System: ÄKTAexplorer 10

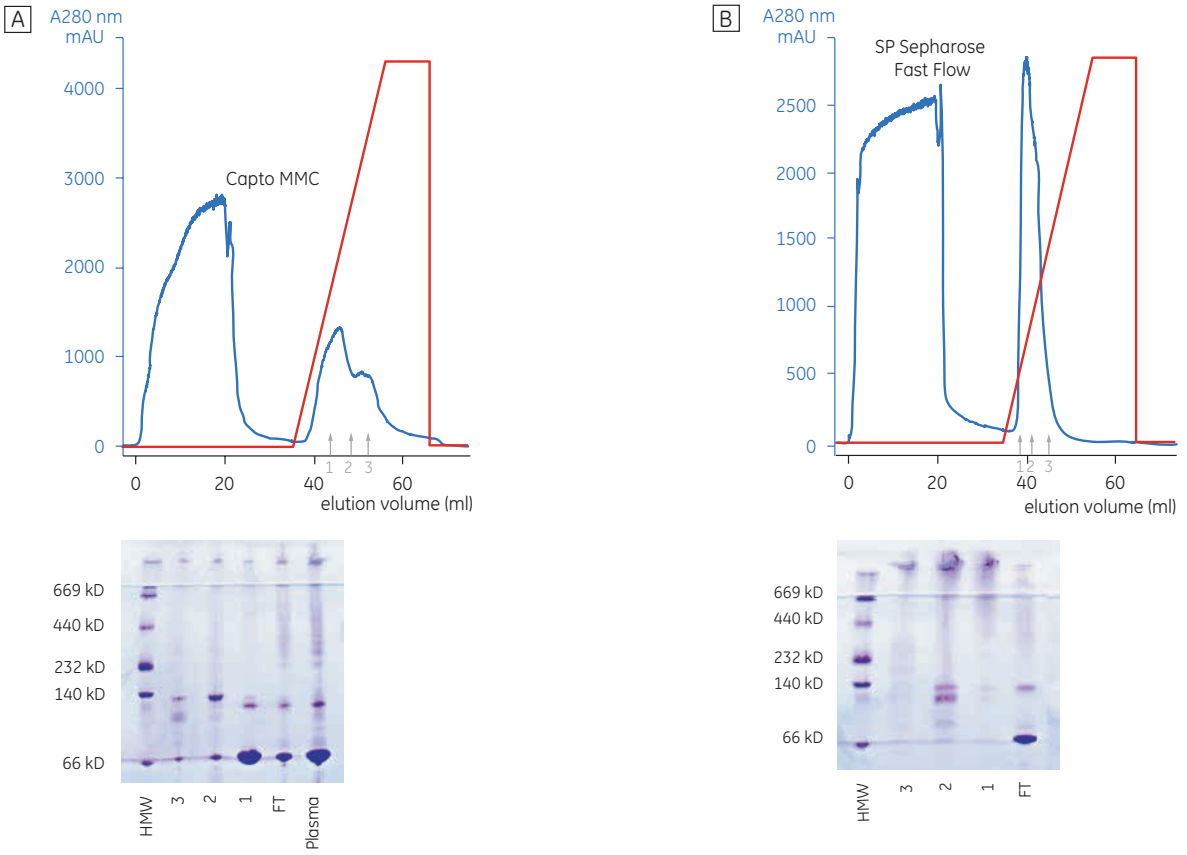


Fig 5. The selectivity of (A) Capto MMC and (B) SP Sepharose Fast Flow was investigated using human blood plasma. The sample was diluted 5 times to give a conductivity of 6 mS/cm and a pH of approximately 6. Fractions (indicated with arrows) and the flowthrough pool (FT) were analyzed on native PhastGel™ Gradient 8-25% and Coomassie™ stained. High molecular weight marker (HMW, GE Healthcare) and unfractionated plasma sample were also applied to the gels. The elution profile on SP Sepharose Fast Flow revealed one peak whereas the elution profile on Capto MMC showed two, possibly three peaks. Native gel electrophoresis also shows that the separation patterns differ between the media. Note: Different scales on the y-axis.

