Instructions 28-4084-14 AD

HiTrap™ MabSelect™, 1 ml and 5 ml
HiTrap MabSelect Xtra™, 1 ml and 5 ml

HiTrap MabSelect and HiTrap MabSelect Xtra are 1 ml and 5 ml ready to use columns, prepacked with MabSelect and MabSelect Xtra, respectively.

The protein A-derived ligand is produced in *Escherichia coli* and is free from components from mammalian origin.

MabSelect is designed for fast purification of monoclonal antibodies from large sample volumes due to its compatibility with high flow rates and high pressure when scaling up.

MabSelect Xtra is designed for maximum binding capacity, which allows binding from samples with high expression levels of the monoclonal antibody.

Both media are ideal for the purification of monoclonal antibodies from lab to process scale.

The design of the HiTrap column, together with the prepacked high flow matrix and high dynamic binding capacity provides fast, simple, and easy separations in a convenient format.

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Please 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 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, 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

Medium properties

MabSelect

The recombinant protein A is produced in *Escherichia coli*. Fermentation and subsequent purification of the protein A are done in the absence of mammalian products. The recombinant protein A has been specially engineered to favor an oriented coupling that gives an affinity medium with enhanced binding capacity for IgG.

The specificity of binding to the Fc region of IgG is similar to that of native protein A, and provides excellent purification in one step. The epoxy-based coupling chemistry ensures low ligand leakage.

MabSelect is designed to tolerate high flow rates and high pressure with its specially developed base matrix. In combination with low ligand leakage, this makes MabSelect well suited for the purification of monoclonal antibodies from lab to process scale.

The characteristics of HiTrap MabSelect are summarized in Table 2.

MabSelect Xtra

MabSelect Xtra addresses the increasing levels of expression found in monoclonal antibody feedstocks. The medium is engineered to give up to 30% higher dynamic binding capacities than other Protein-A based media.

The recombinant protein A used in MabSelect Xtra is produced in *Escherichia coli.* Fermentation and subsequent purification are performed in the absence of mammalian products. The recombinant protein has been specially engineered to favor an oriented coupling that gives an affinity medium with enhanced binding capacity for IgG.

The specificity of binding to the Fc region of IgG is similar to that of native protein A and provides excellent purification in one step. High capacity and the specially engineered base matrix, make MabSelect Xtra well suited for purifying monoclonal antibodies from lab to process scale. The characteristics of HiTrap MabSelect Xtra are summarized in Table 3.

Table 2. Characteristics of HiTrap MabSelect

Matrix	Rigid, highly cross-linked agarose
Average particle size	85 μm
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 30 mg human IgG/ml medium
Recommended flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml columns, respectively
Maximum flow rates[2]	4 ml/min and 20 ml/min for 1 ml and 5 ml columns, respectively
Chemical stability[3]	Stable in all aqueous buffers commonly used in protein A chromatography: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability working short term (Cleaning-in-place, CIP)	 3 to 10 2 to 12
Storage	2°C to 8°C in 20% ethanol

[1] Determined at 10% breakthrough by frontal analysis at 500 cm/h in a column with a bed height of 20 cm, i.e. residence time is 2.4 min. Residence time is equal to bed height (cm) divided by flow velocity (cm/h) during sample loading. Flow velocity (cm/h) is equal to flow rate (ml/h) divided by column cross-sectional area (cm^2).
Note: The dynamic binding capacity may decrease for columns with bed height of 2.5 cm at the recommended flow rate (1 ml/min for 1 ml column or 5 ml/min for 5 ml column), due to too short residence time for optimal binding.

[2] H_2O at room temperature.

[3] No significant change in chromatographic performance after 1 week storage, or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 h (10 mM NaOH).

Table 3. Characteristics of HiTrap MabSelect Xtra

Matrix	Rigid, highly cross-linked agarose
Average particle size	75 µm
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 40 mg human IgG/ml medium
Recommended flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml columns, respectively
Maximum flow rates[2]	4 ml/min and 20 ml/min for 1 ml and 5 ml columns, respectively
Chemical stability[3]	Stable in all aqueous buffers commonly used in protein A chromatography: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability working short term (Cleaning-in-place, CIP)	 3 to 10 2 to 12
Storage	2°C to 8°C in 20% ethanol

1 Determined at 10% breakthrough by frontal analysis at 250 cm/h in a column with a bed height of 10 cm, i.e. residence time is 2.4 min. Residence time is equal to bed height (cm) divided by flow velocity (cm/h) during sample loading. Flow velocity (cm/h) is equal to flow rate (ml/h) divided by column cross-sectional area (cm^2).
Note: The dynamic binding capacity may decrease for columns with bed height of 2.5 cm at the recommended flow rate (1 ml/min for 1 ml column or 5 ml/min for 5 ml column), due to too short residence time for optimal binding.

