GE Healthcare

Instructions 71-7149-00 AN **HiTrap ion exchange columns**

HiTrap SP HP, 1 ml and 5 ml
HiTrap Q HP, 1 ml and 5 ml

HiTrap™ SP HP and HiTrap Q HP are prepacked, ready to use cation and anion exchange columns for method scouting, group separations, sample concentration and sample clean-up of charged biomolecules. HiTrap SP HP and HiTrap Q HP provide fast, reproducible, and easy separations in a convenient format.

The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTAdesign™ or FPLC™ System.

HiTrap

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Code No.	Designation	No. supplied
17-1151-01	HiTrap SP HP	5 x 1 ml
17-1152-01	HiTrap SP HP	5 x 5 ml
17-1153-01	HiTrap Q HP	5 x 1 ml
17-1154-01	HiTrap Q HP	5 x 5 ml

Connectorkit

Connectors supplied	Usage	No. supplied
1/16" male/luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column*	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column**	1
Union 1/16" female/ M6 male	Connection to original FPLC System through bottom of HiTrap column	1
Union M6 female/ 1/16" male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16"	Sealing bottom of HiTrap column	2, 5 or 7

* Union 1/16" female/M6 male is also needed.

** Union M6 female/1/16" male is also needed.

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1. Description

Media properties

SP Sepharose™ High Performance and Q Sepharose High Performance are strong cation and strong anion exchangers respectively. Both remain charged and maintain high capacity over broad pH ranges. The functional groups are coupled to the matrix via chemically stable ether linkages. Characteristics of HiTrap SP HP and HiTrap Q HP, 1 and 5 ml columns are listed in Table 1.

Column

HiTrap Q HP and HiTrap SP HP are 1 ml and 5 ml columns made of polypropylene, which is biocompatible and non-interactive with biomolecules. The top and bottom frits are manufactured from porous polyethylene. It is delivered with a stopper on the inlet and a snap-off end on the outlet.

The separation can be easily achieved using a syringe together with the supplied luer adaptor, a peristaltic pump, or in a chromatography system such as ÄKTA™ or FPLC.

Note: To prevent leakage it is essential to ensure that the adaptor is tight.

The column cannot be opened or refilled.

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Table 1. HiTrap SP HP and HiTrap Q HP columns characteristics

Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) and 1.6 × 2.5 cm (5 ml)
Total ionic capacity	0.14–0.20 mmol (Cl^-)/ml medium (Q) 0.15–0.20 mmol (H^+)/ml medium (SP)
Dynamic binding capacity	SP: approx. 55 mg ribonuclease/ml medium (0.1 M sodium acetate, pH 6.0 at 1 ml/min) Q: approx. 50 mg HSA/ml medium (20 mM Tris-HCl, pH 8.2 at 1 ml/min)
Mean particle size	34 µm
Bead structure	6% highly cross-linked spherical agarose
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
Maximum backpressure	0.3 MPa, 3 bar, 42 psi
Chemical stability	All commonly used buffers
Charged group	SP: – $CH_2CH_2CH_2SO_3$ Q: – $CH_2N^+(CH_3)_3$
pH stability*	
Short term	SP: 3–14, Q: 1–14
Working	SP: 4–13, Q: 2–12
Long term	SP: 4–13, Q: 2–12
Storage temperature	+4° to +30 °C
Storage buffer	SP: 20% ethanol, 0.2 M sodium acetate Q: 20% ethanol
Avoid	SP: Oxidizing agents, cationic detergents and buffers Q: Oxidizing agents, anionic detergents and buffers

* The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

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2. Selection of ion exchanger and conditions

Ion exchange chromatography is based on adsorption and reversible binding of charged sample molecules to oppositely charged groups attached to an insoluble matrix. The pH value at which a biomolecule carries no net charge is called the isoelectric point (pI). When exposed to a pH below its pI, the biomolecule will carry a positive charge and will bind to a cation exchanger (SP). At pH’s above its pI the protein will carry a negative charge and will bind to an anion exchanger (Q). If the sample components are most stable below their pI’s, a cation exchanger should be used. If they are most stable above their pI´s, an anion exchanger is used. If stability is high over a wide pH range on both sides of pI, either type of ion exchanger can be used (Figure 1).

Selection of buffer pH and ionic strength

Buffer pH and ionic strength are critical for the binding and elution of material (both target substances and contaminants) in ion exchange chromatography. Selection of appropriate pH and ionic strength for the start and elution buffers allows the use of three possible separation strategies.

