cytiva

HiTrap MabSelect SuRe

1 mL and 5 mL

Prepacked columns

Instructions for Use

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

Table of contents

1. Product description 3
2. Preferred ligands 5
3. Operation 7
4. Optimization 10
5. Removal of leached ligand from final product 10
6. Cleaning-in-place (CIP) 10
7. Sanitization 12
8. Scaling up 12
9. Adjusting pressure limits 14
10. Storage 16
11. Troubleshooting 16
12. Ordering Information 17

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.

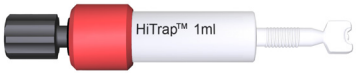

Fig 1. HiTrap, 1 mL column.

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	0.5 MPa (5 bar)	0.5 MPa (5 bar)

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

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 resin 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	Highly cross-linked agarose, spherical	
Particle size, d_{50v}[1]	~ 85 µm	
Ligand	Alkali-tolerant, protein A (*E. coli*)	
Coupling chemistry	Epoxy	
Dynamic binding capacity, Q_{B10}[2]	~ 35 mg IgG/mL resin	
	1 mL column	**5 mL column**
Recommended operating flow rate[3]	0.5 mL/min	2.5 mL/min
Maximum operating flow rate[4]	4 mL/min	20 mL/min
Chemical stability	Stable to commonly used aqueous buffers	
pH stability, operational[5]	3 to 12	
pH stability, CIP[6]	3 to 13.7	
Temperature stability	2°C to 40°C	
Storage	20% ethanol, 2°C to 8°C	

[1] Median particle size of the cumulative volume distribution

[2] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in an XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M NaH_2PO_4, pH 7.4. Flow velocity (cm/h) is equal to flow rate (mL/h) divided by column cross-sectional area (cm^2).

[3] At room temperature in buffers with the same viscosity as water.

[4] At room temperature in H_2O

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

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

Note: *The dynamic binding capacity can be optimized for process development. Increased residence time gives higher dynamic binding capacity.*

2 Preferred ligands

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

Preparation of buffers

Water and chemicals used for buffer preparation must 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 to 3.6

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

Preparation of the sample

1. If needed, adjust the sample to the composition of the start buffer using one of these two methods:
   - Dilute the sample with start buffer.
   - Exchange buffer using a Prepacked columns for desalting, refer to Table 4**.**
2. Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Prepacked columns for desalting

The prepacked columns described in Table 4 are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules (Mr > 5000).

Table 4. Prepacked columns for desalting

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 1.0 mL

[1] Prepacked with Sephadex™ G-25™ Fine, requires a pump or a chromatography system to run.

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

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

[4] Volumes with gravity elution.

[5] Volumes with centrifugation.

Purification

Note: *A blank run, including CIP, is recommended before the first run with antibody feed. This decreases the ligand leakage during the chromatography step.*

Note: *The recommended operating flow rate for HiTrap MabSelect SuRe is 0.5 or 2.5 mL/min for 1 and 5 mL column, respectively.*

1 If the eluted sample needs to be neutralized, add an alkaline buffer as 1 M Tris-HCl, pH 9.0, to the collection tubes.

2 Remove the stopper from the inlet and the snap-off end at the column outlet.

3 Connect the column to the system with 1/16" male connectors (28401081).

Note: *Make a drop-to-drop connection to prevent air from entering the column.*

Note: *Make sure that the connectors are tight to prevent leakage.*

4 Wash with 5 column volumes (CV) of distilled water to remove the ethanol. This prevents precipitation of buffer salts at exposure to ethanol.

Note: *The viscosity for 20% ethanol is higher than for water. For this step, do not use a higher flow rate than the recommended 0.5 or 2.5 mL/min for 1 and 5 mL column, respectively.*

5 Equilibrate the column with start buffer for at least 5 CV, or until the UV baseline, eluent pH, and conductivity are stable.

6 Load sample onto the column.

7 Wash with 5 to 10 CV binding buffer or until the UV trace of the effluent returns to near base line.

8 Elute by linear gradient elution or a step elution:

- *Step elution*
  Elute with 2 to 5 CV elution buffer
- *Linear gradient elution*
  Elute with 0-100% elution buffer in 10 to 20 CV

9 Wash the column with 5 CV elution buffer.

10 Re-equilibrate the column with 5-10 CV binding buffer.

11 If required, clean the column, refer to Section *Cleaning-in-place (CIP).*

12 If required, perform a buffer exchange or a desalting of the collected eluted fractions. Refer to Table 4 for recommended columns.

4 Optimization

Optimizing elution conditions

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. Stepwise 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.

5 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.

6 Cleaning-in-place (CIP)

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

Regular CIP prevents the buildup 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 must 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 resin 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 must be done in between runs when the same column is used for purification of different antibodies.

7 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 must therefore be evaluated to maximize microbial killing and to minimize loss of capacity.*

8 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up. For quick small scale-up of purification, two or three HiTrap columns can be connected in series with a union (18112093) to give increased bed height.

Note: *The back pressure will increase when the columns are connected in series. This can easily be addressed by lowering the flow rate.*

Scaling up is typically performed by keeping bed height and linear flow velocity (cm/h) constant, while increasing bed diameter and volumetric flow rate (mL/min or L/h).

Factors such as clearance of critical impurities may change when column bed height is modified and should be validated using the final bed height.

Bulk resin is available for further scaling up, see *Ordering information*.

MabSelect Sure belongs to 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

A general description of the scaling up procedure is described below.

1. Select bed volume according to required sample load. Keep sample concentration constant.
2. Select column diameter to obtain the desired bed height. The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.
3. The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes.

9 Adjusting pressure limits

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) can damage the column.*

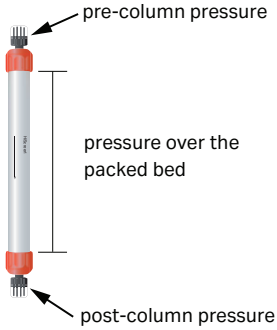

Fig 3. Pre-column and post-column measurements.

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 the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

1

- Replace the column with a piece of tubing.
- Run the pump at the maximum intended flow rate.
- Record 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.
- Record the 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.

Result: The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the total system pressure (P1).

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

10 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.

11 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. De-gas the sample using a vacuum de-gasser.
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.

12 Ordering Information

Product	No. Supplied	Product code
HiTrap MabSelect SuRe	1 × 1 mL	29049104
	5 × 1 mL	11003493
	1 × 5 mL	11003494
	5 × 5 mL	11003495

Related products	No. Supplied	Product code
MabSelect SuRe	25 mL	17543801
	200 mL[1]	17543802
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
	100 × 5 mL[2]	11000329
HiTrap MabSelect	5 × 1 mL	28408253
	1 × 5 mL	28408255
	5 × 5 mL	28408256
HiTrap MabSelect Xtra	5 × 1 mL	28408258
	1 × 5 mL	28408260
	5 × 5 mL	28408261
PD-10 Desalting Column	30	17085101
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702

[1] Larger pack sizes are available.

[2] Pack size available by special 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
Antibody Purification Handbook	18103746
Affinity Chromatography Handbook, Principles and Methods	18102229
Affinity Chromatography Column and Media, Selection Guide	18112186
Solutions for antibody purification, Selection Guide	28935197

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