2 H_2O at room temperature.

3 No significant change in chromatographic performance after 1 week storage, or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 h (10 mM NaOH)

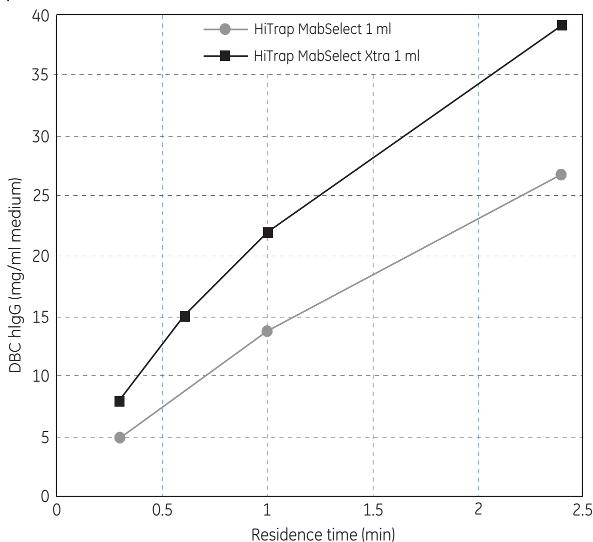

Fig 3. Dynamic binding capacity (DBC) versus residence time for HiTrap MabSelect 1 ml and HiTrap MabSelect Xtra 1 ml. Sample: 1 mg/ml human IgG (hIgG) Gammanorm™ (Octapharma); Binding buffer: 20 mM phosphate, 150 mM NaCl, pH 7.4; Elution buffer: 100 mM sodium citrate, pH 3.0. Also see Fig 4 on page 12.

Note: *Most IgG can be purified using protein A, but for some IgG protein G is the preferred ligand. See Table 4 for relative binding strengths for protein A and protein G.*
See Table 5 for affinity of protein A for selected classes of monoclonal antibodies.

Table 4. Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	–
	IgD	–	–
	IgE	–	–
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	–	++++
	IgG_4	++++	++++
	IgM*	variable	–
Avian egg yolk	IgY†	–	–
Cow		++	++++
Dog		++	+
Goat		–	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		–	+
Llama		–	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM*	variable	–
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG_1	–	+
	IgG_{2a}	–	++++
	IgG_{2b}	–	++
	IgG_3	+	++
Sheep		+/–	++

* Purify using HiTrap IgM Purification HP columns.

† Purify using HiTrap IgY Purification HP columns.

++++ = strong binding

++ = medium binding

– = weak or no binding

Table 5. Affinity of protein A for selected classes of monoclonal antibodies[1]

Antibody	Affinity	Elution pH	Binding pH
Human			
IgG_1	Very high	6.0 to 7.0	3.5 to 4,5
IgG_2	Very high	6.0 to 7.0	3.5 to 4.5
IgG_3	Low-none	8.0 to 9.0	$\leq$ 7.0
IgG_4	Low-high	7.0 to 8.0	3.0 to 6.0
Mouse			
IgG_1	Low	8.0 to 9.0	4.5 to 6.0
IgG_{2a}	Moderate	7.0 to 8.0	3.5 to 5.5
IgG_{2b}	Moderate	7.0	3.0 to 4.0
IgG_3	Low-high	7.0	3.5 to 5.5

[1] This table is compiled from a variety of sources. Comparisons should be understood to be approximate since they are derived from runs conducted under a variety of conditions.

2 Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

Recommended buffers

Binding buffer:	20 mM sodium phosphate, 0.15 M NaCl, pH 7.2
Elution buffer:	0.1 M sodium citrate, pH 3.0–3.6

Note: *When purifying mouse IgG_1 on Protein A media, an increased binding capacity will be achieved by including 2.5 M NaCl in the binding buffer.*

Sample preparation

If necessary, the sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or Desalting PD-10 column, see Table 5.

The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the columns to prevent clogging of the column when loading large sample volumes.

Purification

1. Prepare collection tubes by adding 60 to 200 µl of 1 M Tris-HCl, pH 9.0 per ml of fraction to be collected.
2. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column.
3. Remove the snap-off end at the column outlet. Wash out the ethanol with at least 5 column volumes of distilled water or binding buffer.
4. Equilibrate the column with 10 column volumes of binding buffer at 1 ml/min or 5 ml/min for 1 ml and 5 ml column respectively.

