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HiTrap SP XL, 1 mL and 5 mL
HiTrap Q XL, 1 mL and 5 mL

Instructions for Use

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Abstract

HiTrap™ SP XL and HiTrap Q XL are 1 mL and 5 mL 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 XL and HiTrap Q XL 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 ÄKTA™.

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Important

Read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 mL column.

Fig 2. HiTrap, 5 mL column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa, 72 psi)	5 bar (0.5 MPa, 72 psi)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography resin, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/ 16"	For sealing bottom of HiTrap column	2, 5 or 7

Resin Properties

Q Sepharose™ XL and SP Sepharose XL resins have long chains of dextran coupled to a robust, cross-linked agarose matrix. The dextran chains increase the exposure of the Q or SP charged groups which results in very high loading capacities.

Characteristics of the ion exchangers are listed in Table 2.

Table 2. Characteristics of SP Sepharose XL and Q Sepharose XL.

	Q Sepharose XL	SP Sepharose XL
Bead structure	Cross-linked agarose, with dextran surface extender, spherical	
Type of resin	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Total ionic capacity	0.18–0.26 mmol Cl^-/ mL resin	0.18–0.25 mmol H^+/ mL resin
Particle size, d_{50v}[1]	~ 90 µm	~ 90 µm
Recommended operational flow rate	HiTrap 1 mL: 1 mL/min HiTrap 5 mL: 5 mL/min	
Maximum operational flow rate[2]	4 mL/min resp. 20 mL/min	
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[3], 8 M urea, 20% ethanol, 6 M guanidine hydrochloride, 70% ethanol	
pH stability, operational[4]	2 to 12	4 to 13
pH stability, CIP[5]	2 to 14	3 to 14
pH ligand fully charged	Entire operational range	Entire operational range
Dynamic binding capacity, Q_{B10}	≥ 160 mg BSA/ mL resin[6]	≥ 160 mg Lysozyme/ mL resin[7]
Temperature stability	4°C to 30°C	4°C to 30°C
Avoid	Oxidizing agents, anionic detergents and buffers	Oxidizing agents, cationic detergents and buffers, long exposure to pH < 4
Storage	20% ethanol, 4°C to 30°C	0.2 M sodium acetate in 20% ethanol. 4°C to 30°C

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

[2] At room temperature using buffers with the same viscosity as water.

[3] 1.0 M NaOH should only be used for cleaning purposes.

[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] Dynamic binding capacity at 10% breakthrough by frontal analysis at the 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.

[7] Dynamic binding capacity at 10% breakthrough by frontal analysis at the 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.

2 General considerations

Selection of ion exchanger

Ion exchange chromatography is based on the binding of charged sample molecules to oppositely charged groups attached to an insoluble matrix.

Substances are bound to ion exchangers when they carry a net charge opposite to that of the ion exchanger. This binding is electrostatic and reversible.

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 net charge and will bind to a cation exchanger (SP). At pH's above its pI the biomolecule will carry a negative net charge and will bind to an anion exchanger (Q) (Fig 3).

If the sample components are most stable below their pI's, a cation exchanger must 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 side of the pI, either type of ion exchanger can be used.

Weak ion exchangers have a limited pH working range (Table 3).

Information on the pI and how the net charge on the molecule varies with pH gives valuable information regarding the choice of starting conditions. Electrophoretic titration curves enable the determination of the charge/pH relationship for the molecules present across the pH range of interest.

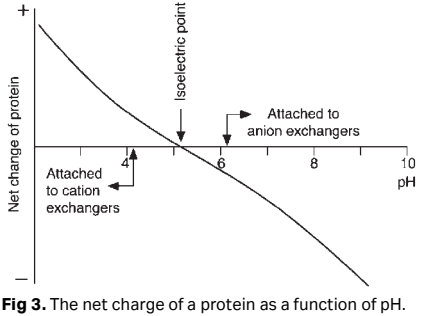

Fig 3. The net charge of a protein as a function of pH.

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.

Strategy 1. Binding and elution of all sample components

Binding is achieved by choosing a start buffer with a low pH for SP Sepharose XL, or high pH for Q Sepharose XL. The ionic strength should be kept as low as possible to allow all components to bind to the ion exchanger (< 5 mS/cm).

This results in a concentration of the target substance and a complete picture of the total sample. The drawback of this strategy is that the binding capacity of the ion exchanger for the target substance depends on the amount of contaminants in the sample. Strongly binding contaminants can also displace bound target protein if a large volume of sample is loaded.

