

HiTrap™ MabSelect SuRe™, 1 ml and 5 ml

HiTrap MabSelect SuRe is a ready-to-use column, prepacked with MabSelect SuRe, an alkali-tolerant protein A-derived medium for capturing antibodies. This prepacked column is well suited for preparative purification of monoclonal antibodies when cleaning of the medium is of importance between the purifications.

The alkali-tolerant protein A-derived ligand allows the use of 0.1 to 0.5 M sodium hydroxide for cleaning-in-place (CIP).

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

HiTrap MabSelect SuRe 1 ml and 5 ml columns are prepacked with MabSelect SuRe. The protein A-derived ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal products. The ligand has been specially engineered to create an affinity medium with enhanced alkali stability and high binding capacity for IgG. The specificity of binding to the Fc region of IgG is similar to that of conventional Protein A and provides excellent purification in one step. Alkali tolerance, high capacity and low ligand leakage plus the rigid base matrix, make MabSelect SuRe well suited for the purification of monoclonal antibodies.

The characteristics of the prepacked column are summarized in Table 2.

Table 2. Characteristics of HiTrap MabSelect SuRe

Matrix	Rigid, highly cross-linked agarose
Average particle size	85 µm
Ligand	Alkali-tolerant, protein A-derived (<i>E. coli</i>)
Coupling chemistry	Epoxy
Dynamic binding capacity ¹	Approx. 30 mg human IgG/ml medium
Recommended flow rates	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rates ²	4 and 20 ml/min for 1 and 5 ml columns, respectively
Chemical stability	Stable in all aqueous buffers commonly used in protein A chromatography.
pH, working range	3 to 12
Cleaning-in-place stability	0.1 to 0.5 M NaOH
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 (cm²).

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 H₂O at room temperature.

2 General considerations

In general, most IgG's can be purified using protein A. However, for some IgG, protein G is the preferred ligand. See Table 3 for relative binding strengths for protein A and protein G.

Table 3. 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

3 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

Sample preparation

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 4.

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 luer 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 syringe. Either using a one-step gradient or linear gradient of 0 to 100% elution buffer in 10 to 20 column volumes, using a pump or chromatography system. Collect fractions into tubes containing 60 to 200 μ l 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 3 column volumes of binding buffer.
- 10 If required perform Cleaning-in-place (CIP) with 5 column volumes of 0.1 to 0.5 M NaOH.
- 11 Re-equilibrate the column with 5 to 10 column volumes binding buffer (or until the column has reached the same pH as the binding buffer).

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

Table 4. 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 ($M_r > 5000$).
PDMiniTrap™ 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		
PDMidTrap™ 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 Sure 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 the application note *Two step purification of monoclonal IgG1 from CHO cell culture supernatant* (28-9078-92), available for download at 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.

MabSelect SuRe is an alkali-tolerant medium allowing the use of NaOH as CIP agent. NaOH is widely accepted for cleaning due to the low cost and the ability to dissolve proteins and saponify fats.

CIP protocol

- 1 Wash the column with 3 column volumes of binding buffer.
- 2 Wash with at least 2 column volumes of 0.1 to 0.5 M NaOH. Contact time 10 to 15 minutes.
- 3 Wash immediately with at least 5 column volumes of binding buffer.

CIP is usually performed immediately after the elution.

Before applying the 0.1 to 0.5 M NaOH solution, we recommend equilibrating the column with a solution of neutral pH in order to avoid the direct contact between low-pH elution buffer and high-pH NaOH solution on the column. Mixing acid and alkaline solutions might cause a rise in temperature in the column.

NaOH concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP. The nature of the sample will ultimately determine the final CIP. However, the general recommendation is to clean the column at least every fifth run when purifying the same antibody.

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.

6 Sanitization

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. MabSelect SuRe is alkali-tolerant allowing the use of NaOH as sanitizing agent. NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins. In addition, NaOH is inexpensive compared with other sanitizing agents.

Sanitization protocol

- 1 Wash the column with 3 column volumes of binding buffer.
- 2 Equilibrate the column with 0.1 to 0.5 M NaOH.
- 3 Use a contact time of at least 15 minutes (see the note below).
- 4 Wash immediately with at least 5 column volumes of binding buffer.

Note: *Higher concentrations of NaOH and/or longer contact time inactivates micro-organisms more effectively. However, these conditions might also lead to a decrease in the dynamic binding capacity. The conditions for sanitization should therefore be evaluated to maximize microbial killing and to minimize loss of capacity.*

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 3. 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) may damage the column.*

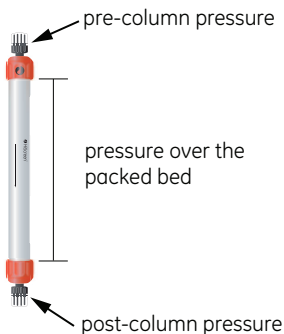


Fig 3. Pre-column and post-column measurements.

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 SuRe in 20% ethanol at 2°C to 8°C. After storage, it is recommended before use to equilibrate with binding buffer and perform a blank run, including CIP.

10 Troubleshooting

Fault	Possible cause/corrective action
High back pressure during the run.	The column is clogged. Perform 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. Might be due to insufficient elution and CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks.	Optimize the elution conditions, the IP 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	No. Supplied	Code No.
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

Related products	No. Supplied	Code No.
MabSelect SuRe	25 ml	17-5438-01
	200 ml ¹	17-5438-02
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml ²	11-0003-29
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
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 <i>(For connection of syringe to top of HiTrap column)</i>	2	18-1112-51
Tubing connector flangeless/M6 female <i>(For connection of tubing to bottom of HiTrap column)</i>	2	18-1003-68
Tubing connector flangeless/M6 male <i>(For connection of tubing to top of HiTrap column)</i>	2	18-1017-98
Union 1/16" female/M6 male <i>(For connection to original FPLC™ System through bottom of HiTrap column)</i>	6	18-1112-57
Union M6 female /1/16" male <i>(For connection to original FPLC System through top of HiTrap column)</i>	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" <i>(For sealing bottom of HiTrap column)</i>	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

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

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