HiScreen™ MabSelect™ VL Prepacked columns

Instructions for Use

HiScreen™ MabSelect™ VL is a ready-to-use chromatography column, prepacked with MabSelect VL resin, an affinity BioProcess™ chromatography resin for capturing bispecific antibodies and antibody fragments containing the kappa light chain.

This prepacked column is well suited for preparative purification of bispecific antibodies and mAb derived fragments. The product also enables separation of homodimers from heterodimers for kappa-lambda bispecific antibodies during the capture step. The alkaline tolerant protein L-derived ligand allows for the use of 0.1 M sodium hydroxide for cleaning-in-place (CIP).

1 Introduction

Important

Read these instructions carefully before using the product.

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.

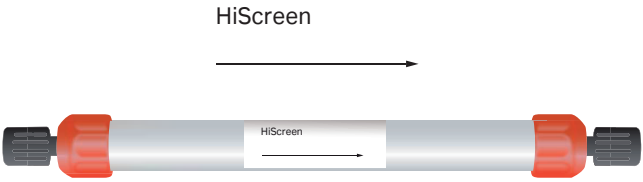
Safety

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

2 Product description

Column description

HiScreen columns are made of biocompatible polypropylene that does not interact with the biomolecules. The columns are delivered with stoppers at the inlet and 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 might be reused for up to 10 feed cycles. If scale-up requires a larger bed height, two columns can be connected in series using a union to give a 20 cm bed height, see *Scale-up, on page 16*.

Column properties

Column volume	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:*

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

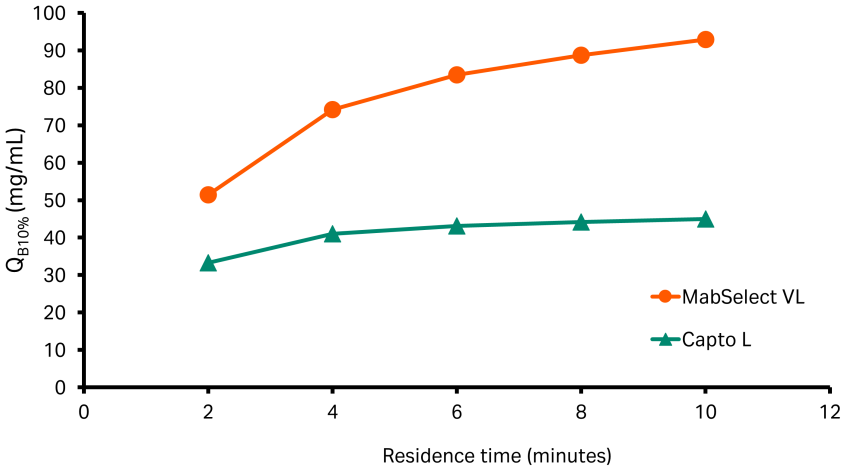
Resin description

MabSelect VL is an affinity BioProcess chromatography resin for capturing bispecific antibodies and antibody fragments containing the kappa light chain. The resin combines a rigid, high-flow agarose base matrix with a new generation of protein L ligand designed to have a higher dynamic binding capacity and higher alkaline tolerance than Capto™ L resin. The ligand of MabSelect VL resin is recombinantly produced in *Escherichia coli* and originates from a domain of protein L from the bacterium *Peptostreptococcus magnus*. Fermentation and subsequent purification are performed in the absence of animal derived products. The ligand has been specifically engineered for improved alkaline stability.

Alkaline tolerance, high dynamic binding capacity at most commonly used residence times, low ligand leakage, and a rigid base matrix make MabSelect VL resin suited for diversified antibody processes involving the purification of mAb derived fragments and bispecific antibodies for clinical applications.

Resin dynamic binding capacity

MabSelect VL resin has a high dynamic binding capacity at most commonly used residence times. The image below shows a comparison between the dynamic binding capacity of MabSelect VL resin and Capto L resin at 10% breakthrough ($Q_{B10\%}$) for IgGκ1, determined in a HiScreen column.



