

MabSelect™ VH3

Affinity chromatography resin

Instructions for Use

MabSelect™ VH3 is 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 the 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. The high binding capacity and the high-flow base matrix are combined to give high productivity in protein A capture chromatography.

MabSelect VH3 resin provides the following:

- high dynamic binding capacity for bispecific antibodies and antibody fragments containing the VH3 sequence family
- good resolution for product-related impurities in the capture of bispecific antibodies
- excellent alkaline stability that allows for regular use of 0.5 M NaOH for cleaning-in-place (CIP), reducing the risk for bioburden incidents, and supporting long resin lifetime
- convenient scale-up to production-sized AxiChrom™ columns

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 and further manufacturing. The product must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

2 Product description

BioProcess resin

BioProcess™ chromatography resins are developed and supported for production-scale chromatography. BioProcess resins cover all purification steps from capture to polishing.

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 for BioProcess resins to assist in process validation and submissions to regulatory authorities.

Resin description

MabSelect VH3 is an affinity 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.

A Regulatory Support File is available for MabSelect VH3. The RSF contains further product data such as characteristics, quality, and chemical stability.

Resin properties

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 <i>E. coli</i>), no interaction with the Fc region and enhanced interaction with the VH3 region
Coupling chemistry	Epoxy
Dynamic binding capacity, $QB_{10\%}$²	~ 70 mg IgG VH3/mL resin at 6 minutes residence time ~ 60 mg IgG VH3/mL resin at 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
Maximum operating flow velocity 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 Dynamic binding capacity 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 HiScreen™ column at 10 cm bed height in PBS buffer, pH 7.4.

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

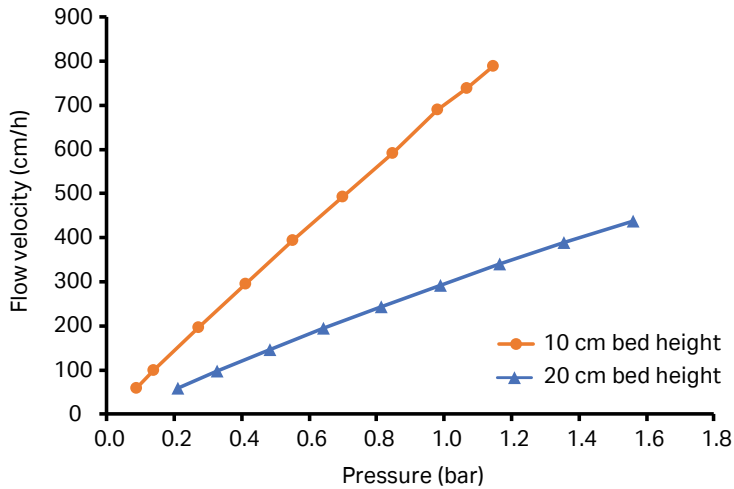
⁴ pH range where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁵ In an AxiChrom column with 30 cm diameter and 20 cm bed height, using a buffer with the same viscosity as water at 20°C.

