HiScreen™ MabSelect PrismA™ X prepacked column

Affinity chromatography

Instructions for Use

HiScreen™ MabSelect PrismA™ X prepacked column is ready-to-use and prepacked with MabSelect PrismA X protein A resin, a BioProcess™ affinity chromatography resin for fast capturing of 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 dynamic binding capacity is 74 mg/mL at 4 min residence time and as high as 80 mg/mL at 6 min residence time.

The prepacked column is 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 HiScreen column design, combined with highly cross-linked matrix and the high dynamic binding capacity of the prepacked resin, provides fast separations in a convenient format for method optimization and small-scale purification.

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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*.

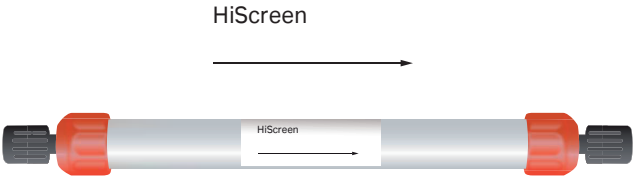
Intended use

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

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and at the outlet. The arrow on the column label shows the recommended flow direction, see image below.



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

The HiScreen column format is suitable for parameter and method optimization, and for robustness testing when developing a new purification process. The small column volume and the bed height enable scalable experiments at relevant process flow rates. Depending on sample characteristics, the column can be reused for up to ten feed cycles. If necessary, two columns can be connected in series with a union to give a 20 cm bed height, see *Chapter 9 Scale-up, on page 17*.

Column properties

Column volume (CV)	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	0.8 MPa (8 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

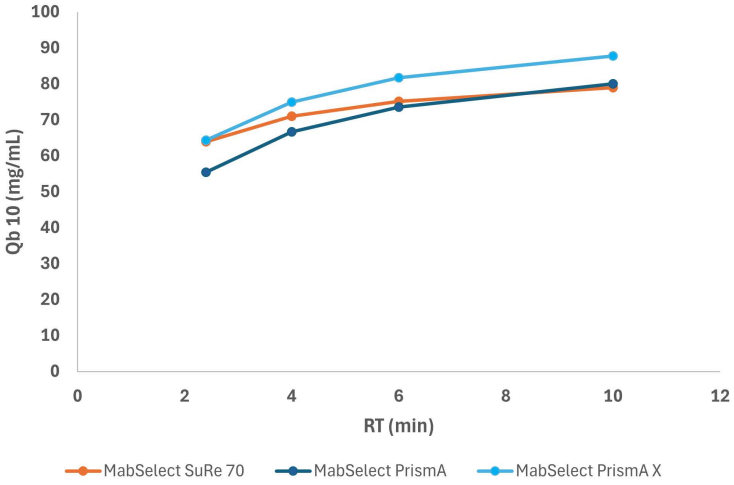
The protein A-derived MabSelect PrismA ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal-derived products. The ligand is specifically engineered to create an affinity resin with enhanced alkaline stability and protease stability. 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 rapid cycling chromatography (RCC), as it has very high dynamic binding capacities at short residence times.

Alkaline stability, high capacity, low ligand leakage, and a rigid base matrix make ideal for the purification of monoclonal antibodies for clinical applications.

The characteristics of the resin are summarized in *Resin properties, on page 6*.

Dynamic binding capacity

MabSelect PrismA X has a high dynamic binding capacity at commonly used residence times. The figure below shows the dynamic binding capacity of the resin compared to MabSelect PrismA and MabSelect™ SuRe 70 at 10% breakthrough ($QB_{10\%}$) for trastuzumab (with a bed height of 10 cm) tested in HiScreen columns.



As MabSelect PrismA X has high dynamic binding at short residence times, the resin is suitable to use in rapid cycling chromatography (RCC) mode to further reduce resin volumes, increase grams of mAb produced per liter of resin, and decrease overall resin costs.

Resin properties

The characteristics of the resin are summarized in the table below.

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 ~ 74 mg trastuzumab/mL resin, 4 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
Recommended flow velocity	Flow values for HiScreen MabSelect PrismA X columns are shown in *Recommended flow values, on page 9*.
Maximum operating flow velocity	220 cm/h (20 cm bed height)[5] 400 cm/h (10 cm bed height)
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 at a mobile phase velocity of 100 cm/h (6 min residence time) and 150 cm/h (4 min residence time) in PBS buffer, pH 7.4.
[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] In an AxiChrom™ 1000 column with 100 cm inner diameter at 20 cm bed height, operating pressure up to 3 bar, using buffers with the same viscosity as water at 20°C.

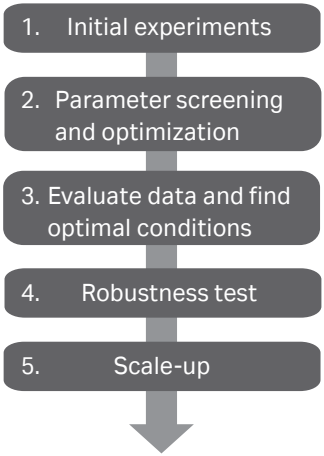
Note: *The dynamic binding capacity can be optimized for process development. Increased residence time gives higher dynamic binding capacity.*

3 Process development

General description

The HiScreen column format is suitable for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume of 4.7 mL and the 10 cm bed height enable scalable experiments at relevant process flow rates. If necessary, two columns can be connected in series with a union to give a 20 cm bed height, see *Chapter 9 Scale-up, on page 17*.

