

MabSelect™ VL

Affinity chromatography

Instructions for Use

MabSelect™ VL is an affinity BioProcess™ chromatography resin that uses a protein L ligand with strong affinity for the variable region of a human antibody's kappa light chain. The resin allows for high productivity and robust processes for affinity capture of bispecific antibodies and antibody fragments containing the kappa light chain. The product is a good capturing alternative for antibody variants that do not bind to protein A.

The product has an improved dynamic binding capacity (DBC) and alkaline stability compared to its predecessor, which makes it well-suited for cost-efficient capture of antibody variants. The product allows for good resolution of product-related impurities in the capture of bispecific antibodies, and it provides a tool for efficient purification of antibody variants to high purity.

MabSelect VL provides:

- high dynamic binding capacity for bispecific antibodies and antibody fragments containing a kappa light chain
- alkaline stability when cleaned with 0.1 M sodium hydroxide, reducing the risk for bioburden incidents
- good resolution for product-related impurities in the capture of bispecific antibodies

1 Introduction

Important

Read these instructions carefully before using the product.

Intended use

The products are intended for research use and further manufacturing, 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 Bioprocess resins

Bioprocess chromatography resins are developed and supported for production-scale chromatography. All Bioprocess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale.

Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. Bioprocess resins cover all purification steps from capture to polishing.

3 Product description

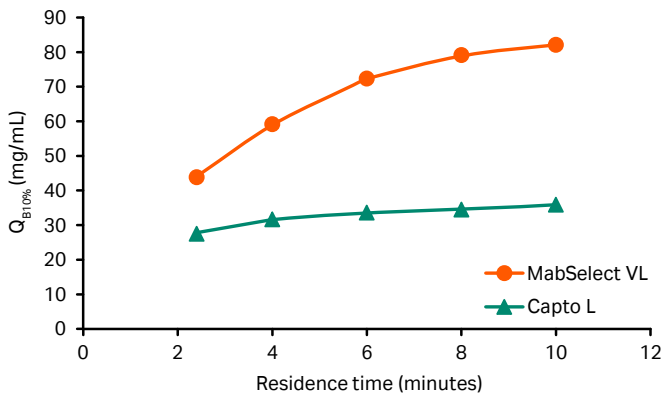
Resin description

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 IgGk1, determined in a Tricorn™ column.



Resin properties

MabSelect VL	
Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50V}¹	~ 60 μm
Ligand	Alkaline stabilized, protein L-derived (<i>E. coli</i>)
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10\%}$²	~ 60 mg IgGκ1/mL resin, 4 minutes residence time ~ 70 mg IgGκ1/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
Operating flow velocity	
Maximum ⁵	300 cm/h
Temperature stability	2°C to 40°C
Storage	2°C to 8°C, 20% ethanol or 2% benzyl alcohol
Delivery conditions	20% ethanol or 2% benzyl alcohol (on request)

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.

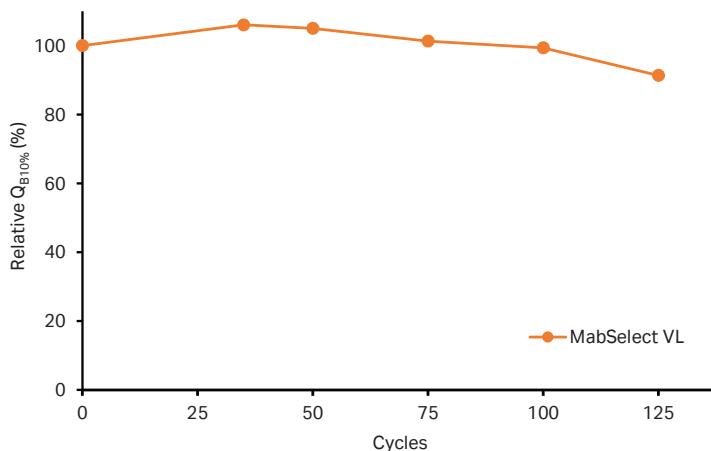
³ pH range where the resin can be operated without significant change in function.

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 20 cm bed height, using a buffer with the same viscosity as water at 20°C.

Alkaline stability

MabSelect VL resin is stable in alkaline conditions. The graph below shows the relative dynamic binding capacity after 125 cycles of alkaline treatment. Capto L resin was not included in this study since it is not stable in 0.1 M NaOH.



Each cycle consisted of:

- 5 column volumes (CV) binding buffer, PBS, pH 7.2
- 5 CV 50 mM sodium citrate, pH 2.5
- 3 CV binding buffer, PBS, pH 7.2
- 3 CV 0.1 M NaOH, 15 minutes contact time
- 5 CV binding buffer, PBS, pH 7.2

The dynamic binding capacity, $Q_{B10\%}$, for IgGk1 was measured regularly during the study.

