HiTrap MabSelect PrismA

Prepacked columns

Instructions for Use

HiTrap™ MabSelect™ PrismA is a ready-to-use column, prepacked with MabSelect PrismA, an affinity BioProcess™ chromatography resin for capturing monoclonal antibodies and Fc-containing recombinant proteins.

This prepacked column is well suited for preparative purifications when cleaning of the resin between the purifications is of importance. The alkali-tolerant protein A-derived ligand allows the use of 0.5 to 1.0 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

Read these instructions carefully before using the products.

Intended use

The products 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 products in a safe way, refer to the Safety Data Sheets.

1 Product description

HiTrap column characteristics

HiTrap 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, on page 3* lists the characteristics of HiTrap columns. HiTrap MabSelect PrismA columns can be operated with a syringe, peristaltic pump, or chromatography system.

Fig 1. HiTrap, 1 mL column.

Fig 2. HiTrap, 5 mL column

Note: *HiTrap columns must not be opened or refilled.*

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 and 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 PrismA 1 mL and 5 mL columns are prepacked with MabSelect PrismA. 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 and protease stability. 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. MabSelect PrismA has very high dynamic binding capacities at most commonly used residence times.

Alkali tolerance, high capacity, and low ligand leakage plus the rigid base matrix, make MabSelect PrismA ideal for the purification of monoclonal antibodies.

This prepacked column is well suited for preparative purifications when cleaning of the resin between the purifications is of importance. The alkali-tolerant protein A-derived ligand allows the use of 0.5 to 1.0 M sodium hydroxide for cleaning-in-place (CIP).

The characteristics of the prepacked column are summarized in *Table 2, on page 5*.

Table 2. Characteristics of HiTrap MabSelect PrismA

Matrix	Rigid, highly cross-linked agarose	
Particle size, d_{50V} [1]	~ 60 µm	
Ligand	MabSelect PrismA ligand (alkali-tolerant, protein A-derived from *E. coli*)	
Coupling chemistry	Epoxy	
Dynamic binding capacity, $Q_{B10\%}$ [2]	~ 40 mg polyclonal IgG/mL resin, 2 minutes residence time[3] ~ 80 mg polyclonal IgG/mL resin, 6 minutes residence time[4]	
Chemical stability	Stable to commonly used aqueous buffers for Protein A chromatography	
pH stability,		
Operational [5]	3 to 12	
CIP [6]	2 to 14	
	1 mL column	**5 mL column**
Recommended operating flow rate [7]	0.5 mL/min	2.5 mL/min
Maximum operating flow rate [7]	4 mL/min	20 mL/min
Temperature stability [8]	2°C to 40°C	
Storage	20% ethanol, 2°C to 8°C	

1 Median particle size of the cumulative volume distribution

2 Determined at 10% breakthrough by frontal analysis in a lab scale column in PBS buffer, pH 7.4.

3 Flow rate 0.5 mL/min (78 cm/h) in a HiTrap 1 mL column with a 2.5 cm bed height.

4 Flow rate 0.8 mL/min (100 cm/h) in a HiScreen™ column with a 10 cm bed height.

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.

7 At room temperature in buffers with the same viscosity as water at 20°C.

8 Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

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

Preferred ligands

In general, most IgGs can be purified using protein A, but for some IgG, protein G is the preferred ligand. Refer to *Table 3, on page 6* 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}	-	++++

Species	Subclass	Protein A binding*	Protein G binding
	IgG_{2b}	-	++
	IgG_3	+	++
Sheep		+/-	++

* ++++ = strong binding; ++ = medium binding; - = weak or no binding

† Purify using HiTrap™ IgM and HiTrap IgY Purification HP columns, respectively.

2 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

Step	Action
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, on page 8*.
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, on page 8* are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).

Table 4. 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]	3.5 mL
	1.75 to 2.5 mL[5]	Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	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

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 PrismA is 0.5 or 2.5 mL/min for 1 and 5 mL column, respectively.*

Step	Action
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.*

Step	Action
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 CV binding buffer.
11	If required, clean the column, refer to *Chapter 5 Cleaning-in-place (CIP) , on page 12*.

Step	Action
12	If required, perform a buffer exchange or a desalting of the collected eluted fractions. Refer to *Table 4, on page 8* for recommended columns.

3 Optimization

Optimizing elution conditions

Determine the highest pH that allows efficient elution of antibody when optimizing the elution conditions. This prevents denaturation of sensitive antibodies caused by exposure to low pH. Elute into an alkaline buffer, for example 1 M Tris-HCl, pH 9.0, to neutralize the fractions.

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.

4 Removal of leached ligand from final product

The PrismA protein A ligand can be analyzed using commercial available protein A immunoassays. The ligand leakage from MabSelect PrismA 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 to remove leached ligand, such as ion exchange chromatography, multimodal exchange chromatography, and size exclusion chromatography.