Resin properties

	MabSelect VL
Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50V}[1]	~ 60 µm
Ligand	Alkaline stabilized, protein L-derived (*E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10\%}$[2]	~ 60 mg IgGk1/mL resin, 4 minutes residence time ~ 70 mg IgGk1/mL resin, 6 minutes residence time
Chemical stability	Stable in commonly used aqueous buffers for protein L chromatography
pH stability	
Operational[3]	2 to 10
CIP[4]	2 to 13
Recommended flow velocity	Flow values for HiScreen MabSelect VL columns are shown in *Recommended flow values, on page 8*.
Maximum operating flow velocity[5]	300 cm/h
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 a lab scale column with a 10 cm bed height in PBS buffer, pH 7.2.
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™ column with 30 cm diameter and a 20 cm bed height, using buffers with the same viscosity as water at 20°C. The maximum operating flow velocity also applies to a HiScreen column.

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

3 Process development

3.1 General description

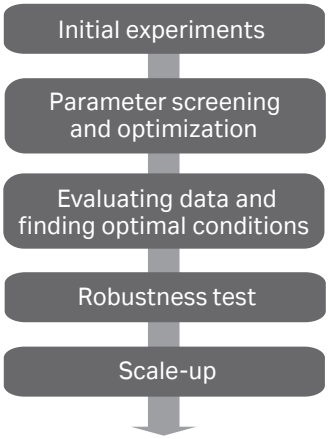
It is important to consider process cost, cleaning of the resin, and environmental contraints early in the development of a purification process.

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 *Scale-up, on page 16*.

Design of Experiments (DoE) is an effective tool for method parameter screening, optimization, and robustness testing of a purification process, 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.

The figure below shows the typical steps during process development.



The robustness of a process is a measure of its capacity to remain unaffected by variations, and shows the process reliability during normal usage. A robustness test evaluates factors potentially causing variability in the process, detected by responses of methods, for example purity and yield. For this purpose, small deliberate variations in the process parameters are introduced.

For scale-up, see *Scale-up, on page 16*.

4 Operation

Buffer preparation

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

For MabSelect VL resin a citrate based buffer system is recommended for elution. The buffering capacity of a citrate buffer is better suited for the recommended pH ranges in comparison to an acetate or phosphate buffer system.

Binding buffer:	20 mM sodium phosphate, 0.15 M NaCl, pH 7.4
Elution buffer:	50 mM sodium citrate, pH 2.5

Sample preparation

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 buffer using a prepacked column for desalting listed in the table in the next section.
2	Filter the sample through a 0.45 µm filter, or centrifugate 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 in the table 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 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 1.0 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 pump or a chromatography system to run.
[3] Prepacked with Sephadex G-25. The column can be run by gravity flow or by centrifugation.
[4] Volumes with gravity elution.
[5] Volumes with centrifugation.

Recommended flow values

The table below lists the recommended values for different operations for HiScreen MabSelect VL.

Operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration[1]	≤ 2.3	≤ 300	≥ 2
Washing[1]	≤ 2.3	≤ 300	≥ 2
Sample loading	0.6 to 2.3	75 to 300	8 to 2
Cleaning-In-Place[2]	≤ 0.78	≤ 100	≥ 6

[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] Cleaning-In-Place must be performed with at least 3 CV and a total contact time of at least 15 min. See also Cleaning-in-place (CIP).

Column tubing

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

Purification

Follow the steps below to perform a 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: *For the recommended operating flow rate for the HiScreen MabSelect VL column, see* *Recommended flow values, on page 8**.*

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 fingertight 1/16" connector (28401081).*
3	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 of water. For this step, do not use a higher flow rate than 1.2 mL/min (150 cm/h).*
4	Equilibrate the column with 5 CV binding buffer.
5	Load the sample onto the column.
6	Wash with 5 to 10 CV binding 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.
9	Wash the column with 3 CV binding buffer.

Step	Action
10	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.*
11	If required, clean the column, refer to *Cleaning-In-Place (CIP), on page 13*.
12	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 8*.

5 Optimization

Optimizing elution conditions

Determine the highest pH that allows efficient elution of antibodies. 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, reducing buffer consumption and cycle times. It might be necessary to decrease the flow rate due to the high protein concentrations in the elution pool.

6 Removal of leached ligand from final product

The MabSelect VL protein L ligand can be analyzed using commercially available protein L immunoassays. For more information, contact Cytiva. Ligand leakage from MabSelect VL is generally low, but 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) and multimodal chromatography (MMC).

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

Methods used for removal of leached ligand from MabSelect PrismA™ are applicable also to removal of leached ligand from MabSelect VL.