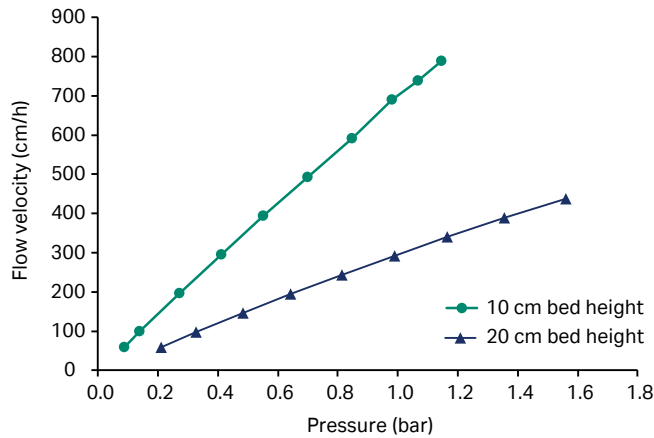
Each DBC cycle consisted of:

- 5 column volumes (CV) binding buffer, PBS, pH 7.2
- sample application 2 mg/mL IgGk1 until 20% breakthrough
- 5 CV 50 mM sodium citrate, pH 2.5
- 3 CV binding buffer, PBS, pH 7.2
- 3 CV 0.1 M NaOH, 15 minutes contact time
- 5 CV binding buffer, PBS, pH 7.2

The dynamic binding capacity, $Q_{B10\%}$, for IgGk1 was measured regularly during the study.

Pressure-flow characteristics

The relationship between the pressure and the flow velocity for MabSelect VL resin is the same as for MabSelect Prisma™ resin. The graph below shows a pressure-flow curve in water, at 20°C, for a 10 cm and 20 cm packed bed of MabSelect PrismaA in an AxiChrom 300 column. The column was equipped with stainless steel bed support, and the packing factor used was 1.18. The additional pressure from test system and tubing has been subtracted.



4 Process development

Recommended formats

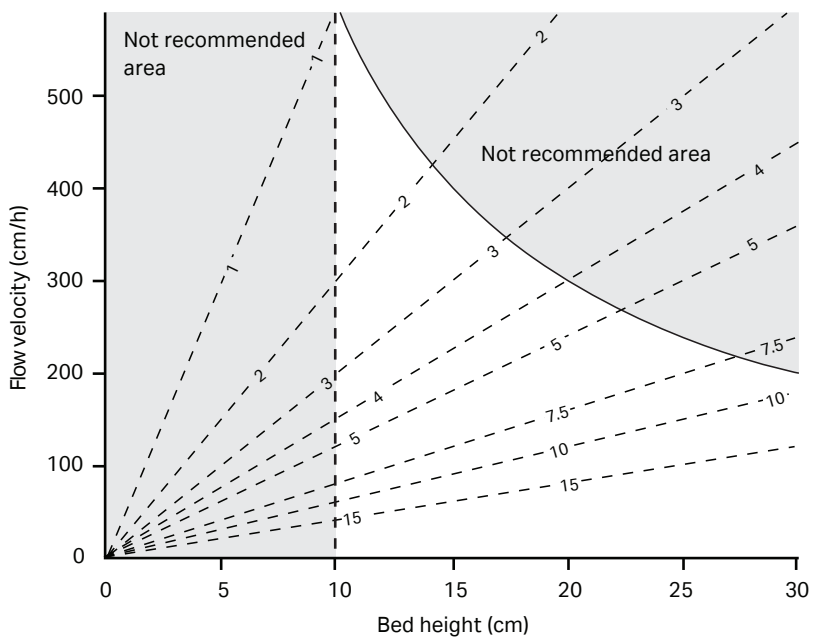
For initial studies on MabSelect VL resin, PreDicator™ plates or PreDicator RoboColumn® units are preferably used. PreDicator plates are 96-well plates, prefilled with chromatography resin, for rapid screening of chromatographic conditions at a small scale. PreDicator RoboColumn units are 200 or 600 µL columns prefilled with chromatography resin, which can be used for dynamic experiments when sample is limited, or for high throughput experiments. For further optimization in small-scale columns, we recommend prepacked HiTrap™ or HiScreen™ columns.

Operating window

Choose a residence time that meets the requirements for dynamic binding capacity and nominal fluid velocity according to the figure below. The other cycle operations including wash, elute, and equilibration steps can be run at maximum operational flow velocity, see [Resin properties, on page 5](#).

