cytiva

HiScreen™ MabSelect™ VH3

Prepacked column

Instructions for Use

HiScreen™ 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 HiScreen 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.

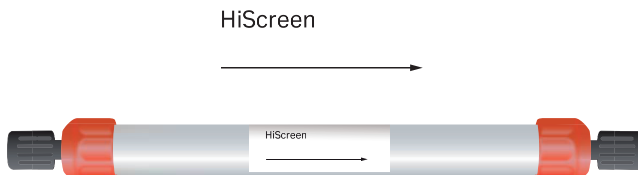
Intended use

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

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 *Scale-up, on page 18*.

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:*

- *properties of the chromatography resin*
- *viscosity of the sample and the liquid*
- *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 4*.

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

The characteristics of the resin are summarized in the table below. The dynamic binding capacity stated can be optimized for process development. Increased residence time gives higher dynamic binding capacity.

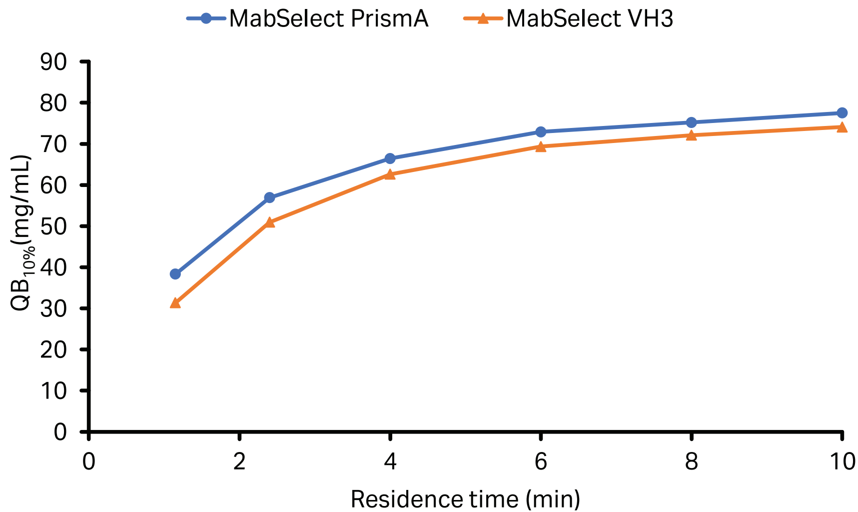
Property	MabSelect VH3
Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50v}[1]	~ 60 µm
Ligand	MabSelect VH3 (alkaline-stabilized, protein A-derived from *E. coli*), no interaction with the Fc region and enhanced interaction with the VH3 region
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10\%}$[2]	~ 70 mg IgG VH3/mL resin, 6 minutes residence time ~ 60 mg IgG VH3/mL resin, 4 minutes residence time
Chemical stability	Stable in commonly used aqueous buffers for protein A chromatography
pH stability	
Operational[3]	3 to 12
CIP[4]	2.5 to 13.7
Recommended flow velocity	Flow values for HiScreen MabSelect VH3 columns are shown in *Recommended flow values, on page 10*.
Maximum operating flow velocity[5]	300 cm/h
Temperature stability	2°C to 40°C
Delivery conditions	20% ethanol

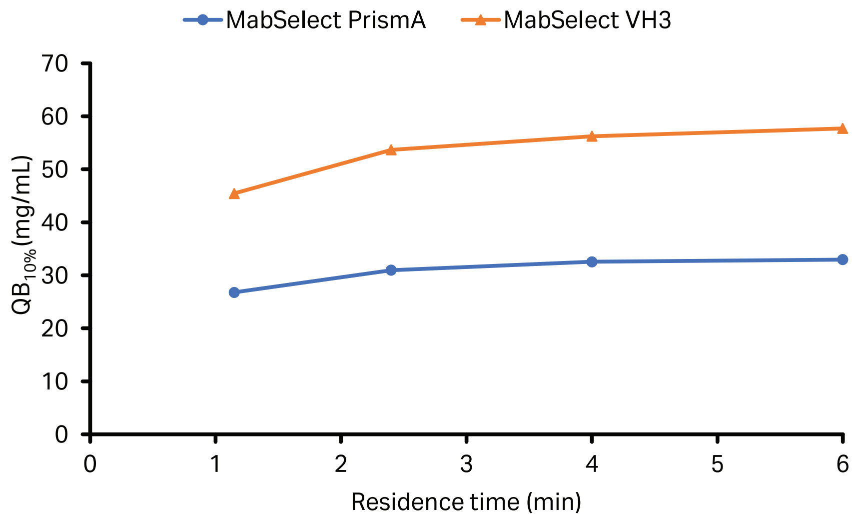
1 Median particle size of the cumulative volume distribution.
2 Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h (6 min residence time) and 150 cm/h (4 min residence time) in PBS buffer, pH 7.4.
3 pH range where the resin can be operated without significant change 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™ 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 value stated also applies to HiScreen MabSelect VH3.

