HiTrap™ MabSelect PrismA™ X prepacked columns

Affinity chromatography

Instructions for Use

HiTrap™ MabSelect PrismA™ X prepacked columns are ready-to-use and prepacked with MabSelect PrismA X protein A resin, a BioProcess™ affinity chromatography resin for capturing high-titer monoclonal antibodies.

The resin is suitable for rapid cycling chromatography (RCC), as it achieves high dynamic binding capacity at short residence times.

The prepacked columns (1 mL and 5 mL) are well-suited for preparative purifications where cleaning of the resin between purifications is important. The alkaline-tolerant protein A-derived ligand allows for regular use of 0.5 to 1.0 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.

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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 Sheet*.

Intended use

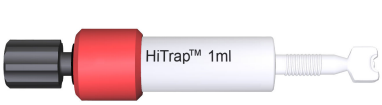
The product is intended for research use only and must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

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 the following:

- 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 following:*

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

Resin description

The protein A-derived ligand of MabSelect PrismA X resin is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal-derived products. The ligand is specifically engineered to create an affinity resin with enhanced alkaline stability and protease stability. The specificity of binding to the Fc region of IgG is similar to that of conventional protein A and provides efficient and reliable purification in one step. MabSelect PrismA X protein A resin is designed for high-

titer antibody processes and has very high dynamic binding capacities. The resin is suitable for rapid cycling chromatography (RCC), as it achieves high dynamic binding capacity at short residence times. Alkaline stability, high capacity, low ligand leakage, and a rigid base matrix make MabSelect PrismA X ideal for the purification of monoclonal antibodies for clinical applications.

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

Resin properties

Property	MabSelect PrismA X	
Matrix	Highly cross-linked agarose	
Particle size, d_{50v}[1]	50 µm	
Ligand	Alkaline-stabilized, protein A-derived (from *E. coli*)	
Coupling chemistry	Epoxy	
Dynamic binding capacity, $QB_{10\%}$[2]	~ 80 mg trastuzumab/mL resin, 6 minutes residence time ~ 55 mg trastuzumab/mL resin, 2 minutes residence time	
Chemical stability	Stable in aqueous buffers commonly used in protein A chromatography	
pH stability		
Operational[3]	3 to 12	
CIP[4]	2 to 14	
	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	

[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 resin can be operated without significant change in function.
[4] pH range where 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 at 20°C.

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

With MabSelect PrismA X protein A resin, it is recommended to use phosphate and acetate buffers for typical monoclonal antibody (mAb) processes. Examples of suitable buffers are shown in the table below. Buffer composition might require optimization. Most mAbs elute using the recommended elution buffer, but 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

Follow the steps below to prepare the sample.

Note: *For CHO cell culture harvests, go directly to step 2.*

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 0.22 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 0.5 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).

Purification protocol

Note: *A blank run, including CIP, is recommended before the first run with sample. This decreases ligand leakage during the chromatography step. For the CIP protocol, see* *CIP protocol, on page 12**.*

Note: *For the recommended operating flow rate for the HiTrap MabSelect PrismA X 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 the column with 5 column volumes (CV) distilled water to remove the storage solution. 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 base-line, eluent pH, and conductivity are stable.
6	Load the sample onto the column.
7	Wash the column 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 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	Clean the column as recommended, see *CIP protocol, on page 12*.
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 for efficient desorption of the target molecule from the column. Doing so prevents denaturation of sensitive molecules due to exposure to low pH. Elute into an alkaline buffer (e.g., 1 M Tris-HCl, pH 9.0) to neutralize the fractions.

Step 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 the final product

Leakage of the protein A ligand of MabSelect PrismA X resin can be analyzed using PrismA ELISA Kit (29707299). MabSelect PrismA protein A free ligand in solution is available for leakage determination and other analytical purposes.

Ligand leakage from the resin is generally low. However, in many applications it is a requirement 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 the application note *Two-step purification of monoclonal IgG1 from CHO cell culture supernatant (CY13148)*, available on *cytiva.com*. Methods used for the removal of leached ligand from MabSelect SuRe™ and MabSelect SuRe LX are also applicable to the removal of leached ligand from MabSelect PrismA X.

6 Cleaning-in-place (CIP)

Introduction

CIP is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the buildup of impurities or contaminants in the packed bed and helps to maintain capacity, flow properties, and general performance. MabSelect PrismA X is an alkaline-stabilized chromatography resin that allows for the use of 0.5 to 1.0 M NaOH for CIP.