Use the figure as a guide when determining suitable bed height and operating flow velocity in terms of residence time and thus capacity and pressure drop.

The figure shows the recommended combinations of bed height and operational nominal flow velocity for MabSelect VL and the resulting residence time in the interval 1 to 15 minutes for any bed height and flow velocity. Pressure drop and packing limitations at large scale are also included. The solid curved line shows the calculated large-scale column pressure restriction which is 2 bar according to specification (300 cm/h at 2 bar and 20 cm bed height). The dashed vertical line indicates that bed heights below 10 cm are not favorable since large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.



5 Recommended screening condition

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 range in comparison to an acetate or phosphate buffer system. The typical elution pH range is 3.5 to 2.5.

Binding buffer:	20 mM sodium phosphate, 0.15 M NaCl, pH 7.4
Washing buffer:	50 mM sodium citrate, pH 5
Elution buffer:	50 mM sodium citrate, pH 3.5 to 2.5

Experimental conditions

Follow the steps below to set up the screening for the optimal conditions.

Step	Action
1	Equilibrate the column with 5 column volumes (CV) of binding buffer.
2	Apply approximately 5 mg sample per mL resin at a residence time > 4 min.
3	Wash the column with 5 CV of washing buffer.
4	Elute the column with a 10 CV linear gradient from, for example, pH 5 to 2.5 using a 50 mM sodium citrate buffer.
5	Collect fractions into titrating diluent (for example, 1.0 M Tris-HCl, pH 8.0, so that the diluent volume equals 5% of the programmed fraction volume).
6	Wash the column with 3 CV of binding buffer.
7	Perform Cleaning-In-Place (CIP) with 5 CV of NaOH (0.1 M), 15 min contact time.
8	Re-equilibrate the column with binding buffer.

To minimize the use of buffer, we recommend optimizing the washing procedure with respect to residence time, volumes, pH, and conductivity.

Optimizing elution conditions

Determine the highest pH that allows efficient desorption of antibody fragments from the column. This prevents denaturation of sensitive antibodies due to low pH exposure.

Stepwise elution is often preferred in large-scale applications because it allows the target monoclonal antibodies to be eluted in a more concentrated form, 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.

Optimizing dynamic binding capacity

Determine the dynamic binding capacity for the target antibody by frontal analysis using real process feedstock. The dynamic binding capacity is a function of the sample residence time and should therefore be defined over a range of different sample residence times.

6 Measurement of ligand leakage

6.1 Introduction

During mAb purification using a chromatography resin with a protein affinity ligand, ligand leakage can occur. To control the production process and the amount of ligand contaminant in the final drug product, leakage of the MabSelect VL ligand can be measured using different immunoassays and commercially available anti-protein L antibodies.

6.2 Gyrolab immunoassay protocol

Introduction

After describing the materials required and sample preparation, an immunoassay protocol using a Gyrolab® system for measuring MabSelect VL ligand leakage in the presence of the drug product is presented. An example of the MabSelect VL ligand standard curve and a description of the anti-protein L antibodies tested conclude the section.

Materials

Protein complex dissociation (PCD) diluent:	100 mM sodium phosphate, 150 mM NaCl, 60 mM SDS, 12.7 mM EDTA, 1.5% bovine serum albumin (BSA)
PVP solution:	1% polyvinylpyrrolidone (PVP40)

- Gyrolab system
- Gyrolab Bioaffy 1000 CD (Art. Nr. P0004253) from Gyros Protein Technologies
- chicken anti-protein L polyclonal IgY (Art. Nr. BRD-0808MZ) from Creative Biolabs
- biotinylation reagent and Alexa Fluor™ 647 reagent (Molecular Probes)
- MabSelect VL ligand (for more information, contact Cytiva sales representative)

Sample preparation

When preparing the sample for the immunoassay, it is important to dissociate the ligand-antibody complex. Heat-treatment is a suitable method for that. Verify that dissociation is satisfactory by performing a dilution series of the sample followed by a spike recovery study where unbound ligand is added to the sample.

We suggest combining heat-treatment with the use of a PCD diluent to prevent non-uniform analyte loss.

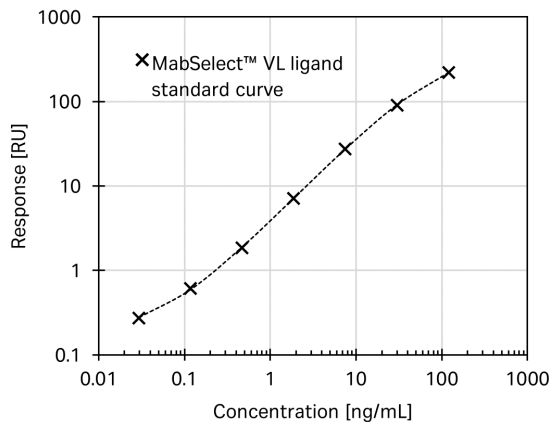
Immunoassay protocol

Note: For each immunoassay it is important to use the MabSelect VL ligand to generate an adequate standard curve.