Pressure-flow characteristics

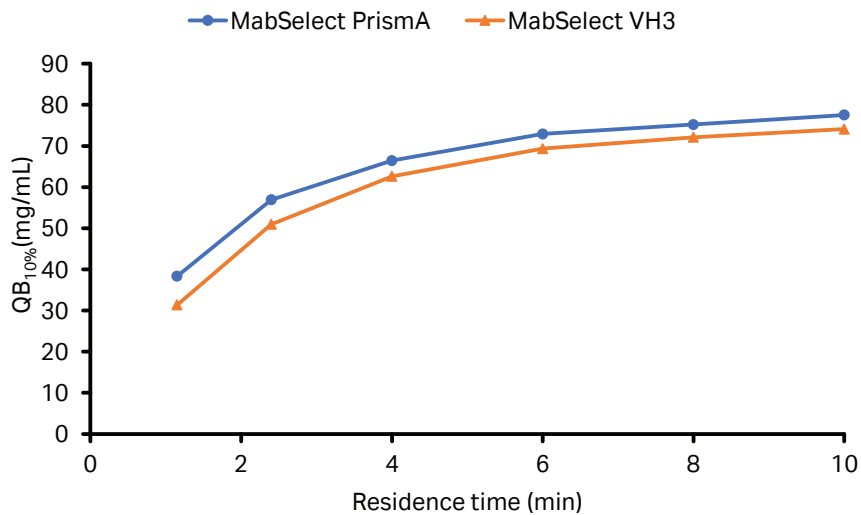
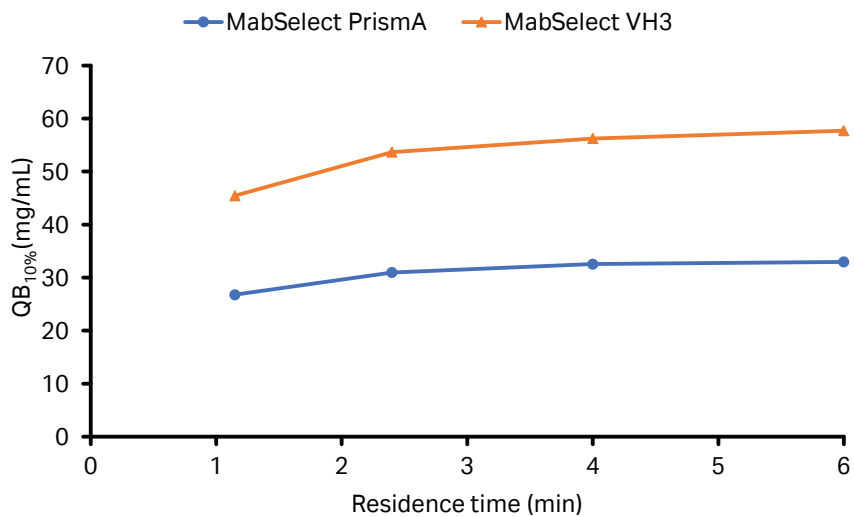
The relationship between the pressure and the flow velocity for the MabSelect VH3 resin is very similar to that of the MabSelect PrismA™ resin.

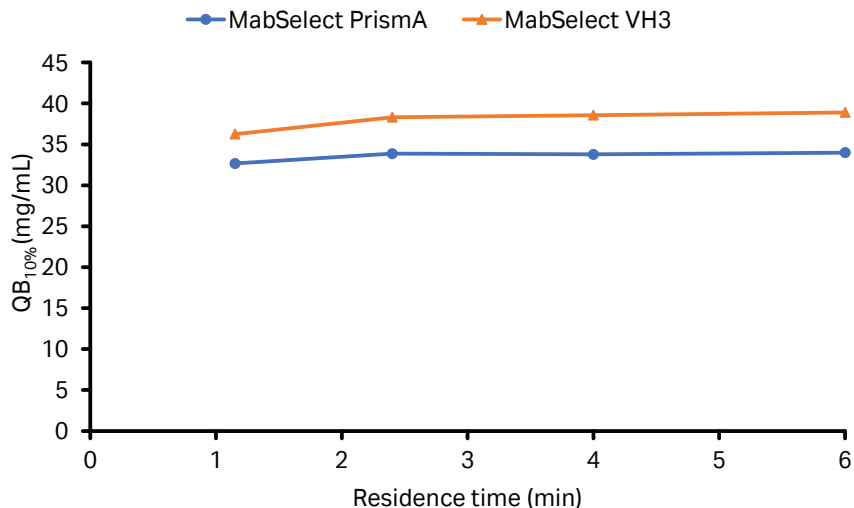
The image below shows a pressure-flow curve in water, at 20°C, for a 10 cm and 20 cm packed bed of MabSelect PrismA in an AxiChrom 300 column. The column was equipped with stainless steel bed support, and the packing factor was 1.18. The additional pressure from the test system and the tubing has been subtracted.



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 (QB_{10%}) for mAb (A), Fab (B), and VHH (C), respectively.