Note: *Starting 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 an ionic strength as possible to avoid binding of sample contaminants. This strategy results in a higher concentration of the target substances.

Strategy 3. Binding of sample contaminants

This is achieved by choosing a start buffer with a pH and an ionic strength that promotes the binding of some or all contaminants but allows the target substance to pass through the column.

The drawback of this approach is that the target substance is not concentrated and the amount of sample that can be applied to the ion exchanger depends 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 Table 3, Figures 2 and 3. In the majority of cases a concentration of at least 10 mM is required to provide adequate buffering capacity. The ionic strength of the buffer needs to 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 is not so low that protein denaturation or precipitation occurs.

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 might take part in the ion exchange process and cause local disturbances in pH.

Table 3. Buffer 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.

Table 4. Buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C)[1]
4.3–5.3	N-Methyl-piperazine	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-Methyl-diethanolamine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyl-diethanolamine	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.

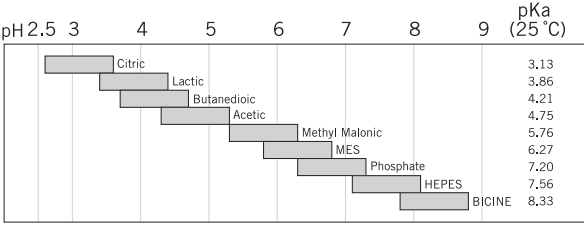

Fig 4. Recommended buffer substances for cation exchange chromatography.

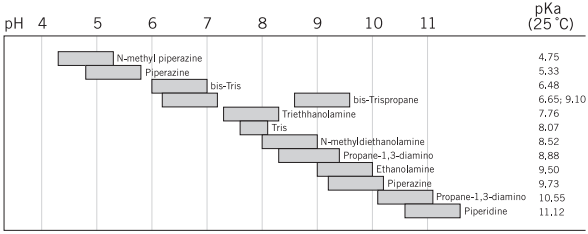

Fig 5. Recommended buffer substances for anion exchange chromatography.

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.

3 Operation

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

Buffer preparation

Water and chemicals used for buffer preparation must be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use. See Tables 3 and 4, Figures 4 and 5 for recommended buffers.

Sample preparation

The sample must be adjusted to the composition of the start buffer by buffer exchange using HiTrap Desalting,

HiPrep™ 26/10 Desalting or PD-10 columns. The sample must be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column (see Table 5).

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 luer connector), or pump tubing, drop-to-drop to avoid introducing 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 HiTrap 1 mL and 5 mL/min for HiTrap 5 mL.
4. Wash with 5 column volumes of elution buffer (start buffer with 1 M NaCl).
5. Finally equilibrate with 5 to 10 column volumes of start buffer.
6. Apply the sample at 1 mL/min for HiTrap 1 mL and 5 mL/min for HiTrap 5 mL using a syringe fitted to the luer connector 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 to 10 column volumes of elution buffer (see page 16 for Choice of gradient type).

9 The purified eluted fractions can be desalted using a HiTrap Desalting, HiPrep 26/10 Desalting or a PD-10 column if necessary.

10 After completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 columns volumes of start buffer. The column is now ready for a new sample.

For a first experiment the following conditions are recommended:

Flow rates:	1 mL/min using HiTrap 1 mL column 5 mL/min using HiTrap 5 mL column
Start buffer:	See Tables 3 and 4, Figures 4 and 5
Elution buffer:	Start buffer + 1 M NaCl
Gradient volume:	20 mL

4 Optimization

If the composition of the sample is unknown, a simple screening test using 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 to 8 (SP) or 5 to 9 (Q), with 0.5 to 1 pH unit intervals between each buffer. Make one series with 1 M NaCl included in the buffers (elution buffer) and the other without NaCl (start buffer).
2. Equilibrate the column with start buffer, see *Purification.*
3. Adjust the sample to the chosen start buffer, see *Sample preparation.*
4. Apply a constant known amount of the sample at 1 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect the eluate.
5. Wash with at least 5 column volumes of start buffer or until no material appears in the effluent. Collect the eluate.
6. Elute bound material with elution buffer. 3 to 5 column volumes are usually sufficient but other volumes might be required dependent on the exact experimental conditions. Collect the eluate.
7. Analyze all eluates (by activity assay for example) and determine the purity and the amount bound to the column.
8. Perform steps 2 to 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 salt concentration is varied in the interval 0 to 0.5 M, with intervals of 0.05 to 0.1 M salt between each buffer.