Step	Action
1	Prepare a relevant standard stock solution of the MabSelect VL ligand.
2	Mix one volume of the standard stock solution with four volumes of PCD diluent, and mix one volume of the eluate sample with four volumes of PCD diluent.
3	Heat-treat both the standard stock solution and the eluate sample at 95°C for at least 30 min.
4	Spin both the standard stock solution and the eluate sample briefly. Mix both the standard stock solution and the sample eluate with equal volumes of freshly prepared 1% PVP.
5	Perform further dilutions in a PCD:1% PVP solution (2:3 ratio).
6	Generate the standard curve points by serial dilution in the PCD:PVP solution.
7	Dilute the eluate sample four times with PCD:PVP solution. Further dilutions with the PCD:PVP solution for the sample can be performed as required.
8	Perform an analysis on a Gyrolab system: <ul style="list-style-type: none">a. Set up the Gyrolab system following the instructions from Gyros Protein Technologies.b. Use a Gyrolab Bioaffy 1000 CD and chicken anti-protein L polyclonal IgY from Creative Biolabs.c. Use biotinylated antibodies for capture (0.1 mg/mL) and Alexa Fluor 647-conjugated antibodies for detection (25 nM).d. Label the antibodies prior to the immunoassay according to the Gyros Protein Technologies protocol.

MabSelect VL ligand standard curve

Below is an example of the MabSelect VL ligand standard curve (0.03–120 ng/mL) generated on a Gyrolab Bioaffy 1000 CD using the recommended anti-protein L antibodies from Creative Biolabs.



Recommended anti-protein L antibodies

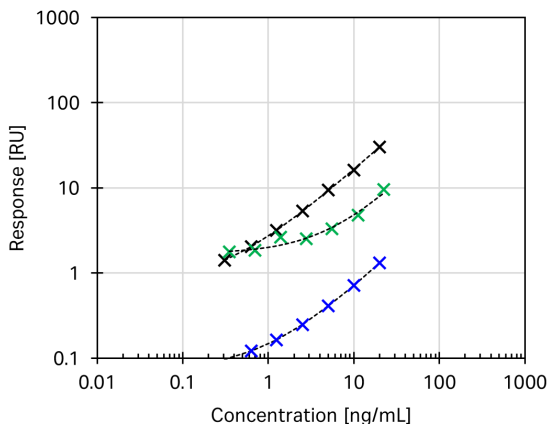
We recommend using the chicken anti-protein L polyclonal IgY from Creative Biolabs. However, antibodies from the other providers listed in the table below have been used successfully in Gyrolab immunoassays. They can also be recommended for other immunoassay protocols.

Antibody type	Available from	Art. Nr.
Chicken anti-protein L polyclonal IgY	Creative Biolabs	BRD-0808MZ
	Innovative Research	ICHAPROLAP1MG
	Invitrogen	pa1-72066

Below is the ligand standard curve (0.3–20 ng/mL) generated on a Gyrolab Bioaffy 1000 CD using the anti-protein L antibodies listed in the table. For all antibodies, the antibody concentration was 0.1 mg/L for capture and 50 nM for detection.

Legend to the colors used in the diagram:

- black: antibodies from Creative Biolabs
- green: antibodies from Innovative Research
- blue: antibodies from Invitrogen



6.3 Considerations for quantitative immunoassays

The Gyrolab immunoassay protocol can be used for automation of the immunoassay steps and for screening commercially available antibodies. A similar protocol can be used for setting up immunoassays in other formats, such as ELISA, using the recommended commercially available antibodies.

Immunoassay set-up recommendations

Consider the following for valid immunoassay results:

- Dissociate the MabSelect VL ligand from the drug molecule during sample preparation. This can be done using a PCD diluent. Heat-treatment alone or incubation at a low pH might also work.
- Wash thoroughly between steps.
- Optimize the antibody concentration and the incubation time for each step.
- Prevent the reaction site from drying between steps.
- Use blocking agents to minimize background signals.
- Determine the dynamic range for the immunoassay.
- Collect samples, store them under suitable conditions and avoid multiple freeze-thaw cycles.

7 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.

8 Packing columns

8.1 General packing information

Definitions

The bed height of a gravity settled bed often differs from the bed height of a bed settled at a given flow (consolidated). Therefore, the compression factor (CF) must be separated from the packing factor (PF).