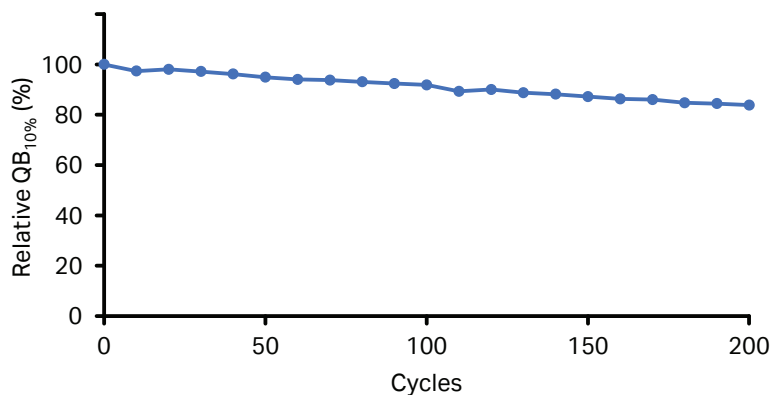
A**B**

c

Alkaline stability

Alkaline stability for MabSelect VH3 resin was evaluated in a repeated CIP cycling study with 0.5 M NaOH (15 minutes contact time). The mock study without sample load was performed on a Tricorn™ 5/50 column for 200 cycles. Every tenth cycle, the relative remaining binding capacity (DBC) was calculated. After 200 cycles, the relative remaining DBC was approximately 84%, demonstrating excellent alkaline stability.

The graph below shows the relative remaining dynamic binding capacity.



Each cycle of the mock study was characterized by the following:

- **Equilibration:** 2 column volumes (CV) of binding buffer, PBS, pH 7.4
- **Absence of sample load**
- **Elution:** 5 CV 50 mM sodium acetate, pH 3.5
- **Wash:** 1.5 CV binding buffer, PBS, pH 7.4
- **CIP:** 3 CV 0.5 M NaOH, 15 minutes contact time
- **Wash:** 8.5 CV binding buffer, PBS, pH 7.4

Every tenth cycle of the study, a frontal analysis was performed. The dynamic binding capacity, $QB_{10\%}$, for regular mAb containing the VH3 sequence family was measured by loading a sample of 2 mg/mL mAb (VH3), 6 min residence time, until 20% breakthrough was reached.

3 Process development

Recommended formats

For initial studies on MabSelect VH3, PreDicator™ plates or PreDicator RoboColumn units are the preferred choices. PreDicator plates are 96-well plates that are prefilled with chromatography resin, for rapid screening of chromatographic conditions at a small scale. PreDicator RoboColumn units are 200 µL or 600 µL columns that are prefilled with chromatography resin, for dynamic experiments with limited sample sizes or for high-throughput experiments. For further optimization in small-scale columns, we recommend prepacked HiTrap™ or HiScreen columns.

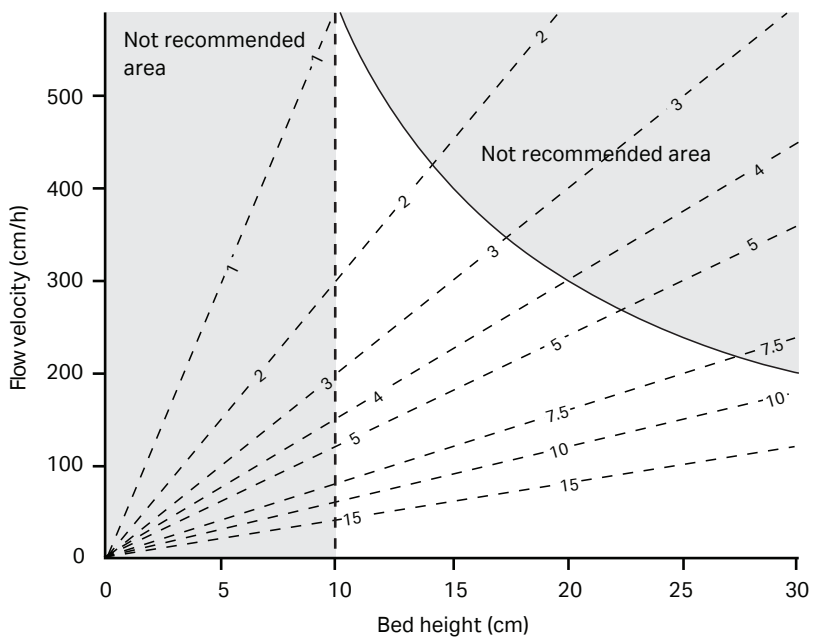
Operating window

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, elution, and equilibration steps can be run at maximum operational flow velocity, see [Resin properties, on page 4](#).