Column: Superdex™ 200 10/300 GL
Sample: A) Flowthrough fraction from the Capto adhere step
B) Eluted fraction from the Capto adhere step
Sample load: 50 µl each
Buffer: 0.01 M sodium phosphate, 3 mM potassium phosphate, 140 mM sodium chloride, pH 7.4
Flow rate: 0.5 ml/min (78 cm/h)
System: ÄKTAexplorer

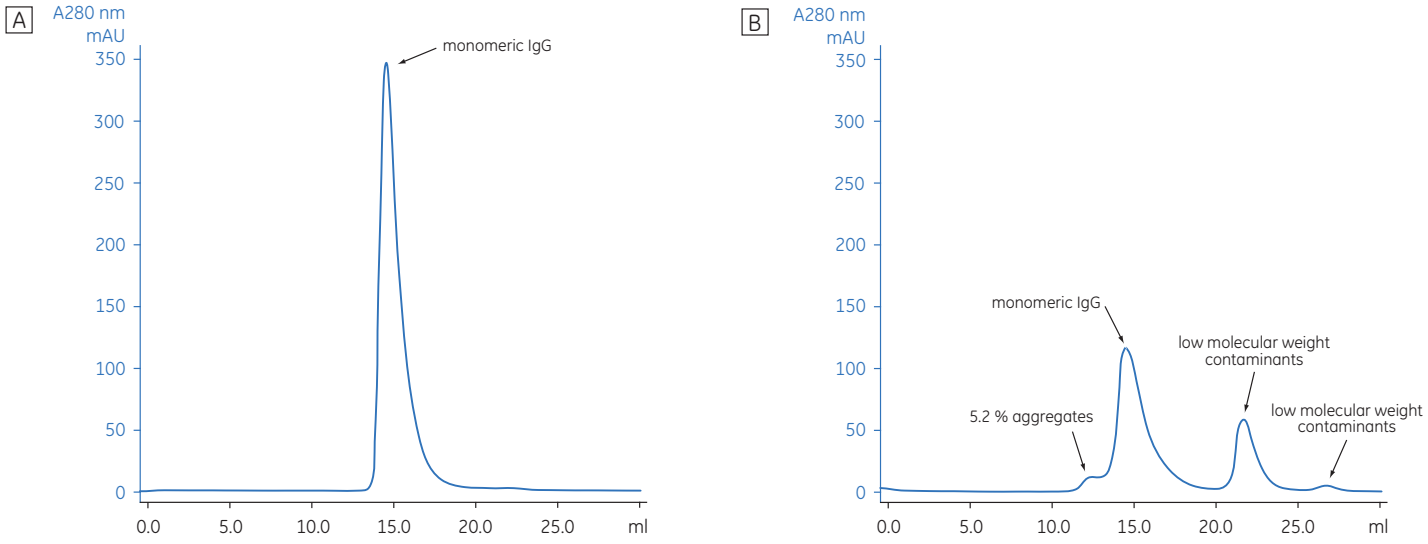


Fig 6. (A) Gel filtration analysis of flowthrough pool from Capto adhere containing monomeric IgG. (B) Gel filtration analysis of eluted pool from Capto adhere containing 5.2% aggregates, monomeric IgG, and low molecular weight contaminants.

Further optimization

HiTrap Capto IEX Selection Kit is an excellent tool for conducting the screening of different Capto chromatography media as well as more detailed optimization studies. Different types of ion exchange ligands can easily be compared and the effects of buffer composition, pH, and sample loading can be studied with small sample quantities. Design of Experiments (DoE) is an effective tool for investigating the effects of several parameters in order to establish optimal conditions.