5. Apply the sample, using a syringe fitted to the Luer connector or by pumping it onto the column.
6. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
7. Elute with 2 to 5 column volumes of elution buffer using a one-step gradient or linear gradient of 0–100% elution buffer in 10 to 20 column volumes. Use a pump or chromatography system when a linear gradient is applied. Collect fractions into tubes containing 60 to 200 µl of 1 M Tris-HCl, pH 9.0 per ml of fraction to be collected. The eluted fractions can be buffer exchanged using HiTrap Desalting, HiPrep 26/10 Desalting or using a Desalting PD-10 column.
8. Regenerate the column with 5 column volumes of elution buffer.
9. Wash the column with at least 3 column volumes of binding buffer

3 Optimization

When optimizing binding conditions, one important parameter to check is the flow rate, as the residence time is important for binding capacity, see Figure 3 on page 7 and Figure 4 below.

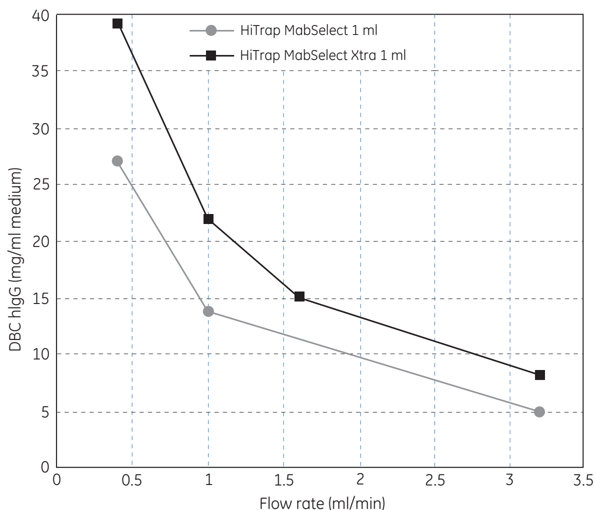

Fig 4. Dynamic binding capacity (DBC) versus flow rate for HiTrap MabSelect 1 ml and HiTrap MabSelect Xtra 1 ml. Sample: 1 mg/ml human IgG (hIgG) Gammanorm (Octapharma); Binding buffer: 20 mM phosphate, 150 mM NaCl, pH 7.4; Elution buffer: 100 mM sodium citrate, pH 3.0. Also see Figure 3 on page 7.

Optimizing elution

When optimizing elution conditions, determine the highest pH that allows efficient elution of antibody from the column. This will prevent denaturing sensitive antibodies due to exposure to low pH.

Step-wise elution allows the target antibody to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. It might be necessary to decrease the flow rate due to the high concentrations of protein in the eluted pool.

Whatever conditions are chosen, HiTrap MabSelect and HiTrap MabSelect Xtra columns can be operated with a syringe, peristaltic pump, or chromatography system.

Table 6. Prepacked columns for desalting and buffer exchange

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5–15 ml	7.5–20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25–1.5 ml	1.0–2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0–2.5 ml[1] 1.75–2.5 ml[2]	3.5 ml[1] Up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1– 0.5 ml[1] 0.2–0.5 ml[2]	1.0 ml[1] Up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5–1.0 ml[1] 0.75–1.0 ml[2]	1.5 ml[1] Up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

4 Removal of leached ligand from final product

The ligand leakage from MabSelect and MabSelect Xtra is generally very low. However, in some monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions, such as cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography.

The optimal conditions for removal of leached ligand must be evaluated for each individual antibody.

More details can be found in instructions for MabSelect (71-5021-91) and MabSelect Xtra media (11-0026-02), available for download at www.gelifesciences.com/protein-purification.

5 Cleaning-in-place (CIP)

CIP is the removal of very tightly bound, precipitated or denatured substances from the medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the column, reduce the capacity of the medium and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure, and reduce flow rate.

Regular CIP prevents the build up of contaminants and helps to maintain the capacity, flow properties, and general performance of HiTrap MabSelect and HiTrap MabSelect Xtra. When an increase in back pressure is seen, the column should be cleaned. We recommend performing a blank run, including CIP, before the first purification is started to wash out leached protein A.

The CIP protocols below should be used as guidelines for formulating a cleaning protocol specific for the sample applied to the column. The frequency of use will depend on the nature of the sample but it is recommended to use a CIP procedure at least every 5 cycles during normal use.

Depending on the nature of the contaminants, different protocols may have to be used in combination. If fouling is severe, the protocols may have to be further optimized.

To prevent cross-contamination between different antibodies, CIP should be done in between runs when the same column is used for purification of different antibodies.