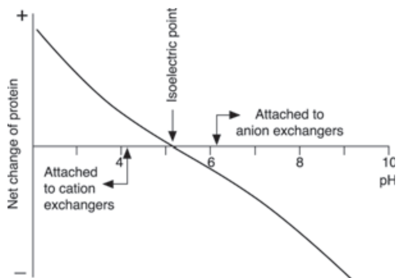

Fig 1. The net charge or a protein as a function of pH.

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Strategy 1. Binding and elution of all sample components

Binding is achieved by choosing a start buffer with a low pH for HiTrap SP HP or a high pH for HiTrap Q HP. The ionic strenght should be kept as low as possible to allow all components to bind to the ionic exchange (<5 mS/cm). This results in a concentration of the target substance and a complete picture of the whole sample. The drawback of this strategy is that the binding capacity of the ion exchanger for the target substance is dependent on the amount of contaminant in the sample. Strongly binding contaminants can also displace bound target protein if a large volume of sample is loaded.

Note: Start conditions are subject to the stability of the sample components.

Strategy 2. Enrichment of target protein

This is achieved by choosing a start buffer with a pH optimized to allow maximal binding of target protein, and as high as possible ionic strength to suppress binding of sample contaminants. This strategy results in a concentration of the target substances.

Strategy 3. Binding of sample contaminants

This is achieved by choosing a start buffer with a pH and ionic strength that promotes the binding of some or all contaminating substances but allows the substance of interest to pass through the column. The drawback of this approach is that the target substance is not concentrated and the sample volume applied to the ion exchanger is dependent on the amount of contaminants in the sample.

Start buffer

The concentration of buffer required to give effective pH control varies with the buffer system. A list of suitable buffers and suggested starting concentrations is shown in Tables 2 and 3, Figs 2 and 3. In the majority of cases a concentration of at least 10 mM is required to ensure adequate buffering capacity. The ionic strength of the buffer should be kept low (< 5 mS/cm) so as not to interfere with sample binding. Salts also play a role in stabilizing protein structures in solution and it is important the ionic strength should not be so low that protein denaturation or precipitation occurs.

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The buffering ion should carry the same charge as the ion exchange group and should have a pKa within 0.5 pH units of the pH used in the separation. Buffering ions of opposite charge may take part in the ion exchange process and cause local disturbances in pH.

Starting pH

Cation exchangers (SP): At least 1 pH unit below the pI of substance to be bound.
Anion exchangers (Q): At least 1 pH unit above the pI of substance to be bound.

Table 2. Buffers for cation exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25 °C)[1]
1.4–2.4	Maleic acid	20	Na^+	1.92
2.6–3.6	Methyl malonic acid	20	Na^+ or Li^+	3.07
2.6–3.6	Citric acid	20	Na^+	3.13
3.3–4.3	Lactic acid	50	Na^+	3.86
3.3–4.3	Formic acid	50	Na^+ or Li^+	3.75
3.7–4.7; 5.1–6.1	Succinic acid	50	Na^+	4.21; 5.64
4.3–5.3	Acetic acid	50	Na^+ or Li^+	4.75
5.2–6.2	Methyl malonic acid	50	Na^+ or Li^+	5.76
5.6–6.6	MES	50	Na^+ or Li^+	6.27
6.7–7.7	Phosphate	50	Na^+	7.20
7.0–8.0	HEPES	50	Na^+ or Li^+	7.56
7.8–8.8	BICINE	50	Na^+	8.33

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

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Table 3. Buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25 °C)[1]
4.3–5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8–5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5–6.5	L-Histidine	20	Cl^-	6.04
6.0–7.0	bis-Tris	20	Cl^-	6.48
6.2–7.2; 8.6–9.6	bis-Tris propane	20	Cl^-	6.65; 9.10
7.3–8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6–8.6	Tris	20	Cl^-	8.07
8.0–9.0	N-Methyldiethanolamine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyldiethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl^-	8.88
8.4–9.4	Propane 1,3-Diamino	20	Cl^-	8.88
9.0–10.0	Ethanolamine	20	Cl^-	9.50
9.2–10.2	Piperazine	20	Cl^-	9.73
10.0–11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6–11.6	Piperidine	20	Cl^-	11.12

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

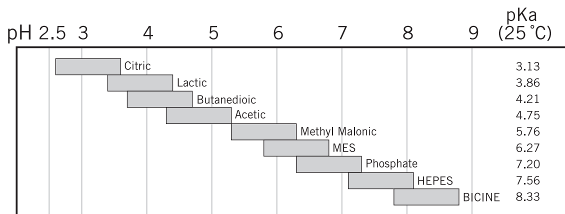

Fig 2. Recommended buffer substances for cation exchange chromatography.

