HiTrap™ MabSelect™ VH3

Prepacked columns

Instructions for Use

HiTrap™ MabSelect™ VH3 is a ready-to-use column, prepacked with MabSelect VH3, an affinity chromatography resin with an engineered protein A ligand that interacts only with the variable heavy chain (VH) of the VH3 sequence family of human antibody. Traditional protein A interaction with the fragment crystallizable (Fc) region of antibodies is knocked out, allowing for efficient separation of bispecific antibodies (bsAbs) and antibody fragments (Fabs, scFvs, and VHHs) that contain the VH3 sequence family.

This prepacked column is well-suited for preparative purifications where cleaning of the resin between purifications is important. The alkaline-stabilized, protein A-derived ligand allows for regular use of 0.5 M NaOH for cleaning-in-place (CIP).

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

1 Introduction

Important

Read these instructions carefully before using the product.

Safety

For safe use and handling of the product, refer to the Safety Data Sheets.

Intended use

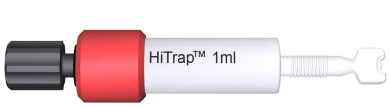
The product is intended for research use only and must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

2 Product description

Column description

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. The columns can be operated with:

- a syringe
- a peristaltic pump
- a chromatography system

HiTrap 1 mL	HiTrap 5 mL
	

Note: *Do not open or refill HiTrap columns.*

Column properties

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 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:*

- *the properties of the chromatography resin*
- *the viscosity of the sample and the liquid*
- *the type of column tubing used*

Resin description

MabSelect VH3 is an affinity BioProcess™ chromatography resin for capturing antibodies and antibody fragments containing the variable heavy chain of the VH3 sequence family. The VH3 sequence family is the most common VH class for antibodies in commercialized biologics.

The MabSelect VH3 resin ligand is specifically engineered to create affinity only for the variable region of the heavy chain (VH3). Traditional protein A ligand resins have affinity for both the Fc region and the Fab VH3 region of human antibodies. With the MabSelect VH3 resin ligand, Fc interaction is knocked out and Fab VH3 interaction is enhanced. In bioprocessing, affinity ligands with single interaction to the Fab VH3 region have advantages over dual interaction affinity ligands as separation of unwanted mispaired antibodies and fragments from the target bispecific antibodies might be more efficient.

The characteristics of the resin are summarized in *Resin properties, on page 5*.

A Regulatory Support File (RSF) is available for MabSelect VH3. The RSF contains further product data such as characteristics, quality, and chemical stability.

Resin properties

Property	HiTrap MabSelect VH3	
Matrix	Rigid, highly cross-linked agarose	
Particle size, d_{50v}[1]	~ 60 µm	
Ligand	MabSelect VH3 (alkaline-stabilized, protein A-derived from *E. coli*), no interaction with the Fc region and enhanced interaction with the VH3 region	
Coupling chemistry	Epoxy	
Dynamic binding capacity, $Q_{B10\%}$[2]	~ 70 mg IgG VH3/mL resin, 6 minutes residence time ~ 40 mg IgG VH3/mL resin, 2 minutes residence time	
Chemical stability	Stable in common aqueous buffers for protein A chromatography	
pH stability		
Operational[3]	3 to 12	
CIP[4]	2.5 to 13.7	
	1 mL column	**5 mL column**
Recommended operating flow rate[5]	0.5 mL/min	2.5 mL/min
Maximum operating flow rate[5]	4 mL/min	20 mL/min
Temperature stability	2°C to 40°C	
Storage	2°C to 8°C, 20% ethanol	
Delivery conditions	20% ethanol	

1 Median particle size of the cumulative volume distribution.
2 Determined at 10% breakthrough by frontal analysis in a HiTrap 1 mL column in 20 mM sodium phosphate, 0.15 M sodium chloride, pH 7.4. Flow rate 0.5 mL/min (78 cm/h) and 0.16 mL/min (25 cm/h).
3 pH range where the resin can be operated without significant change in function.
4 pH range where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function.
5 At room temperature in buffers with the same viscosity as water.

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

3 Operation

Buffer preparation

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

Recommended buffers

Examples of suitable buffers are shown in the table below. Buffer composition might require optimization. 20 mM sodium citrate can be used for screening purposes to determine a suitable elution pH.

Binding buffer	Wash buffer	Elution buffer
20 mM sodium phosphate, 0.15 M sodium chloride, pH 7.4	50 mM sodium acetate, pH 6.0	50 mM sodium acetate, pH 3.5

Prepare the sample

Step	Action
1	If needed, adjust the sample to the composition of the binding buffer, using one of the following methods: • Dilute the sample with binding buffer. • Exchange the buffer using a prepacked column for desalting listed in the table in the next section.
2	Immediately before loading the sample onto the column, filter the sample through a 0.45 µm filter or centrifugate the sample. This prevents clogging and increases column lifespan when loading large sample volumes.