The figure below shows the typical steps during process development.



Early on in the development of a purification process, aspects like process cost, resin cleaning, and environmental constraints need to be considered.

Design of Experiments (DoE) is an effective tool for method parameter screening, optimization, and robustness testing of a purification process. For more information, refer to handbook *Design of Experiments in Protein Production and Purification* (*cytiva.com/handbooks*).

A common approach in DoE is to define a reference experiment (center point) and to perform representative experiments around that point. Some initial experiments are required to define the center point and the variable ranges.

Robustness is the ability of a process to perform reliably and deliver the desired outcomes, even in the presence of minor variability or uncertainties in the input parameters or conditions. A robustness test evaluates factors that can cause differences in the method response, such as purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see *Chapter 9 Scale-up, on page 17*.

4 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

The table below lists the recommended flow values for the HiScreen MabSelect PrismA X column during specific operations.

Operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration[1]	≤ 3.1	≤ 400	≥ 1.5
Washing[1]	≤ 3.1	≤ 400	≥ 1.5
Sample loading[1]	0.78 to 1.55	100 to 200	6 to 3
CIP[2]	≤ 0.93	≤ 120	≥ 5

1 The flow rates stated are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, such as 20% ethanol, lower flow rates must be used.
2 CIP must be performed with at least 3 CV and a total contact time of at least 15 min, see also *CIP protocol, on page 15*.

Purification protocol

Follow the steps below to perform a purification. For the recommended operating flow values for the HiScreen MabSelect PrismA X column, see the table in *Recommended flow values, on page 9*.

Note: *A blank run, including CIP, is recommended before the first run with sample. This decreases the ligand leakage during the chromatography step. For the CIP protocol, see CIP protocol on page 15.*

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 stoppers and connect the column to the system. ***Note:*** *Make a drop-to-drop connection to prevent air from entering the column.* ***Note:*** *Use a fingertight 1/16" connector (28401081).*
3	Wash the column with 5 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 of water. For this step, do not use a higher flow rate than 0.85 mL/min (110 cm/h).*
4	Equilibrate the column with 5 CV binding buffer.
5	Load the sample onto the column.
6	Wash the column with 5 to 10 CV wash buffer, or until the UV trace of the effluent returns to near baseline.
7	Elute by linear gradient elution or 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.
8	Wash the column with 5 CV elution buffer.

Step	Action
9	Re-equilibrate the column with 5 to 10 CV binding buffer, or until the UV signal, eluent pH, and conductivity reach the required values. ***Note:*** *Do not exceed the maximum recommended flow rate or back pressure for the column.*
10	Clean the column as recommended, see *CIP protocol, on page 15*.
11	If required, perform a buffer exchange or a desalting of the collected eluted fractions using a recommended column listed in *Prepacked columns for desalting, on page 9*.

5 Optimization

Optimizing elution conditions

Determine the highest pH that allows efficient desorption of the target molecule from the column. Doing so prevents denaturation of sensitive molecules caused by low pH exposure. Elute into an alkaline buffer, for example 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 the high protein concentrations in the eluate.

6 Removal of leached ligand from the final product

Leakage of the MabSelect PrismA protein A ligand 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.

7 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.

Flow values for HiScreen MabSelect PrismA X columns are shown in *Recommended flow values, on page 9*.

8 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 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. But these conditions can 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 HiScreen MabSelect PrismA X columns are shown in *Recommended flow values, on page 9*.

9 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 a bed height of 20 cm, two HiScreen 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 22*.

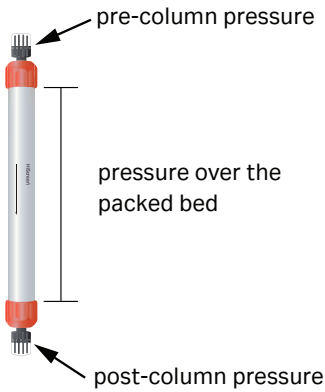
10 Adjusting pressure limits

Introduction

The pressure generated by the flow through a column affects the packed bed and the column hardware, see the following image. Increased pressure is generated when running the column 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 column-specific limit in* *Recommended flow values, on page 9**.*



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.*

11 Storage

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

Before use after storage, it is recommended to equilibrate with binding buffer and perform a blank run, including CIP.

12 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.

13 Ordering information

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

Products

Product	Pack size	Product code
HiScreen MabSelect PrismA X	1 × 4.7 mL	17550115

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
HiTrap MabSelect PrismA X	1 × 1 mL	17550151
	5 × 1 mL	17550152
	1 × 5 mL	17550153
	5 × 5 mL	17550154
HiTrap Desalting columns	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep Desalting columns	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting columns	30 columns	17085101
PD MidiTrap G-25	50 columns	28918008
PD MiniTrap G-25	50 columns	28918007

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