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

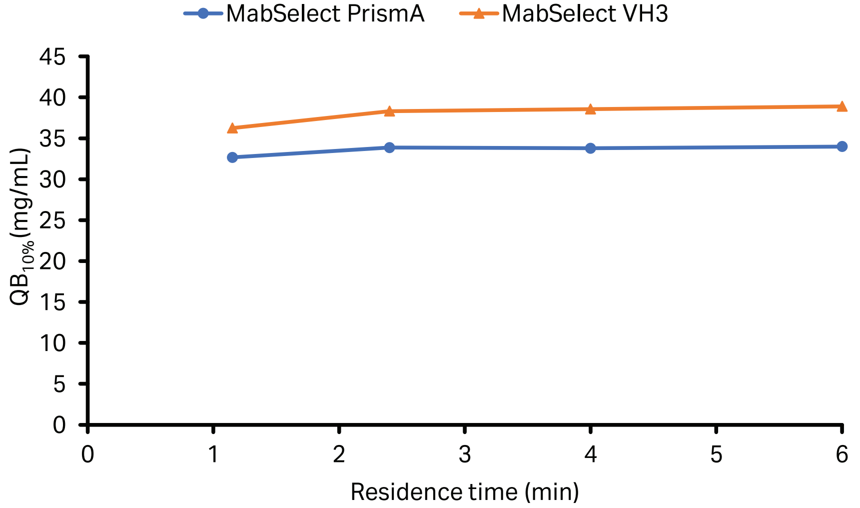
Dynamic binding capacity

The MabSelect VH3 resin has a high dynamic binding capacity at commonly used residence times. In the figures below, the dynamic binding capacity of MabSelect VH3 is compared to that of MabSelect PrismA™ at 10% breakthrough (Q_{B10}) for mAb (**A**), Fab (**B**), and VHH (**C**), respectively.


A
MabSelect PrismA
MabSelect VH3
90
80
70
60
50
40
30
20
10
0
QB10%(mg/mL)
0
2
4
6
8
10
Residence time (min)


B
MabSelect PrismA
MabSelect VH3
70
60
50
40
30
20
10
0
QB10% (mg/mL)
0
1
2
3
4
5
6
Residence time (min)

c

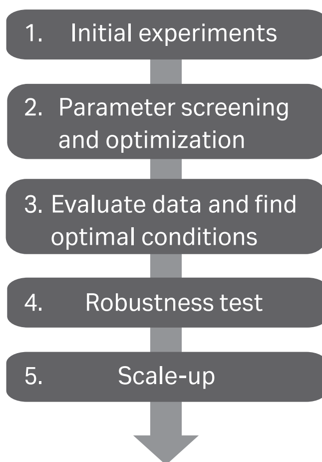


3 Process development

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 *Scale-up, on page 18*.

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, 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 *Scale-up, on page 18*.

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

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

The table below lists the recommended flow values for the HiScreen MabSelect VH3 column during specific operations.

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	2 to 8
CIP[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] CIP must be performed with at least 3 CV and a total contact time of at least 15 min. See also *CIP, on page 15*.

Purify the sample

Follow the steps below to perform a purification. For the recommended operating flow values for the HiScreen MabSelect VH3 column, see the table in *Recommended flow values, on page 10*.

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

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 with 5 CV 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 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	If required, clean the column, see *CIP, 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 10*.

5 Optimization

Optimizing elution conditions

Determine the highest pH that allows efficient desorption of antibody from the column. This prevents denaturation of sensitive antibodies caused by low pH exposure. 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 antibody, 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 final product

Leakage from MabSelect VH3 is generally low. However, in many 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.

7 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.

8 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.

9 Scale-up

Introduction

After optimizing the method at laboratory scale, the process is ready for scale-up. For quick scale-up of purification, two HiScreen 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 need to be validated using the final bed height.

Bulk resin is available for further scaling up, see *Ordering information, on page 23*.

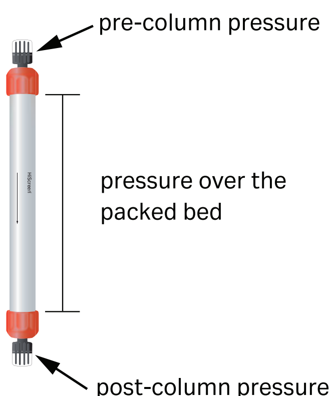
10 Adjusting pressure limits

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 10*.



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 3*.

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 3* 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 HiScreen MabSelect VH3 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 the first run after storage, it is recommended to equilibrate with binding buffer and to 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 VH3	1 × 4.7 mL	17549315

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
HiTrap MabSelect VH3	1 × 1 mL	17549351
	5 × 1 mL	17549352
	1 × 5 mL	17549353
	5 × 5 mL	17549354
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

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