Settling flow	Flow that consolidates the resin suspension (slurry).	
Packing flow	Flow that compresses the bed after it has settled.	
Conditioning flow	Flow applied after column packing to get a uniform bed over the entire column height.	
L_{settled}	Settled bed height/Gravity settled bed height. Bed height measured after settling the resin by gravity.	
L_{cons}	Consolidated bed height. Bed height measured after settling the resin at a given flow velocity.	
L_{packed}	Packed bed height.	
CF	Compression factor	$CF = L_{\text{settled}} / L_{\text{packed}}$
PF	Packing factor	$PF = L_{\text{cons}} / L_{\text{packed}}$
A_C	Cross-sectional area of the column	
V_C	Column volume	$V_C = L_{\text{packed}} \times A_C$
C_{slurry}	Slurry concentration	

Slurry preparation

Slurry preparation can be performed manually, or mechanically, for example by using a Media Wand™ or Media Handling Unit. Shaking gives good results, but is often not practical for larger volumes. Use soft stirrers without sharp edges for stirring. Media Wand suspends the resin directly in the container and transfers the slurry to the slurry tank in a single operation. This makes it suitable for large-scale packing.

Measure the slurry concentration accurately to get the correct amount of resin for packing to target bed height or compression. Let the resin settle overnight in 20% ethanol in a measuring cylinder to determine the slurry concentration or use the Slurry Concentration Kit, see [Ordering information, on page 36](#).

Compression factor for MabSelect VL

The compression factor (CF) is used to calculate the required resin volume (V) for packing a desired bed height:

$$V = (A_C \times L_{\text{packed}} \times CF) / C_{\text{slurry}}$$

CF for gravity settled MabSelect VL in 20% ethanol is 1.10.

8.2 Packing laboratory scale columns

Recommended laboratory scale columns

Column	Inner diameter (mm)	Bed volume ¹ (mL)	Bed height (cm)
Tricorn 5/100	5	2	10
Tricorn 10/100	10	8	10
HiScale™ 10/40	10	8 to 20	max. 25
HiScale 16/40	16	20 to 70	max. 35
HiScale 26/20	26	53 to 106	max. 20
HiScale 26/40	26	53 to 186	max. 35
HiScale 50/20	50	196 to 393	max. 20
HiScale 50/40	50	196 to 687	max. 35

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

Materials

- MabSelect VL
- plastic spoon or spatula
- P4 glass filter funnel
- vacuum suction equipment
- filtering flask
- measuring cylinder
- packing solution

For **Tricorn** columns:

- Tricorn column
- Tricorn packing tube
- Tricorn 5 medium filter kit, or Tricorn 10 medium filter kit

For **HiScale** columns:

- HiScale column
- HiScale packing tube (not needed for lower bed heights)

Make sure that all materials and equipment are at room temperature before starting to pack.

Equipment

An ÄKTA™ system or a stand-alone pump can be used for packing, depending on the flow rate required.

To avoid column drainage during packing, a pressure relief valve can be attached to the outlet valve of the system. Setting a low back pressure of 0.02 MPa (0.2 bar, 2.9 psi) is sufficient.

Equilibration to packing solution

Follow the step below to equilibrate and suspend the resin in packing solution to the recommended slurry concentration.

Step	Action
1	Attach a glass filter funnel to a filtering flask.
2	Suspend the resin by shaking the measuring cylinder, and pour the slurry into the P4 glass filter funnel.
3	Wash 5 times with 2 CV of packing solution. Gently stir with a spatula between additions.
4	Pour the washed resin from the glass filter funnel into a beaker.
5	Add packing solution to obtain the recommended slurry concentration for the column used.

The resin is ready to be packed in the laboratory-scale column used.

Packing Tricorn columns

Column	Tricorn 5	Tricorn 10
Bed height (cm)	10	10
Packing solution	20% ethanol with 0.2 M NaCl	
Slurry concentration (%)	60	60
Packing flow velocity (cm/h)	1070	764
Packing flow rate (mL/min)	3.5	10