7 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 packed column, reduce the capacity, or contaminate the subsequent runs. HiScreen MabSelect VL chromatography resin allows the use of up to 0.1 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:

- after each time with real feed
- when an increase in 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-term storage

Note: *An acid regeneration (pH 2.3) before CIP is recommended if the antibodies were not completely eluted.*

CIP protocol

Follow the steps below to perform a CIP.

Step	Action
1	Wash the column with 3 CV binding buffer.
2	Wash with at least 3 CV NaOH (up to 0.1 M), 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 optimization

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

The conditions for CIP must be designed for efficient CIP and minimized loss of capacity. The nature of the feed material ultimately determines 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 might have to be combined.

CIP recommendation

CIP is usually performed immediately after the elution. Before applying the alkaline NaOH CIP solution, it is recommended to equilibrate the column with a solution of neutral pH 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.

8 Sanitization

Overview

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

Sanitization protocol

Follow the steps below to sanitize the column.

Step	Action
1	Wash the column with 3 CV binding buffer.
2	Wash the column with at least 3 CV NaOH (up to 0.1 M).
3	Use a contact time of at least 15 minutes for 0.1 M NaOH.
4	Wash immediately with at least 5 CV sterile and filtered binding buffer at pH 7 to 8.

Note: *Higher concentrations of NaOH and longer contact times inactivate 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.*

9 Scale-up

Overview

After optimizing the method at laboratory scale, the process is ready for scale-up. For quick small scale-up of purification, two or three HiScreen MabSelect VL columns can be connected in series to give 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 need to be validated using the final bed height.

Bulk resin is available for further scaling up, see *Ordering information, on page 21*.

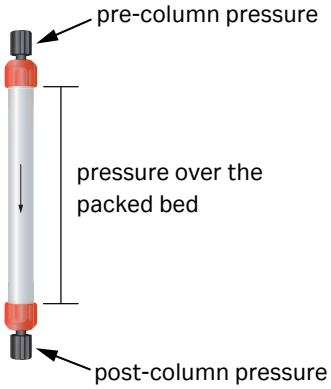
10 Adjusting pressure limits

Overview

The pressure resulting from a flow through a column affects the packed bed and the column hardware, as shown in the image below. Pressure increases in connection with:

- high flow rate
- high-viscosity buffer or sample
- low temperature
- flow restrictor
- long and narrow tubing

Note: *Exceeding the flow limit can damage the column, see* *Recommended flow values, on page 8**.*



ÄKTA avant and ÄKTA pure

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

The maximum pressure for the packed bed depends on the resin characteristics and the sample/liquid viscosity. The pressure value also depends on the tubing that is used between the column and the instrument.

Systems with a pressure sensor in the pump

Follow the steps below to adjust the pressure limit in the software for systems with a pressure sensor in the pump.

Step	Action
1	Replace the column with a piece of tubing.
2	Run the pump at the maximum intended flow rate.
3	Record the pressure as total system pressure, P1.
4	Disconnect the tubing and run the pump at the same flow rate used in *step 2* .
5	Note that there will be a drip from the column valve.
6	Record the pressure as P2.
7	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit, see *Column properties, on page 3*.
8	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 minus the total system pressure (P1).

Note: *Repeat the procedure each time parameters change.*

11 Storage

Store HiScreen MabSelect VL 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.

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.
	Adapter net/filter is clogged.	Clean or replace the adapter net/filter.
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, 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, or perform CIP more frequently.
Gradual increase in CIP peaks	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, 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, see *cytiva.com*.

Products

Product	Quantity	Product code
HiScreen MabSelect VL	1 × 4.7 mL	17542015

Related products

Related product	Quantity	Product code
MabSelect VL	25 mL	17542001
	200 mL	17542002
	1 L	17542003
	5 L	17542004
	10 L	17542005
HiTrap MabSelect VL	1 × 1 mL	17542051
	5 × 1 mL	17542052
	1 × 5 mL	17549853
	5 × 5 mL	17549854
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	30 columns	17085101
PD MiniTrap G-25	50 columns	28918007
PD MidiTrap G-25	50 columns	28918008

Related documentation

All items below are available at *cytiva.com*.

Related documentation	Reference
Application notes	
Capture of human single-chain Fv (scFv) fusion protein on Capto L affinity medium	CY13389
High-throughput process development for design of cleaning-in-place protocols	CY14702
Data file	
MabSelect VL	CY26149
Handbook	
Affinity Chromatography, Vol. 1: Antibodies	CY13981
Selection guide	
Columns and resins for antibody purification and immunoprecipitation	CY12803

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