Note: *It is recommended to perform an acidic strip (pH 3) before CIP in order to remove impurities and target molecules that were not completely eluted.*

It is recommended to perform a CIP in the following situations:

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

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, this 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 impurities or contaminants, a combination of protocols might be required, for example, 0.5 M NaOH every cycle and 1.0 M NaOH every 10 cycles.

For challenging cases where CIP with NaOH is not sufficient to restore the column performance, it is recommended to use an extended protocol including wash with 100 mM thioglycerol, pH 8.5 followed by CIP with 0.5 to 1.0 M NaOH. For more details, refer to the application note *High-throughput process development for design of cleaning-in-place protocols (CY14702)*, available on cytiva.com.

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 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 binding buffer at pH 7 to 8.

7 Sanitization

Introduction

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. The resin 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. For more information, refer to the application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*, available on cytiva.com.

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 0.5 M or 1.0 M NaOH. Use a contact time of at least 1 h.
3	Wash immediately with at least 5 CV sterile binding buffer at pH 7 to 8.

Flow values for HiTrap MabSelect PrismA X columns are shown in *Recommended flow values, on page 7*.

8 Scale-up

Introduction

After optimizing the method at laboratory scale, consider scaling up to purify larger volumes of feed material. To evaluate purification performance at an increased bed height, two or three HiTrap columns can be connected in series.

Note: *Back pressure increases when columns are connected in series. Decrease back pressure by lowering the flow rate.*

Points of consideration:

- Scaling up typically involves maintaining the bed height and linear flow velocity (cm/h), while increasing the bed diameter and the volumetric flow rate (mL/min or L/h).
- Keep the residence time constant to maintain the dynamic binding capacity.
- Make sure that the chosen velocity does not exceed the large scale pressure or flow limitation.
- Factors such as clearance of critical impurities might change when column bed height is modified and need to be validated using the final bed height.

Bulk resin is available for further scaling up, see *Related products, on page 19*.

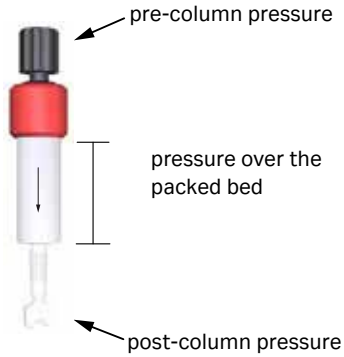
9 Adjusting pressure limits

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 chromatography systems

The system monitors the pressures automatically. The following table describes which pressures are monitored by each system.

System	Pressures monitored
ÄKTA pure™ without column valve **V9-C**	• system pressure • pre-column pressure

System	Pressures monitored
ÄKTA™ avant and ÄKTA pure with column valve **V9-C**	• system pressure • pre-column pressure • pressure over the packed bed, *Δp*

The pre-column pressure limit is the column hardware pressure limit. The limits are described in *Column properties, on page 4*.

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

Systems without multiple pressure sensors

Systems without multiple pressure sensors only measure the system pressure. For optimal system functionality, adjust the pressure limit in the software as follows:

Step	Action
1	**a.** Replace the column with either a piece of tubing with a large inner diameter or a connector with zero dead-volume. Keep all tubing connected to the instrument, including the tubing running to and from the column. **b.** Run the pump at the maximum intended flow rate. **c.** 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	**a.** Disconnect the tubing and run the pump at the maximum intended flow rate. ***Note:*** *The column valve will drip.* **b.** Record the pressure as **P2**.
3	**a.** Calculate the new pressure limit as the sum of **P2** and the column hardware pressure limit. See *Column properties, on page 4* for the hardware pressure limit for the given column. **b.** Replace the pressure limit in the software with the calculated value.

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

10 Storage

Store the columns in 20% ethanol at 2°C to 8°C. Do not freeze. Make sure that the columns are sealed, to prevent them from drying out.

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 PrismA X	1 × 1 mL	17550151
	5 × 1 mL	17550152
	1 × 5 mL	17550153
	5 × 5 mL	17550154

Related products

Product	Pack size	Product code
MabSelect PrismA X	25 mL	17550101
	200 mL	17550102
	1 L	17550103
	5 L	17550104
	10 L	17550105
HiScreen™ MabSelect PrismA X	1 × 4.7 mL	17550115
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 units	17085101
PD MidiTrap G-25	50 columns	28918008
PD MiniTrap G-25	50 columns	28918007

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