Use the figure as a guide when determining a 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 VH3 and the resulting residence time in the interval 1 to 15 minutes for any bed height and flow velocity. The 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 a bed height below 10 cm is not favorable because large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.



4 Conditions screening

Recommended buffers

For MabSelect VH3 resin, we recommend the use of phosphate and acetate buffers. However, 20 mM sodium citrate can be used for screening purposes to determine a suitable elution pH, see the *Screening protocol* below.

Screening protocol for elution pH

The steps below are a starting point for screening to determine suitable elution conditions for the target molecule, using a low sample load, for example, 5 to 10 mg/mL of resin.

Phase	Solution
Equilibration	5 CV 20 mM sodium phosphate, 0.15 M NaCl, pH 7.4
Sample application	Small amount of antibody sample, 6 min residence time
Wash	5 CV 20 mM sodium phosphate, 0.5 M NaCl, pH 7.2
Elution	10 to 20 CV 20 mM sodium citrate, linear gradient, pH 6.5 to pH 2.5
Regeneration	3 CV 20 mM sodium citrate, pH 2.5
CIP	3 CV 0.5 M NaOH
Re-equilibration	5 CV 20 mM sodium phosphate, 0.15 M NaCl, pH 7.4

If the results are not optimal, we recommend screening various residence times, wash parameters, and elution parameters.

General recommendations

We recommend optimizing the wash procedure with respect to the following:

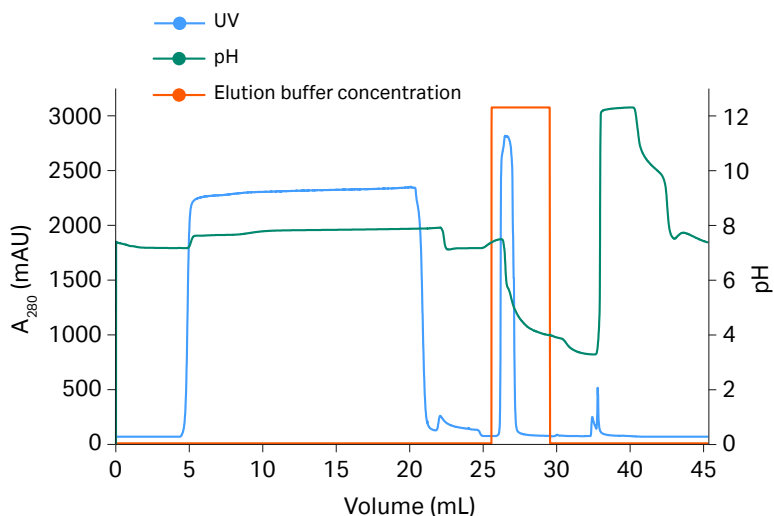
- residence time
- volumes
- pH
- conductivity

Optimizing elution and wash conditions

Determine the highest pH that allows for efficient desorption of the target molecule from the column. Doing so prevents denaturation of sensitive molecules due to exposure to low pH.

For large-scale applications, it is recommended to set up a step elution protocol after suitable elution conditions have been determined. Sodium acetate is the recommended elution buffer, but other buffers can be used. The composition of the mobile phase is adapted in steps to the target elution conditions. Target molecules are thus eluted in a more concentrated form, with lower buffer consumption and shorter cycle times. It might be necessary to decrease the flow rate due to high protein concentrations in the eluate.

The graph below shows an example of loading approximately 20 mL of a clarified cell culture harvest containing 2.46 g/L mAb with the VH3 sequence family onto a Tricorn 5/50 column (1 mL) packed with MabSelect VH3 resin. Bound mAb was eluted in 50 mM sodium acetate, pH 3.5 with a yield of 98% mAb.