Note: *The safest and fastest way to prevent cross-contamination is always to use a new column when starting to purify a new antibody.*

CIP protocol for precipitated or denatured substances using HiTrap MabSelect

1. Wash with 2 column volumes of 10 mM NaOH, contact time approx. 30 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

or

1. Wash with 2 column volumes of 6 M guanidine hydrochloride, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

CIP protocol for hydrophobically bound substances using HiTrap MabSelect

1. Wash the column with 2 column volumes of a non-ionic detergent (e.g. conc. 0.1%), contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

or

1. Wash the column with 3 to 4 columns volumes of 70% ethanol, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

or

1. Wash the column with 3 to 4 column volumes of 30% isopropanol, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

CIP protocol for precipitated or denatured substances using HiTrap MabSelect Xtra

1. Wash with 2 column volumes of 50 mM NaOH in 1.0 M NaCl, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

or

1. Wash with 2 column volumes of 50 mM NaOH in 0.5 M Na_2SO_4, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

or

1. Wash with 2 column volumes of 6 M guanidine hydrochloride in 10 mM NaOH, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

CIP protocol for hydrophobically bound substances using HiTrap MabSelect Xtra

1. Wash the column with 2 column volumes of a non-ionic detergent (e.g., conc. 0.1%), contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8.

or

1. Wash the column with 3 to 4 column volumes of 70% ethanol, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

or

1. Wash the column with 3 to 4 column volumes of 30% isopropanol, contact time approx. 10 min.
2. Wash immediately with at least 5 column volumes of filtered binding buffer at pH 7 to 8. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

6 Sanitization

Sanitization reduces microbial contamination of the chromatographic bed to a minimum.

Sanitization protocols for HiTrap MabSelect and HiTrap MabSelect Xtra

1. Equilibrate the column with 0.1 M acetic acid in 20% ethanol.
2. Allow to stand for 1 hour, and wash with at least 5 column volumes of sterile binding buffer.

or

1. Equilibrate the column with 70% ethanol.
2. Allow to stand for 12 hours, and wash with at least 5 column volumes of sterile binding buffer.

7 Scaling up

For quick scale-up of purification, two or three HiTrap columns can be connected in series (back pressure will increase). If further scale-up is necessary bulk media is available.

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 Fig 5. 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 and Table 3) may damage the column.*

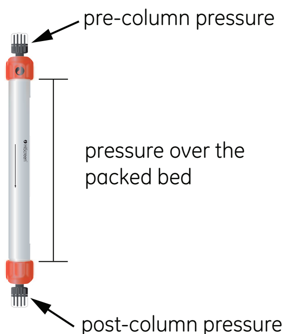

Fig 5. Pre-column and post-column measurements.

ÄKTA avant

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 media 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 may 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

Store HiTrap MabSelect and HiTrap MabSelect Xtra in 20% ethanol at 2°C to 8°C.

10 Troubleshooting

Fault	Possible cause/corrective action
High back pressure during the run.	The column is clogged. Take a new column or perform a cleaning-in-place (CIP).
Unstable pressure curve during sample application.	Remove air bubbles that might have been trapped in the sample pump. Degas the sample using a vacuum degasser.
Gradual broadening of the eluate peak.	Might be due to insufficient elution and CIP caused by contaminants accumulating in the column. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual decrease in yield.	Too high sample load. Decrease the sample load.
Precipitation during elution.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks.	Might be due to insufficient elution or CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High ligand leakage during the first purifications.	Perform a blank run, including CIP, before the first purification cycle on a new column.

11 Ordering information

Product	Pack size	Code No.
HiTrap MabSelect	5 × 1 ml	28-4082-53
	1 × 5 ml	28-4082-55
	5 × 5 ml	28-4082-56
HiTrap MabSelect Xtra	5 × 1 ml	28-4082-58
	1 × 5 ml	28-4082-60
	5 × 5 ml	28-4082-61

Related products	Pack size	Code No.
MabSelect	25 ml	17-5199-01
	200 ml[1]	17-5199-02
MabSelect Xtra	25 ml	17-5269-07
	200 ml[1]	17-5269-02
MabSelect SuRe	25 ml	17-5438-01
	200 ml[1]	17-5438-02
HiTrap MabSelect SuRe	1 × 1 ml	29-0491-04
	5 × 1 ml	11-0034-93
	1 × 5 ml	11-0034-94
	5 × 5 ml	11-0034-95
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml[2]	11-0003-29
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[1] Larger pack sizes are available.
[2] Pack size available by special order.

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16” female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16” male *(For connection to original FPLC System through top of HiTrap column*)	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” *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Literature	Code No.
Antibody Purification Handbook	18-1037-46
Solutions for antibody purification, Selection Guide	28-9351-97
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Column and Media, Selection Guide	18-1121-86

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