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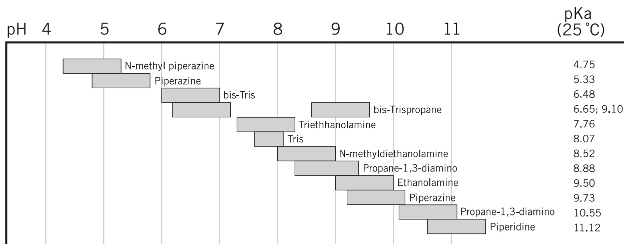

Fig 3. Recommended buffer substances for anion exchange chromatography.

The columns can be operated with a syringe, peristaltic pump or a chromatography system.

3. Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 µm filter immediately before use. See Tables 2 and 3, Figs 2 and 3 for recommended buffers.

Sample preparation

The sample should be adjusted to the composition of the start buffer. This can be done by either diluting the sample with start buffer or by buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 column. The sample should be filtered through a 0.22 µm or 0.45 µm filter or centrifuged immediately before it is applied to the column. See Table 4.

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Table 4. Prepacked columns for desalting and buffer exchange.

Code No	Column	Loading volume	Elution volume	Comments	Application
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 ml	Prepacked with Sephadex™ G-25 Superfine. Requires a syringe or pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15–20 ml	Prepacked with Sephadex G-25 Fine. Requires a pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0851-01	PD-10 Desalting	2.5 ml	3.5 ml	Prepacked with Sephadex G-25. Requires only gravity to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0855-01	NICK™	0.1 ml	0.4 ml	Prepacked with Sephadex G-25.	For separation of proteins (M_r>5000) and nicktranslated DNA from radiolabelled nucleotides not shorter than 120 mers, and similar separations.
17-0853-01 17-0854-01 17-0852-01	NAP™-5 NAP-10 NAP-25	0.5 ml 1.0 ml 2.5 ml	1.0 ml 1.5 ml 3.5 ml	Prepacked with Sephadex G-25 DNA grade. Requires only gravity to run.	For purification of proteins (M_r>5000), DNA and oligo-nucleotides greater than 10 bases in length.

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Purification

1. Fill the syringe or pump tubing with start buffer (low ionic strength). Remove the stopper and connect the column to the syringe (with the provided adaptor), "drop to drop" to avoid introducting air into the column.
2. Remove the snap-off end at the column outlet.
3. Wash out the preservatives with 5 column volumes of start buffer, at 1 ml/min for the 1 ml column and 5 ml/min for the 5 ml column.
4. Wash with 5 column volumes of elution buffer (start buffer with 1 M NaCl).
5. Finally equilibrate with 5–10 column volumes of start buffer.
6. Apply the sample at 1 or 5 ml/min for the 1 ml and 5 ml columns respectively, using a syringe fitted to the luer adaptor or by pumping it onto the column.
7. Wash with at least 5 column volumes of start buffer or until no material appears in the effluent.
8. Elute with 5–10 column volumes of elution buffer (see section "Choice of gradient type").
9. The purified fractions can be desalted using a HiTrap Desalting, HiPrep 26/10 Desalting or a PD-10 columns if necessary.
10. After the completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5–10 column volumes of start buffer. The column is now ready for a new sample.

For a first experiment the following conditions are recommended:

Flow rate:	1 ml/min using HiTrap 1 ml column 5 ml/min using HiTrap 5 ml column
Start buffer:	See Tables 2 and 3
Elution buffer:	Start buffer + 1 M NaCl
Gradient volume:	20 ml

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Note: If a P1-pump is used a max flow rate of 1–3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose High Performance media.

4. Optimization

If sample composition is unknown, a simple screening test with the aid of a syringe or pump can be performed to optimize starting pH and ionic strength.