Note: For optimization of elution conditions such as gradient slope, we recommend the use of a column containing a final bed height during scale-up. 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 or www.gelifesciences.com/hitrap.

HiTrap IEX Selection Kit is recommended for comparative studies with other chromatography media. This kit includes a total of seven different IEX Sepharose Fast Flow and IEX Sepharose XL media prepacked in HiTrap 1 ml columns.

Scale-up

The high capacity combined with high flow rate and low back pressure makes Capto chromatography media particularly useful for larger scale purification. Purifications can be scaled up and method development work performed using either prepacked HiTrap 5 ml columns or prepacked HiScreen™ columns (10 cm bed length, 4.7 ml) containing the same media. Two HiScreen columns can easily be connected in series to increase the bed height to 20 cm. Larger quantities of media for further scale-up are also available, see ordering information. All media included in HiTrap Capto IEX Selection Kit are BioProcess™ media with full technical and regulatory support.

Storage

HiTrap Capto Q, HiTrap Capto DEAE, HiTrap Capto MMC, and HiTrap Capto adhere should be stored in 20% ethanol at 4°C to 30°C.

HiTrap Capto S should be stored in 20% ethanol, 0.2 M sodium acetate at 4°C to 30°C.

Ordering information

Product	Quantity	Code number
HiTrap Capto IEX Selection Kit	5 × 1 ml	28-9343-88
Related products	**Quantity**	**Code number**
HiTrap Capto Q	5 × 1 ml	11-0013-02
HiTrap Capto Q	5 × 5 ml	11-0013-03
HiTrap Capto S	5 × 1 ml	17-5441-22
HiTrap Capto S	5 × 5 ml	17-5441-23
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
HiTrap Capto DEAE	5 × 5 ml	28-9165-40
HiTrap Capto MMC	5 × 1 ml	11-0032-73
HiTrap Capto MMC	5 × 5 ml	11-0032-75
HiTrap Capto adhere	5 × 1 ml	28-4058-44
HiTrap Capto adhere	5 × 5 ml	28-4058-46
HiScreen Capto Q	1 × 4.7 ml	28-9269-78
HiScreen Capto S	1 × 4.7 ml	28-9269-79
HiScreen Capto DEAE	1 × 4.7 ml	28-9269-82
HiScreen Capto MMC	1 × 4.7 ml	28-9269-80
HiScreen Capto adhere	1 × 4.7 ml	28-9269-81
Capto Q	25 ml	17-5316-10
Capto Q	100 ml[1]	17-5316-02
Capto S	25 ml	17-5441-10
Capto S	100 ml[1]	17-5441-01
Capto DEAE	25 ml	17-5443-10
Capto DEAE	100 ml[1]	17-5443-01
Capto adhere	25 ml	17-5444-10
Capto adhere	100 ml[1]	17-5444-01
Capto MMC	25 ml	17-5317-10
Capto MMC	100 ml[1]	17-5317-02
HiTrap IEX Selection Kit	7 × 1 ml	17-6002-33
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 26/10 Desalting	4 × 53 ml	17-5087-02

[1] Other quantities are also available. Please contact your local representative or visit www.gelifesciences.com/protein-purification or www.gelifesciences.com/bioprocess

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 products.

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

Related literature	Code number
Data File: Capto Q Capto ViralQ, Capto S and Capto DEAE	11-0025-76
Data File: Capto MMC	11-0035-45
Data File: Capto adhere	28-9078-88
Handbook: Ion Exchange Chromatography & Chromatofocusing, Principles and methods	11-0004-21
Ion Exchange Chromatography Columns and Media, Selection guide	18-1127-31
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

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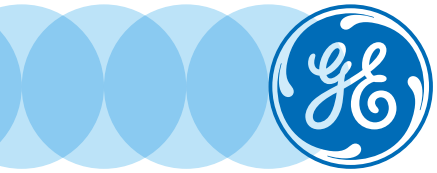

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