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

Prepacked columns

Instructions for Use

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 ÄKTA™.

cytiva.com

71714900 AS

Table of contents

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, please 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	0.5 MPa (5 bar, 72.5 psi)	0.5 MPa (5 bar, 72.5 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

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 2.

BioProcess

SP Sepharose HP and Q Sepharose HP are part of the BioProcess™ range of resins. 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.

Table 2. HiTrap SP HP and HiTrap Q HP columns characteristics

Matrix	Cross-linked agarose, spherical	
Particle size, d_{50V}[1]	~ 34 µm	
Charged group	SP: – $CH_2CH_2CH_2SO_3$ Q: – $CH_2N^+(CH_3)_3$	
Ionic capacity	SP: 0.15 to 0.20 mmol H^+/mL resin Q: 0.14 to 0.20 mmol Cl^-/mL resin	
Dynamic binding capacity	SP: ~ 55 mg ribonuclease/mL resin[2] Q: ~ 70 mg BSA/mL resin[3]	
Maximum operating flow rate[4]	HiTrap 1 mL: 4 mL/min HiTrap 5 mL: 20 mL/min	
Recommended operating flow rate	HiTrap 1 mL: 1 mL/min HiTrap 5 mL: 5 mL/min	
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[5], 1.0 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol	
	Q	**SP**
pH stability, operational[6]	2 to 12	4 to 13
pH stability, CIP[7]	2 to 14	3 to 14
pH ligand fully charged	Entire operational range	Entire operationalrange
Avoid	Oxidizing agents, cationic detergents and buffers	Oxidizing agents, anionic detergents and buffers
Storage	20% ethanol, 0.2 M sodium acetate 4°C to 30°C	20% ethanol 4°C to 30°C
Avoid	Oxidizing agents, cationic detergents and buffers	Oxidizing agents, anionic detergents and buffers

1 Median particle size of the cumulative volume distribution.

2 Dynamic binding capacity measured at 1 mL/min (1 min residence time) using 0.1 M sodium acetate, pH 6.0.

3 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 150 cm/h in a PEEK 4.6/50 column at 5 cm bed height (2 min residence time) for BSA in 50mM Tris-HCl, pH 8.0.

[4] At room temperature with the same viscosity as water.

[5] 1.0 M NaOH must only be used for cleaning purposes.

[6] pH range where resin can be operated without significant change in function.

[7] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

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 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 sides of pI, either type of ion exchanger can be used, see Figure 3.

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.

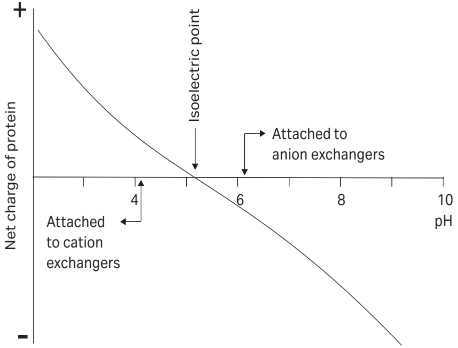

Fig 3. The net charge of a protein as a function of pH.

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 strength must 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 Table 3 and 4, and Figure 4 and 5. In the majority of cases a concentration of at least 10 mM is required for adequate buffering capacity. The ionic strength of the buffer must 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 not be so low that protein denaturation or precipitation occurs.

The buffering ion should carry the same charge as the ion exchange group and must have a pKa within 0.5 pH units of the pH used in the separation. Buffering ions of opposite charge can 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 3. 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.

Table 4. 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.

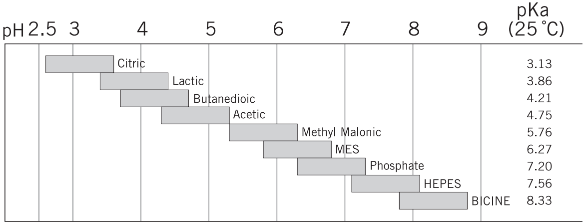

Fig 4. Recommended buffer substances for cation exchange chromatography.