1. Set up a series of buffers with different pH´s, in the range 4–8 (SP) or 5–9 (Q), with 0.5–1 pH unit intervals between each buffer. Make one series with 1 M NaCl included in the buffers (regeneration buffer) and the other without NaCl (start buffer).
2. Equilibrate the column, see Purification.
3. Adjust the sample to the chosen start buffer, see Sample preparation.
4. Apply a known constant amount of the sample at 1 or 5 ml/min for the 1 ml and 5 ml columns respectively. Collect eluate.
5. Wash with at least 5 column volumes start buffer or until no material appears in effluent. Collect eluate.
6. Elute bound material with elution buffer. 3–5 column volumes is usually sufficient. Other volumes may be required, depending on the chosen operational conditions. Collect eluate.
7. Analyze all eluates for example by activity assay and SDS-PAGE and determine the purity and the amount bound to the column.
8. Perform steps 2–7 for the next buffer pH.
9. Decide which pH should be used for the selected purification strategy.
10. To decide on starting ionic strength conditions, a similar screening is done, but the buffer pH is held constant and the ionic strength is varied in the interval 0–0.5 M, with intervals of 0.05 to 0.1 M salt between each buffer.

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Further optimization

The recommendations given above will give a sound basis for developing an efficient purification step. Details of how flow rate, sample loading, particle size and elution scheme may be optimized to meet the special needs can be found in the handbook, Ion Exchange Chromatography & Chromato-foucsing, Principles and Methods, Code No. 11-0004-21.

GE Healthcare supplies a wide range of ion exchange chromatography media for purification of biomolecules at all scales. See Ordering information and visit www.gehealthcare.com/hitrap.

5. Choice of gradient type

1. Stepwise gradients are easy to produce and require minimal equipment. Eluted peaks are very sharp and elution volumes minimal. However, care must be exercised in the design of the steps and the interpretation of results for substances eluted by a sharp change in pH or small differences in ionic strength. Peaks tend to have sharp fronts and pronounced tailing since they frequently contain more than one component.
2. Continuous salt gradients are the most frequently used type of elution. Many types of gradient forming systems are available. Two buffers of differing ionic strength, the start and elution buffer (start buffer + 1 M NaCl or higher buffer salt concentration), are mixed together and if the volume ratio is changed linearly, the ionic strength changes linearly.

Note: Another, but less common, method to desorb bound material is to increase (SP) or decrease (Q) the pH of the eluent. Continuous pH gradients are difficult to produce at constant ionic strength, since simultaneous changes in ionic strength, although small, also occur (buffering capacities are pH dependent).

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Elution with stepwise ionic strength gradients

Stepwise elution is the sequential use of the same buffer at different ionic strengths. It is technically simple and fast, and is suitable for syringe operation. It is often used for sample concentration and sample clean-up. Stepwise elution gives small peak volumes and the resolution depends on the difference in elution power between each step.

1. Choose starting conditions as outlined under Optimizing starting conditions.
2. Equilibrate the column, see Purification.
3. Adjust the sample to the choosen starting pH and ionic strength, see Sample preparation.
4. Apply the sample at 1 or 5 ml/min for the HiTrap 1 ml or 5 ml column respectively. Collect eluate.
5. Wash with at least 5 column volumes of start buffer or until no material appears in effluent. Collect eluate.
6. Elute with the first step ionic strength buffer. The volumes required for stepwise elution depend on the operating conditions. However, 3–5 column volumes is usually sufficient. Collect eluate.
7. Elute with next ionic strength buffer. Collect eluate.
8. After completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5–10 column volumes of start buffer. The column is now ready for a new sample.

Elution with continuous ionic strength gradients

Continuous salt gradient elution is the most frequently used type of elution in ion exchange chromatography. It is very reproducible and leads to improved resolution, since zone sharpening occurs during elution. Continuous gradients can be prepared in different ways, depending on available equipment.

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- A peristaltic pump and a gradient mixer e.g. pump P-1, gradient mixer GM-1.
- A one pump system, e.g. ÄKTAprime™ plus.
- A two pump system, e.g FPLC or ÄKTA.

1. Choose starting conditions as outlined under Optimizing starting conditions.
2. Equilibrate the column, see Purification.
3. Adjust the sample to the chosen starting pH and ionic strength, see Sample preparation.
4. Apply the sample at 1 or 5 ml/min for the HiTrap 1 or 5 ml column respectively. Collect eluate.
5. Wash with 5–10 column volumes of start buffer or until no material appears in effluent.
6. Start the gradient elution. A gradient volume of 10–20 column volumes and an increase in ionic strength to 0.5 M NaCl is usually sufficient.
7. Regenerate the column by washing with 5 column volumes of start buffer with 1 M NaCl followed by 5–10 column volumes of start buffer. The column is now ready for a new sample.