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

5 Cleaning-in-place (CIP)

General description

CIP removes very tightly bound, precipitated or denatured substances from the resin. The accumulated contaminants can affect the chromatographic properties of the prepacked column, reduce the capacity, or contaminate the subsequent runs. NaOH is widely accepted for cleaning due to the low cost and the ability to dissolve proteins and saponify fats. MabSelect PrismA is a highly alkali-tolerant chromatography resin that allows the use of up to 0.5 M to 1.0 M NaOH for CIP.

CIP must be performed regularly to prevent the enrichment of the contaminants and to maintain the capacity, flow properties, and general performance of the prepacked columns.

It is recommended to perform a CIP:

- After every time with real feed.
- When an increase in the back pressure is noticed.
- If a reduced column performance is observed.
- To prevent possible cross-contamination, when the same column is used for purification of different proteins.
- Before first time use or after long time storage.

CIP protocol

Step	Action
1	Wash the column with 3 column volumes (CV) of binding buffer.
2	Wash with at least 3 CV 0.5 to 1.0 M NaOH with a contact time of 15 minutes.
3	Wash immediately with at least 5 CV sterile and filtered binding buffer at pH 7 to 8.

CIP is usually performed immediately after the elution. Before applying the alkaline NaOH CIP solution, it is recommended to equilibration of 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 feed material will ultimately determine the final CIP. However, the general recommendation is to clean the column every cycle during normal use. Depending on the nature of the contaminants, different protocols may have to be combined, for example 0.5 M NaOH every cycle and 1.0 M NaOH every 10 cycles.

6 Sanitization

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

Step	Action
1	Wash the column with 3 column volumes (CV) of binding buffer.
2	Wash the column with at least 3 CV 0.5 to 1.0 M NaOH.
3	Use a contact time of at least 15 minutes for 0.5 to 1.0 M NaOH (see also the note below).
4	Wash immediately with at least 5 CV sterile and filtered binding buffer at pH 7 to 8.

Note: *Higher concentrations of NaOH and/or longer contact time inactivates microorganisms 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

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 *Chapter 11 Ordering information , on page 20*.

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

Step	Action
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.

8 Adjusting pressure limits

The pressure generated by the flow through a column affects the packed bed and the column hardware, refer to *Figure 3, on page 17*. Increased pressure is generated when running/ using one or a combination of the following conditions:

- High flow rate
- High viscosity for buffers or sample
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit can damage the column., refer to* *Table 2, on page 5*.

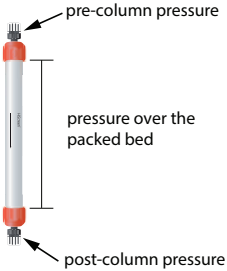

Fig 3. Pre-column and post-column measurements.

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 refer to *Table 1, on page 3*.

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:

Step	Action
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, on page 3*). • 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.*

9 Storage

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

10 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Use lower flow rate.
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	Remove air bubbles that might have been trapped in the sample pump. If possible, degas the sample using a vacuum degasser.
Gradual broadening of the eluate peak	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	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, and/or perform CIP more frequently.
Precipitation during elution	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High back pressure during CIP	Proteins are precipitated in the column.	Optimize elution conditions and/or run acid regeneration (pH 3 or less) before CIP. Use lower flow rate.
High ligand leakage during the first purifications	New column.	Perform a blank run, including CIP, before the first purification cycle on a new column.

Problem	Possible cause	Corrective action
Reduced column performance despite optimized elution and CIP	Column longevity, which depends mainly on the sample type and sample preparation.	Change to a new column.

11 Ordering information

Table 5. Products

Product	Quantity	Product code
HiTrap MabSelect PrismA	1 × 1 mL	17549851
	5 × 1 mL	17549852
	1 × 5 mL	17549853
	5 × 5 mL	17549854

Table 6. Related products

Product	Quantity	Product code
MabSelect PrismA	25 mL	17549801
	200 mL[1]	17549802
HiScreen MabSelect PrismA	1 x 4.7 mL	17549815
PreDictor™ MabSelect PrismA, 6 µL	4 × 96-well filter plates	17549830
PreDictor MabSelect PrismA, 20 µL	4 × 96-well filter plates	17549831
PreDictor MabSelect PrismA, 50 µL	4 × 96-well filter plates	17549832
HiTrap MabSelect SuRe™	1 × 1 mL	29049104
	5 × 1 mL	11003493
	1 × 5 mL	11003494
	5 × 5 mL	11003495

Product	Quantity	Product code
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting Column	30	17085101

[1] Larger pack sizes are available.

Table 7. Accessories

Product	Quantity	Product code
1/16" male/luer female	2	18111251
(For connection of syringe to top of HiTrap column)		
Tubing connector flangeless/M6 female	2	18100368
(For connection of tubing to bottom of HiTrap column)		
Tubing connector flangeless/M6 male	2	18101798
(For connection of tubing to bottom of HiTrap column)		

Table 8. Related literature

Product	Product code
Antibody Purification Handbook	18103746
Affinity Chromatography Handbook, Principles and Methods	18102229
Selection Guide Columns and resins for antibody purification and immunoprecipitation	28935197

cytiva

cytiva.com

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

29275114 AC V:4 06/2020