Prepacked columns for desalting

The prepacked columns in the table below are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).

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 MidiTrap™ G-25[3]	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	Up to 1.0 mL
PD MiniTrap™ G-25[3]	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL

1 Prepacked with Sephadex™ G-25 Fine. The column requires a pump or a chromatography system to run.
2 Prepacked with Sephadex G-25 Superfine. The column requires a syringe, a pump, or a chromatography system to run.
3 Prepacked with Sephadex G-25 Medium. The column can be run by gravity flow or by centrifugation.
4 Volumes with gravity elution.
5 Volumes with centrifugation.

Column tubing

Choose a column tubing kit with an inner diameter (0.25, 0.50, or 0.75 mm) that fits column and application. A smaller inner diameter results in a higher back pressure, while a larger inner diameter results in broader peaks.

Recommended flow values

To allow proper binding, the flow rate during sample application must not be too high.

A good starting point is 0.5 mL/min for the HiTrap 1 mL column and 2.5 mL/min for the HiTrap 5 mL column.

During column equilibration and wash steps higher flow rates can be used, up to 4 mL/min for the HiTrap 1 mL column and 20 mL/min for the HiTrap 5 mL column (600 cm/h).

Purify the sample

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

Note: *For the recommended operating flow rate for the HiTrap MabSelect VH3 columns, see* *Resin properties, on page 5**.*

Step	Action
1	If the eluted sample needs to be neutralized, add an alkaline buffer like 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 fingertight connectors 1/16" male, narrow. ***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 of 20% ethanol is higher than that for water. For this step, do not use a higher flow rate than the recommended.*
5	Equilibrate the column with binding buffer for at least 5 CV or until UV baseline, eluent pH, and conductivity are stable.
6	Load the sample onto the column.
7	Wash with 5 to 10 CV wash buffer or until the UV trace of the effluent returns to near base line.
8	Elute by linear gradient elution or by step elution: • *Step elution* Elute with 2 to 5 CV elution buffer • *Linear gradient elution* Elute with 0% to 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, see *CIP, on page 11*.
12	If required, perform a buffer exchange or a desalting of the collected eluted fractions using one of the recommended columns listed in *Prepacked columns for desalting, on page 7*.

4 Optimization

Optimizing elution conditions

Determine the highest pH that allows efficient desorption of antibody from the column. Doing so prevents denaturation of sensitive antibodies due to exposure to low pH. Elute into an alkaline buffer, for example 1 M Tris-HCl, pH 9.0, to neutralize the fractions.

Stepwise elution gives a high concentration of the target molecule, with less buffer consumption and shorter cycle times. It might be necessary to decrease the flow rate due to high protein concentrations in the eluate.

5 Removal of leached ligand from final product

Leakage from MabSelect VH3 is generally low. However, in many monoclonal antibody applications it is required to remove leached ligand from the final product.

Techniques to remove leached ligand include:

- ion exchange chromatography (IEX)
- multimodal chromatography (MMC)
- size exclusion chromatography (SEC)

For an example of the removal of leached ligand and antibody aggregates, refer to application note *Two step purification of monoclonal IgG1 from CHO cell culture supernatant* (CY13148).

For more information about the removal of leached MabSelect VH3 ligand, refer to the RSF. Measurement of ligand leakage is described in the instructions for bulk resins.

6 CIP

Introduction

CIP removes very tightly bound, precipitated, or denatured substances from the resin. The accumulated contaminants can affect the chromatographic properties of the packed column, reduce the capacity, or contaminate the subsequent runs. MabSelect VH3 is an alkaline-stabilized chromatography resin that allows for the use of up to 0.5 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 packed columns.

It is recommended to perform a CIP:

- before first-time use or after long-term storage
- after each cycle with real feed
- when a deterioration of column performance is observed, such as an increase in back pressure
- to prevent cross-contamination, when the same column is used for purification of different proteins

Note: *An acid regeneration (pH 3) before CIP is recommended to remove impurities and target molecules that were not completely eluted.*

CIP optimization

NaOH concentration, contact time, and frequency are typically the main parameters to vary during CIP optimization. Longer contact times increase CIP efficiency. However, these conditions can also lead to a decrease in dynamic binding capacity.

CIP conditions must be designed for efficiency and minimal loss of capacity. The characteristics of the feed material determine the final CIP. However, the general recommendation is to clean the column every cycle during normal use. Depending on the type of contaminants, a combination of protocols might be required.

CIP recommendation

CIP is usually performed immediately after elution. Before applying the alkaline NaOH CIP solution, it is recommended to equilibrate the column with a neutral pH solution. This is to prevent direct contact between low pH elution buffer and high pH NaOH solution inside the column. Mixing acid and alkaline solutions might cause a temperature rise in the column.

CIP protocol

Follow the steps below to perform a CIP.

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

7 Sanitization

Introduction

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. MabSelect VH3 is alkaline-stabilized allowing for the use of NaOH as sanitizing agent. Depending on concentration, NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins.