6. Determination of binding capacity

The amount of sample which can be applied to a column depends on the capacity of the column and the degree of resolution required. The capacity is dependent on the sample composition, choosen starting conditions of pH and ionic strength and the flow rate at which the separation is done. The influence of flow rate and pH on the capacity for some model proteins are shown in Figure 4.

Samples were applied until 5% of the start material appeared in the eluent. The column was then washed with 10 ml start buffer (20 mM Tris-HCl, pH 8.2 or 9.0) before elution with elution buffer (20 mM Tris-HCl, 1.0 M NaCl, pH 8.2 or 9.0).

1. Equilibrate the column, see Purification.
2. Adjust the sample to the chosen starting pH and ionic strength, see Sample preparation.
3. Determine the concentration of the specific proteins by UV, SDS- PAGE, ELISA or other appropriate techniques.

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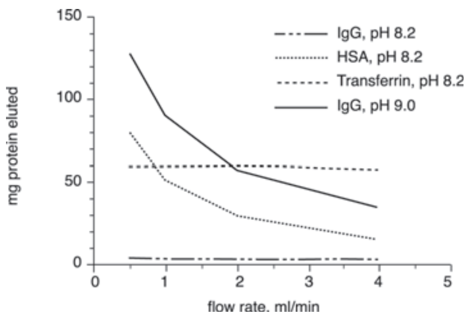

Fig 4. Binding capacity of human IgG, HSA and human transferrin at different pH´s on HiTrap Q HP, 1 ml.

4. Apply the sample solution to the column with a pump or a syringe, at a flow rate equal to the flow rate to be used in the purification method. Collect fractions and continue sample application until the column is saturated.
5. Wash the column with 5–10 column volumes start buffer or until no material appears in the effluent.
6. Elute bound proteins with 3–5 column volumes of elution buffer (start buffer with 1 M NaCl) and collect eluate.
7. Analyse fractions and eluates from steps 4 and 6 for the specific protein and determine the breakthrough profile (sample concentration as a function of the amount of sample applied). The dynamic capacity is the amount that can be applied without any significant breakthrough. The total capacity for the specific protein is determined from step 6.

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7. Scaling up

For quick scale-up of purifications (back pressure will increase), two or three HiTrap ion exchange columns of the same type can be connected in series. For further scale-up SP Sepharose High Performance and Q Sepharose High Performance are available in prepacked HiLoad™ columns or bulk media packs. See Ordering Information.

8. Storage

HiTrap SP HP: Rinse with water and then wash with 5 column volumes of 20% ethanol, 0.2 M sodium acetate.

HiTrap Q HP: Rinse with water and then with 5 column volumes of 20% ethanol.

Seal the column with the supplied stoppers. The recommended storage temperature is +4 to +30 °C.

9. Ordering Information

Product	No. Supplied	Code No.
HiTrap SP HP	5 x 1 ml	17-1151-01
HiTrap SP HP	5 x 5 ml	17-1152-01
HiTrap Q HP	5 x 1 ml	17-1153-01
HiTrap Q HP	5 x 5 ml	17-1154-01

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Related products	No. Supplied	Code No.
HiTrap IEX Selection Kit	7 x 1 ml	17-6002-33
HiTrap Desalting	5 x 5 ml	17-1408-01
HiTrap Desalting	100 x 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02
PD-10 Desalting column	30	17-0851-01
HiLoad 16/10 SP Sepharose High Performance	1 x 20 ml	17-1137-01
HiLoad 26/10 SP Sepharose High Performance	1 x 53 ml	17-1138-01
HiLoad 16/10 Q Sepharose High Performance	1 x 20 ml	17-1064-01
HiLoad 26/10 Q Sepharose High Performance	1 x 53 ml	17-1066-01
SP Sepharose High Performance	75 ml	17-1087-01
Q Sepharose High Performance	75 ml	17-1014-01

* Special pack size delivered on specific order.

Accessories	No. Supplied	Code No.
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 ÄKTAdesign	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 the product.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

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Realated literature

Ion Exchange Chromatography & Chromatofocusing Handbook, Principles and Methods	1	11-0004-21
Ion Exchange Columns and Media, Selection Guide	1	18-1127-31
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-81

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