Step	Action
1	Wet the filters with ethanol and assemble the column according to <i>Tricorn Empty High Performance Columns (CY17144)</i> .
2	Attach a packing connector and a packing tube to the top of the column tube. Fasten the column tube in a stand.
3	Fill the column with slurry suspended in packing solution, and top up with packing solution.
4	Assemble a bottom piece to the top of the packing tube. Make sure that no air is trapped under the filter.
5	Connect the column top to the pump, and start a downflow with packing solution. The packing flow velocity is shown in the previous table.
6	Let the flow continue for 10 minutes.
7	Turn off the flow and place a stop plug in the column bottom.
8	Disconnect the packing tube and remove any excess resin 2.4 cm from the top of the tube using a pipette.
9	Top up the column with packing solution.
10	Attach the top adapter. Make sure no air is trapped under the filter.
11	Turn the adapter downwards until it is 1 to 2 mm above the resin bed to displace any air in the adapter tubing.
12	Connect the top adapter to the pump. Make a drop-to-drop connection to prevent air from entering the column.
13	Start a downflow with packing solution. The packing flow velocity is shown in the previous table.
14	Let the flow continue for 5 minutes.
15	Mark the bed height and stop the pump.
16	Turn the adapter down to the resin bed and then given an extra 1/3 turn. Lock the top adapter by pushing down the lock ring.
17	Measure the bed height to determine packing efficiency.

The column is ready to for efficiency testing.

Packing HiScale 10 columns

HiScale 10 columns are packed with a settling flow and a packing flow followed by mechanical compression. A mechanical compression value in mm is given instead of a packing factor.

Table 8.1: Main features of the packing method for HiScale 10

Column	HiScale 10/40		
Bed height (cm)	10	20	25
Packing solution	0.4 M NaCl in ultrapure water		
Slurry concentration (%)	50	40	40
Settling flow velocity (cm/h)	306	200	200
Settling flow rate (mL/min)	4	2.6	2.6
Packing flow velocity (cm/h)	917	527	527
Packing flow rate (mL/min)	12	6.9	6.9
Mechanical compression (mm)	2	1	1

Step	Action
1	Assemble the column according to the column instructions, <i>HiScale columns (10, 16, 26, 50) and accessories (28967470)</i> .
2	Attach a packing tube to the top of the column tube if needed to achieve the required bed height. Fasten the column tube in a stand. Connect the bottom adapter to the system.
3	Prime the bottom net with a slow upflow (30 cm/h) of packing solution. Make sure that the net is thoroughly wetted. Remove any air bubbles that are trapped under the net by light suction with a syringe.
4	Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter will dip into the slurry to avoid air under the net.
5	Connect the top adapter to the pump and prime with a slow downflow. To remove air from the adapter, hold the adapter with the net facing upwards.
6	Attach the top adapter to the top of the packing tube. Tighten all seals firmly.
7	Start a downflow with packing solution. The settling flow velocity is shown in the table at the beginning of the section.

Step	Action
8	Let the flow continue until the bed consolidates. Do not let the bed consolidate for too long because that will make reading the bed height less accurate.
9	Turn off the flow. Disconnect the packing tube and the packing connector over a beaker or a sink.
10	Reassemble the top adapter. Make sure no air is trapped under the net.
11	Turn the adapter down until it is 10 mm above the resin bed to displace any air in the adapter tubing. Make sure that the bed surface is not disturbed.
12	Tighten the O-ring of the adapter and start a packing flow velocity for 3 CV. The packing flow velocity is shown in the table at the beginning of this section.
13	After 3 CV, use the graduation marks on the column to measure the bed height. A light source can facilitate the measurement of the bed height. Note the bed height as the bed height might expand when the flow is turned off.
14	Turn off the packing flow and turn the top adapter clockwise, smoothly and slowly, until the bed height noted in step 13 is reached.
15	Compress the bed further according to the mechanical compression value stated in the table at the beginning of this section.

The column is ready for efficiency testing.

Packing HiScale 16, 26, and 50 columns

HiScale 16, 26, and 50 columns are packed with a settling flow and mechanical compression followed by a conditioning flow.

Table 8.2: Main features of the packing method for HiScale 16

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Packing solution	20% ethanol with 0.4 M NaCl	0.4 M NaCl in ultrapure water	
Slurry concentration (%)	55	55	55
Settling flow velocity (cm/h)	200	200	358
Settling flow rate (mL/min)	6.7	6.7	12
Packing factor (PF)	1.12	1.10	1.02
Conditioning flow velocity (cm/h)	500	400	230
Conditioning flow rate (mL/min)	16.8	13.4	7.7

Table 8.3: Main features of the packing method for HiScale 26

Column	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Packing solution	20% ethanol with 0.4 M NaCl	0.4 M NaCl in ultrapure water	
Slurry concentration (%)	55	55	58
Settling flow velocity (cm/h)	200	200	362
Settling flow rate (mL/min)	17.7	17.7	32
Packing factor (PF)	1.12	1.12	1.06
Conditioning flow velocity (cm/h)	500	400	230
Conditioning flow rate (mL/min)	44.2	35.4	20.4