Note: *Microorganisms are inactivated more effectively by using higher NaOH concentrations and longer contact times. However, these conditions can also lead to a decrease in dynamic binding capacity. Therefore, sanitization conditions must be evaluated to maximize microbial killing and to minimize loss of dynamic binding capacity.*

Sanitization protocol

The steps below are a starting point for sanitizing the column.

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

8 Scale-up

Introduction

After optimizing the method at laboratory scale, the process is ready for scale-up. For quick scale-up of purification, two or three HiTrap MabSelect VH3 columns can be connected in series for increased bed height.

Note: *Back pressure increases when columns are connected in series. Decrease back pressure 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 might change when column bed height is modified and require validation using the final bed height.

Bulk resin is available for further scaling up, see *Ordering information, on page 19*.

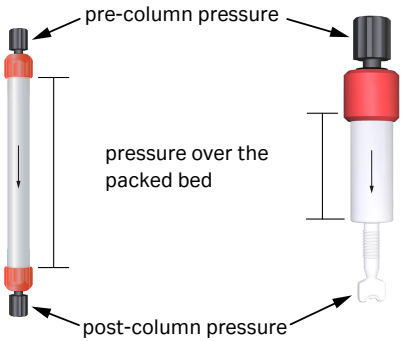
9 Adjusting pressure limits

Introduction

The pressure generated by the flow through a column affects the packed bed and the column hardware, see image below. Increased pressure is generated when using one or more of the following:

- high flow rates
- high-viscosity buffers or samples
- low temperatures
- a flow restrictor
- long and narrow tubing

Note: *Exceeding the flow limit can damage the column, see the table in* *Resin properties, on page 5*.



ÄKTA avant and ÄKTA pure

The system monitors the pressures (pre-column pressure and pressure over the packed bed, Δp) automatically. The pre-column pressure limit is the column hardware pressure limit, see the table in *Column properties, on page 3*.

The maximum pressure for the packed bed depends on resin characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the system.

Systems with a pressure sensor in the pump

For optimal system functionality, adjust the pressure limit in the software 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**. ***Note:*** *The actual pressure over the packed bed (Δp) during a run is equal to the measured pressure minus the total system pressure **P1**.*
2	• Disconnect the tubing and run the pump at the maximum intended flow rate. ***Note:*** *The column valve will drip.* • Record the pressure as **P2**.
3	• Calculate the new pressure limit as the sum of **P2** and the column hardware pressure limit, see the table in *Column properties, on page 3*. • Replace the pressure limit in the software with the calculated value.

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

10 Storage

Store HiTrap MabSelect VH3 in 20% ethanol at 2°C to 8°C. Before use after storage, it is recommended to equilibrate with binding buffer and perform a blank run, including CIP.

11 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Decrease the flow rate.
	In-line filter is clogged.	Replace the in-line filter.
	Column is clogged.	Perform CIP.
Unstable pressure curve during sample loading	Air bubbles trapped in sample pump.	Remove any air bubbles from the sample pump.
		Degas the sample using a vacuum degasser or an air trap.
Gradual broadening of the eluate peak	Insufficient elution and CIP caused by contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
Gradual decrease in yield	Sample load is too high.	Decrease the sample load.
	Precipitation during elution.	Optimize the elution conditions.
	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
Gradual increase in CIP peaks	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
High ligand leakage during the first purification cycle	Column is new.	Perform a blank run, including CIP, before the first purification cycle on a new column.

12 Ordering information

For additional information, refer to *cytiva.com*.

Products

Product	Pack size	Product code
HiTrap MabSelect VH3	1 × 1 mL	17549351
	5 × 1 mL	17549352
	1 × 5 mL	17549353
	5 × 5 mL	17549354

Related products

Product	Pack size	Product code
MabSelect VH3	25 mL	17549301
	200 mL	17549302
	1 L	17549303
	5 L	17549304
	10 L	17549305
	60 L	17549306
HiScreen™ MabSelect VH3	1 × 4.7 mL	17549315
PreDictor RoboColumn MabSelect VH3, 200 µL	1 × 8 columns	17549333
PreDictor RoboColumn MabSelect VH3, 600 µL	1 × 8 columns	17549334
PreDictor™ MabSelect VH3, 2 µL	4 × 96-well filter plates	17549330
PreDictor MabSelect VH3, 20 µL	4 × 96-well filter plates	17549331
PreDictor MabSelect VH3, 50 µL	4 × 96-well filter plates	17549332
MabSelect VH3 Validation column (10/200)	15.7 mL	17549370
HiTrap Desalting columns	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep 26/10 Desalting columns	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting columns	30 columns	17085101

Page intentionally left blank

Give feedback on this document

Visit cytiva.com/techdocfeedback or scan the QR code.

cytiva.com

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

29736926 AA V:6 11/2023