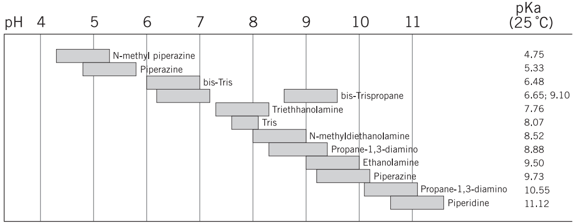

Fig 5. Recommended buffer substances for anion exchange chromatography.

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 must be of high purity. It is recommended to filter the buffers by passing them through a 0.22 µm filter immediately before use. See Table 3 and 4, and Figure 4 and 5 for recommended buffers.

Sample preparation

The sample must 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. See Table 5. The sample must be filtered through a 0.22 µm or 0.45 µm filter or centrifuged immediately before it is applied to the column.

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 chromatography system 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.

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), “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 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 to 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 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 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 to 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 3 and 4
Elution buffer:	Start buffer + 1 M NaCl
Gradient volume:	20 mL

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

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 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 (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 of start buffer or until no material appears in effluent. Collect eluate.

6. Elute bound material with elution buffer. 3 to 5 column volumes is usually sufficient. Other volumes might 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 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 ionic strength 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,* Product code 11000421.

Cytiva supplies a wide range of ion exchange chromatography resins for purification of biomolecules at all scales, see “Ordering information” and visit *cytiva.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)*.

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 “Optimization”.

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 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 to 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 to 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:

- A peristaltic pump and a gradient mixer e.g., pump P-1, gradient mixer GM-1.
- An ÄKTA chromatography system.

1 Choose starting conditions as outlined under “Optimization”.

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

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, 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 6.

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)

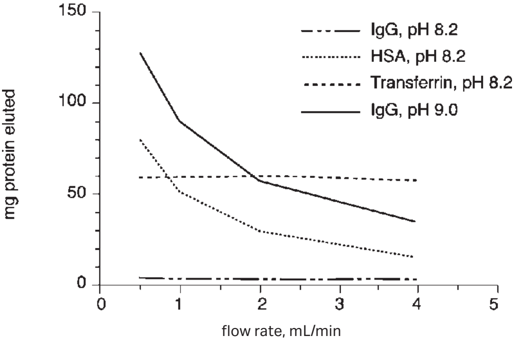

Fig 6. Binding capacity of human IgG, HSA and human transferrin at different pH's on HiTrap Q HP, 1 mL. Equilibrate the column, see "Purification".

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

4 Wash the column with 5 to 10 column volumes of start buffer or until no material appears in the effluent.

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

6 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.

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 bulk resin packs, see "Ordering information".

8 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.*

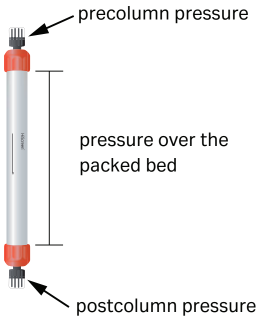

Fig 7. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn 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*.

9 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°C to 30°C.

10 Ordering information

Product	No. Supplied	Product code
HiTrap SP HP	1 × 1 mL	29051324
	5 × 1 mL	17115101
	5 × 5 mL	17115201
HiTrap Q HP	1× 1 mL	29051325
	5 × 1 mL	17115301
	5 × 5 mL	17115401

Related products	No. Supplied	Product code
HiTrap IEX Selection Kit	7 × 1 mL	17600233
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
SP Sepharose High Performance	75 mL	17108701
Q Sepharose High Performance	75 mL	17101401

[1] Special pack size delivered on specific 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 Resin, Selection Guide	18112731
HiTrap Column Guide	18112981
Prepacked chromatography columns for ÄKTA design systems	28931778

Page intentionally left blank

cytiva.com/hitrap

For local office contact information, visit cytiva.com/contact.

71714900 AS 11/2020