The VH3 interaction, particularly the single interaction that is present in bispecific antibodies (bsAbs) and antibody fragments (Fabs, VHH, etc.), is sensitive to high salt concentrations. Use high-salt washes with caution and optimize them thoroughly to prevent product loss. Lowering the salt concentration resolves the issue, but it might take time to determine the optimal salt concentration for host cell protein (HCP) clearance without product loss. Therefore, it is recommended to always determine the DBC at the salt concentration intended for use during the wash phases. Afterwards, set the load during a purification run to 70% to 80% of the DBC determined.

The protocol below lists all steps and conditions used in the purification above and is the recommended protocol.

Phase	Buffer	Volume	Residence time
Equilibration	PBS, pH 7.4	5 CV	4 min
Sample application	mAb feed: 80% of QB _{10%}	20 mL	6 min
Column wash 1	PBS, pH 7.4	1.5 CV	6 min
Column wash 2	20 mM sodium phosphate, 0.5 M NaCl, pH 7.2	3.5 CV	6 min
Column wash 3 ¹	20 mM sodium phosphate, pH 6.5	2 CV	6 min
Elution	50 mM sodium acetate, pH 3.5	5 CV	6 min
Column strip	100 mM acetic acid	2 CV	4 min
Column CIP	0.5 M NaOH	3 CV	5 min
Re-equilibration	PBS, pH 7.4	7 CV	5 min

¹ Intermediate washes at a pH < 6.5 are not recommended as they can lead to product loss.

Optimizing dynamic binding conditions

The dynamic binding capacity for the target antibody should be determined by frontal analysis using real process feedstock. The dynamic binding capacity is a function of the sample residence time and therefore needs to be defined for different sample residence times. The recommended residence time for MabSelect VH3 resin for optimal binding capacity and resolution is 6 minutes.

5 Quantification of ligand leakage

Introduction

Ligand leakage can occur, when purifying antibody-based therapeutics using a chromatography resin with a protein affinity ligand. To control the production process and the amount of ligand contaminant in the final drug product, quantification of the MabSelect VH3 ligand leakage using the VH3 ligand ELISA kit (29737000) is recommended.

ELISA kit

The VH3 ligand ELISA kit is specifically designed for MabSelect VH3 protein A resin. For the most accurate results, it is important to use a resin-matched standard for assay calibration. The VH3 ligand ELISA kit contains all reagents required for the assay, including the MabSelect VH3 ligand.

The VH3 ligand ELISA kit provides the following:

- parts per billion (ppb) sensitivity
- drug product tolerance up to 30 mg/mL
- assay range from 0.018 to 4.5 ng/mL

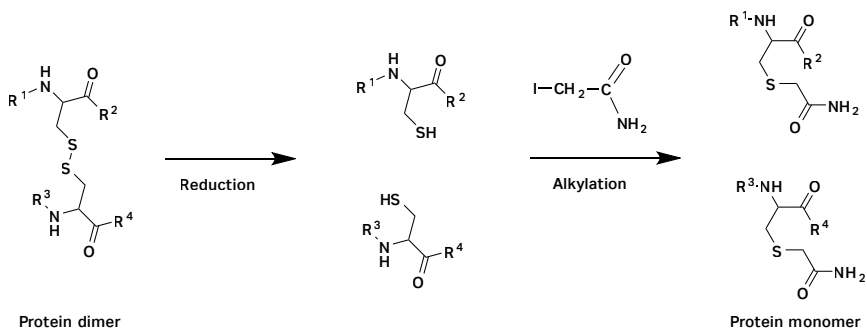
For more information about the VH3 ligand ELISA kit, refer to the *Data file (CY34384)* or *Instructions for Use (29741900)*.

MabSelect VH3 ligand ELISA kit standard

The MabSelect VH3 ligand consists of an engineered protein A domain that is multimerized into a hexamer with a C-terminal cysteine for immobilization purposes. The ligand is available for purchase under a non-transferable license for analytical purposes in protein A leakage quantification. For more information, contact your local Cytiva representative.