Table 8.4: Main features of the packing method for HiScale 50

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Packing solution	20% ethanol with 0.4 M NaCl		0.4 M NaCl in ultrapure water
Slurry concentration (%)	55	55	55
Settling flow velocity (cm/h)	200	200	367
Settling flow rate (mL/min)	65.4	65.4	120
Packing factor (PF)	1.12	1.12	1.02
Conditioning flow velocity (cm/h)	350	300	230
Conditioning flow rate (mL/min)	114.5	98.2	75.3

Step	Action
1	Assemble the column according to the column instructions, <i>HiScale columns (10, 16, 26, 50) and accessories (28967470)</i> .
2	Attach a packing connector and a packing tube to the top of the column tube if needed to achieve the required bed height. Fasten the column tube in a stand. Connect the bottom adapter to the system.
3	Prime the bottom net with a slow upflow (30 cm/h) of packing solution. Make sure that the net is thoroughly wetted. Remove any air bubbles that are trapped under the net by light suction with a syringe.
4	Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter will dip into the slurry to avoid air under the net.
5	Connect the top adapter to the pump and prime with a slow downflow. To remove air from the adapter, hold the adapter with the net facing upwards.
6	Attach the top adapter to the top of the packing tube. Tighten all seals firmly.
7	Start a downflow with packing solution. The settling flow velocity is shown in the tables at the beginning of the section.

Step	Action
8	Let the flow continue until the bed consolidates. Do not let the bed consolidate for too long because that will make reading the bed height less accurate.
9	Use the graduation marks on the column to measure the bed height. A light source can facilitate the measurement of the bed height.
10	Turn off the flow. Disconnect the packing tube and the packing connector over a beaker or a sink.
11	Reassemble the top adapter. Make sure no air is trapped under the net.
12	Turn the adapter down until it is 10 mm above the resin bed to displace any air in the adapter tubing. Make sure that the bed surface is not disturbed.
13	Tighten the O-ring of the adapter, and turn the end cap down to the bed height measured at step 9.
14	Calculate the final bed height by dividing the consolidated bed height with the desired packing factor, $L_{\text{packed}} = L_{\text{cons}}/\text{PF}$. Packing factors for the different column sizes and bed heights are shown in the tables at the beginning of the section.
15	Turn the top adapter clockwise, smoothly and slowly, until the desired bed height is reached.
16	Start a downwards flow with packing solution. The conditioning flow velocity is shown in the tables at the beginning of the section.
17	Let the flow run for 10 column volumes.

The column is ready for efficiency testing.

8.3 Packing large-scale columns

Overview

MabSelect VL can be packed in pilot- and large-scale columns. There are several possible packing procedures, depending on the column and equipment used.

Refer to the instructions for the relevant column for complete packing instructions. Also refer to [cytiva.com/column-packing-for-mabselect-prisma-resin](https://www.cytiva.com/column-packing-for-mabselect-prisma-resin) describing packing methods for similar resins in large-scale columns.

Intelligent Packing in AxiChrom columns

When packing AxiChrom 50 to 200 columns with an ÄKTA system, Intelligent Packing control is managed by the UNICORN™ system control software. For AxiChrom 300 to 1600 columns, Intelligent Packing is performed by the AxiChrom Master, a separate unit that comprises a touchscreen-operated user interface, or from the UNICORN software on the ÄKTA process™ system.

In the Intelligent Packing wizard, packing methods are created by entering values for the following packing variables:

- column
- resin
- packing factor
- slurry concentration
- target bed height

Recommended large-scale columns

Column	Inner diameter (mm)	Bed volume ¹ (L)	Bed height (cm)
AxiChrom ²	50 to 200	0.2 to 13	max. 40
AxiChrom ²	300 to 1600	7 to 804	max. 40
BPG ³	100 to 300	0.8 to 28	max. 40
Chromaflo™ standard ⁴	400 to 800	13 to 151	max. 30

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

² Intelligent Packing method can be used.

³ The pressure rating of BPG 450 is too low to use with MabSelect resins.

⁴ Larger pack stations might be required at larger diameters.

All large-scale columns can be supplied as variable bed height columns. Do not choose large-diameter columns if the bed height is low.

Packing solution

Recommended packing solutions for MabSelect VL are:

- water
- 20% ethanol
- sodium chloride solution

Packing factors for MabSelect VL

When packing BPG and AxiChrom columns, the packing factor (PF) is used to calculate the target bed height after the consolidation step. MabSelect VL settles differently in different solutions. Adding NaCl to the packing solution slows the settling of the resin beads and allows them to settle less tightly. As little as 10 mM NaCl changes the consolidated bed height with 2% and the gravity settled bed height with 16%, compared to water. The table below shows typical packing factors for MabSelect VL in different solutions for optimal bed performance, where the bed is consolidated at 60 cm/h.