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 can be optimized to

meet the special needs can be found in the Handbook, Ion Exchange Chromatography, Principles and Methods, see *Ordering information.*

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 on 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).

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 For a first experiment the following conditions are recommended:.
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 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect eluate.

5 Wash with at least 5 column volumes of start buffer or until no material appears in the 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 to 5 column volumes are 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 to 10 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.

1 Choose starting conditions as outlined under For a first experiment the following conditions are recommended:.

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 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect eluate.

5 Wash with at least 5 column volumes of start buffer or until no material appears in the effluent.

6 Start the gradient elution. A gradient volume of 10 to 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 to 10 column volumes of start buffer. The column is now ready for a new sample.

5 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, chosen 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.

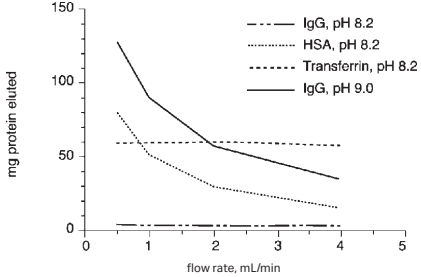

Fig 6. Binding capacity of human IgG, HSA and human transferrin at different pH's on HiTrap Q HP, 1 mL.

Samples were applied until 5% of the start material appeared in the eluent. The column was then washed with 10 mL 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.

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 to 10 column volumes of start buffer or until no material appears in the effluent.

6 Elute bound proteins with 3 to 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) and collect eluate.

7 Analyze 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.

6 Scaling up

For quick scale-up of purification, two or three HiTrap ion exchange columns of the same type can be connected in series. (back pressure will increase). For further scale-up the BioProcess™ resins, Q Sepharose XL and SP Sepharose XL, are available as prepacked HiPrep 16/10 columns and as bulk resin packs.

BioProcess chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale- Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

7 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure 7. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) can damage the column.*

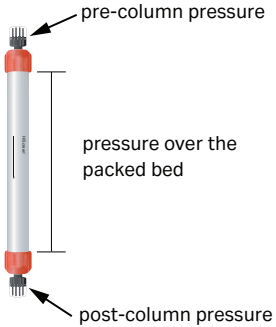

Fig 7. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on resin characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software can be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

8 Storage

HiTrap Q XL: Rinse with water then wash with 5 column volumes 20% ethanol at 1 mL/min (HiTrap 1 mL column) or at 5 mL/min (HiTrap 5 mL column) to prevent microbial growth.

HiTrap SP XL: Rinse with water then wash with 5 column volumes 20% ethanol containing 0.2 M sodium acetate at 1 mL/min (HiTrap 1 mL column) or at 5 mL/min (HiTrap 5 mL column). Seal the column with the supplied stoppers.

The recommended storage temperature is 4°C to 30°C.

Table 5. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting [1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting [2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting [3]	1.0 to 2.5 mL [4] 1.75 to 2.5 mL [5]	3.5 mL Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL [4] 0.2 to 0.5 mL[5]	1.0 mL Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL [4] 0.75 to 1 mL [5]	1.5 mL Up to 1 mL

[1] Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatographysystem to run.

[2] Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

[3] Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

[4] Volumes with gravity elution.

[5] Volumes with centrifugation.

9 Ordering information

Product	Quantity	Product code
HiTrap Q XL	5 × 1 mL	17515801
HiTrap Q XL	5 × 5 mL	17515901
HiTrap SP XL	5 × 1 mL	17516001
HiTrap SP XL	5 × 5 mL	17516101

Related products	Quantity	Product code
HiTrap IEX Selection Kit	7 × 1 mL	17600233
HiPrep Q XL 16/10	1 × 20 mL	28936538
HiPrep SP XL 16/10	1 × 20 mL	28936540
Q Sepharose XL	300 mL	17507201
S Sepharose XL	300 mL	17507301
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
	100 × 5 mL[1]	11000329
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting column	30	17085101

[1] Special package size delivered on specific customer order.

Accessories	Quantity	Product code
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18111251
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap* column)	2	18100368
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18101798
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18111257
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28401081
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11000464
Fingertight stop plug, 1/16"	5	11000355

Related literature	Product code
Ion Exchange Chromatography Handbook, Principles and Methods	11000421
Ion Exchange Columns and Media, Selection Guide	18112731
Prepacked Chromatography columns for ÄKTA design, Selection Guide	28931778
HiTrap Column Guide	18112981

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