GE Healthcare

Instructions 11-0034-89 AD **HiTrap affinity columns**

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 ideal for preparative purification of monoclonal antibodies when cleaning of the medium is of importance between the purifications.

The novel, alkali-tolerant protein A-derived ligand allows the use of 0.1–0.5 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.

HiTrap

Code No.	Product	No. supplied
11-0034-93	HiTrap MabSelect SuRe	5 × 1 ml
11-0034-94	HiTrap MabSelect SuRe	1 × 5 ml
11-0034-95	HiTrap MabSelect SuRe	5 × 5 ml

Connectorkit

Connector supplied	Usage	No. supplied
1/16” male/luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column*	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column**	1
Union 1/16” female/ M6 male	Connection to original FPLC™ System through bottom of HiTrap column	1
Union M6 female/ 1/16” male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16”	Sealing bottom of HiTrap column	2, 5 or 7

* Union 1/16” female/M6 male is also needed.

** Union M6 female/1/16” male is also needed.

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1. Description

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 ideal for the purification of monoclonal antibodies.

The characteristics of the prepacked column are summarized in Table 1.

Column properties

HiTrap columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. The columns have porous top and bottom frits that allow high flow rates.

Columns can be operated with either a syringe and the supplied Luer adapter, a peristaltic pump, or a chromatography system such as ÄKTAdesign™.

Note: HiTrap columns cannot be opened or refilled.

Note: To prevent leakage, ensure that the connector is tight.

Table 1. Characteristics of HiTrap MabSelect

Matrix	Rigid, highly cross-linked agarose
Average particle size	85 µm
Ligand	Alkali-tolerant, protein A-derived *(E. coli)*
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 30 mg human IgG/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate columns, respectively	1 and 5 ml/min for 1 and 5 ml
Maximum flow rates columns, respectively	4 and 20 ml/min for 1 and 5 ml
Maximum back pressure[2]	0.3 MPa, 3 bar
Chemical stability[3] commonly used in	Stable in all aqueous buffers protein A chromatography.
pH, working range	3–12
Cleaning-in-place stability)	0.1–0.5 M NaOH
Storage	+4°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]H_2O at room temperature.

2. General considerations

In general, one can purify most IgG's using protein A, but for some IgG protein G is the preferred ligand, see Table 2 for relative binding strengths for protein A and protein G.

Table 2. Relative binding strengths for protein A and protein G.

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	varible	–
	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		+/–	++

* Purified using HiTrap IgM Purification HP columns.
† Purified 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 3.

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 60–200 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 adapter), 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 adapter or by pumping it onto the column.

6. Wash with 5–10 column volumes of binding buffer or until no material appears in the effluent.
7. Elute with 2–5 column volumes of elution buffer using a syringe. Either using a one-step gradient or linear gradient of 0–100% elution buffer in 10–20 column volumes, using a pump or chromatography system. Collect fractions into tubes containing 60–200 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 3 column volumes of binding buffer.
10. If required perform Cleaning-in-place (CIP) with 5 column volumes of 0.1–0.5 M NaOH.
11. Re-equilibrate the column with 5-10 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 5. Prepacked columns for desalting and buffer exchange.

Code No	Column	Loading volume	Elution volume	Comments	Application
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 ml	Prepacked with Sephadex™ G-25 Superfine. Requires a syringe or pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15–20 ml	Prepacked with Sephadex G-25 Fine. Requires a pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0851-01	PD-10 Desalting	2.5 ml	3.5 ml	Prepacked with Sephadex G-25. Requires only gravity to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0855-01	NICK™	0.1 ml	0.4 ml	Prepacked with Sephadex G-25.	For separation of proteins (M_r>5000) and nicktranslated DNA from radiolabeled nucleotides not shorter than 120-mers, and similar separations.
17-0853-01 17-0854-01 17-0852-01	NAP™-5 NAP-10 NAP-25	0.5 ml 1.0 ml 2.5 ml	1.0 ml 1.5 ml 3.5 ml	Prepacked with Sephadex G-25 DNA grade. Requires only gravity to run.	For purification of proteins (M_r>5000), DNA and oligo-nucleotides greater than 10 bases in length.

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.gehealthcare.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 0.1–0.5 M NaOH. Contact time 10–15 minutes.
3. Wash immediately with at least 5 column volumes of binding buffer.

CIP is usually performed immediately after the elution.

Before applying the 0.1–0.5 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 5 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 0.1–0.5 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. Storage

Store HiTrap MabSelect SuRe in 20% ethanol at +4 to +8 °C. After storage, it is recommended before use to equilibrate with binding buffer and perform a blank run, including CIP.

9. Further information

Please read these instructions carefully before using MabSelect SuRe media. For further information visit www.gehealthcare.com/hitrap or www.gehealthcare.com or contact your local GE Healthcare representative.

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	5 × 1 ml	11-0034-93
HiTrap MabSelect SuRe	1 × 5 ml	11-0034-94
HiTrap MabSelect SuRe	5 × 5 ml	11-0034-95

Related products	No. Supplied	Code No.
MabSelect SuRe	25 ml	17-5438-01
MabSelect SuRe	200 ml*	17-5438-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml†	11-0003-29
HiTrap MabSelect	5 × 1 ml	28-4082-53
HiTrap MabSelect	1 × 5 ml	28-4082-55
HiTrap MabSelect	5 × 5 ml	28-4082-56
HiTrap MabSelect	Xtra 5 × 1 ml	28-4082-58
HiTrap MabSelect	Xtra 1 × 5 ml	28-4082-60
HiTrap MabSelect	Xtra 5 × 5 ml	28-4082-61
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml 1	7-5087-02

* Larger pack sizes are available.
† Pack size available by special order.

Accessories	No. Supplied	Code No.
1/16” male/luer female*	2	18-1112-51
Tubing connector flangeless/M6 female*	2	18-1003-68
Tubing connector flangeless/M6 male*	2	18-1017-98
Union 1/16” female/M6 male*	6	18-1112-57
Union M6 female /1/16” male*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16” male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16”†	5	11-0004-64
Fingertight stop plug, 1/16”‡	5	11-0003-55

* One connector included in each HiTrap package.

† Two, five, or seven stop plugs female included in HiTrap packages depending on the product.

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

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
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

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