Solution	Packing factor
Water	1.18
20% ethanol	1.18
0.4 M NaCl	1.20

For the MabSelect VL resin the packing factor would be 1.18 while the compression factor would be 1.10.

8.4 Evaluation of column packing

Frequency

Test the column efficiency to evaluate the packing quality:

- after completion of a packing procedure
- at regular intervals during the working life of the column
- when a deterioration in separation performance is observed

Column efficiency testing

The recommended method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Note: Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

The calculated plate number depends on the test conditions and must only be used as a reference value. It is important that the test conditions and the equipment are the same so that the results are comparable.

Note: Changing the solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, and chromatography system influences the results.

For more information about column efficiency testing, consult the application note *Column efficiency testing (CY13149)*.

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for the column use.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

L = bed height (cm)
 N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)^2$$

V_R = volume eluted from the start of sample application to the peak maximum
 W_h = peak width measured as the width of the recorded peak at half of the peak height
 V_R and W_h are in the same unit

The concept of reduced plate height is often used for comparing column performance. The reduced plate height, h , is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is acceptable.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be $0.8 < A_s < 1.5$.

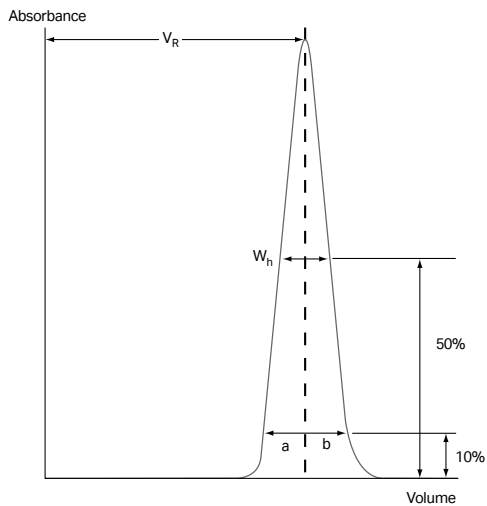
A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height
 b = descending part of the peak width at 10% of peak height

The figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_S values are calculated.



9 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. 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.

10 Sanitization

Overview

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. 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.*

11 Storage

Store unused resin in its container at a temperature of 2°C to 8°C. Make sure that the screw top is fully tightened.

Equilibrate packed columns in buffer containing 20% ethanol or 2% benzyl alcohol to prevent microbial growth.

After storage, equilibrate with binding buffer and perform a blank run, including CIP, before use.

12 Scale-up

After optimizing the antibody fractionation at laboratory scale, the process can be scaled up to pilot and process scale.

- Keep the residence time constant to maintain the dynamic binding capacity.
- Select bed volume according to required binding capacity.
- Select column diameter according to the volume throughput requirements. Then determine the bed height to give the desired residence time. Bed heights of 10 to 25 cm are generally considered appropriate.

Note: *The back pressure increases proportionally with increasing bed height at constant nominal velocity.*

- Verify the purification step with the new bed height, if it has changed.
- Keep sample concentration and elution conditions constant.

For the selection of the appropriate window of operation for MabSelect VL, refer to [Operating window, on page 8](#).

13 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.

14 Ordering information

For additional information, see [cytiva.com](https://www.cytiva.com).

ReadyToProcess™ columns are available on request, see *ReadyToProcess columns* (CY1797).

Product	Quantity	Product code
MabSelect VL	25 mL	17542001
	200 mL	17542002
	1 L	17542003
	5 L	17542004
	10 L	17542005
MabSelect VL in benzyl alcohol	1 L	17542023
	5 L	17542024
	10 L	17542025
PreDicator MabSelect VL, 2 µL	4 × 96-well filter plates	17542030
PreDicator MabSelect VL, 20 µL	4 × 96-well filter plates	17542031
PreDicator MabSelect VL, 50 µL	4 × 96-well filter plates	17542032
PreDicator RoboColumn, 200 µL	8 columns	17542033
PreDicator RoboColumn, 600 µL	8 columns	17542034
HiScreen MabSelect VL	1 × 4.7 mL	17542015
HiTrap MabSelect VL	1 × 1 mL	17542051
	5 × 1 mL	17542052
	1 × 5 mL	17542053
	5 × 5 mL	17542054
Tricorn 5 Medium Filter Kit	1 × 5 units	29258132
Tricorn 10 Medium Filter Kit	1 × 5 units	29258131
Slurry Concentration Kit	1 unit	29096100

Product	Quantity	Product code
Accessory kit for HiScale 10	1 unit	29360581
Accessory kit for HiScale 16	1 unit	28966367
Accessory kit for HiScale 26	1 unit	28966374
Accessory kit for HiScale 50	1 unit	28966375

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