MabSelect VH3 ligand

The MabSelect VH3 ligand has a blocked, carboxymethylated thiol in the C-terminal to prevent dimerization. Thiol blocking as shown below is done to measure ligand leakage more accurately as the dimerized ligand gives a different immunoassay response.



Specifications

Criteria/Test	Method of analysis	Limit
Concentration (mg/mL)	SEC	2.0 to 2.4
Purity (%)	SEC	94
Identity (MW; Da)	LC-MS	40596 to 40602

Storage and handling guidelines

Store the vial at -20°C upon receipt. For extended storage, -80°C is recommended. Prepare single-use aliquots to avoid freeze-thaw cycles. Prior to analysis, a solution of PBS + 0.05% (v/v) Tween-20 can be used to dilute the ligand further.

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.

7 Packing columns

7.1 General packing information

Definitions

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

L_{settled}	Settled bed height/gravity-settled bed height Bed height measured after letting the resin settle by gravity
L_{cons}	Consolidated bed height Bed height measured after letting the resin settle 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 a Media Handling Unit. Shaking gives good results, but is often impractical 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. A Media Wand is 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 VH3

The compression factor (CF) is used to calculate the resin volume (V) that is required to pack a certain bed height:

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

CF for gravity settled MabSelect VH3 in 20% ethanol is 1.10.

7.2 Packing laboratory-scale columns

Recommended laboratory-scale columns

The following table lists the main properties of the recommended laboratory-scale columns.

Column	Inner diameter (mm)	Bed volume* (mL)	Bed height (cm)
Tricorn 5/100	5	2	10
Tricorn 5/150	5	2 to 3	max. 15
Tricorn 5/200	5	2 to 4	max. 20
Tricorn 10/100	10	8	10
Tricorn 10/150	10	8 to 12	max. 15
Tricorn 10/200	10	8 to 16	max. 20
HiScale™ 10/40	10	8 to 20 [†]	max. 25 [†]
HiScale 16/20	16	20 to 40	max. 20
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

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

† Packing methods for bed heights up to 25 cm are provided.

Materials

- MabSelect VH3
- plastic spoon or spatula
- P4 glass filter funnel
- vacuum suction equipment
- filter 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 packing the column.

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.

Equilibrate to packing solution

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

Step	Action
1	Attach a glass filter funnel to a filter flask.
2	Suspend the resin by shaking the measuring cylinder and pour the slurry into the glass filter funnel.

Step	Action
3	Wash the resin 5 times with 2 CV 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 slurry concentration that is recommended for the column used.

7.2.1 Packing Tricorn columns

Main parameters for different column sizes

The table below lists the main packing parameters for Tricorn columns.

Column	Tricorn 5/100	Tricorn 10/100
Bed height (cm)	10	10
Slurry/packing solution	0.4 M NaCl in 20% ethanol	
Slurry concentration (%)	50	50
Packing velocity (cm/h)	300	300
Packing flow rate (mL/min)	0.98	3.9
Flow conditioning		
Conditioning flow velocity (cm/h)	300	300
Conditioning flow rate (mL/min)	0.98	3.9

Pack a Tricorn column

Step	Action
1	Wet the filters with packing solution and assemble the column according to <i>Tricorn Empty High Performance columns</i> available on cytiva.com .
2	Attach a packing connector and a packing tube on 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.

Step	Action
4	Assemble a bottom piece with a wetted filter to the top of the packing tube.
	Note: <i>Make sure that no air is trapped under the filter.</i>
5	Connect the column top to the pump and start a downflow with packing solution. The packing flow velocity is shown in Main parameters for different column sizes, on page 20 . Let the flow run for 10 min.
6	Turn off the flow and attach a stop plug to the column bottom.
7	Disassemble the packing tube and remove the top 2.4 cm of resin, using a pipette.
8	Top up the column with packing solution.
9	Attach the top adapter.
	Note: <i>Make sure that no air is trapped under the filter.</i>
10	Turn the adapter down until it is 1 to 2 mm above the resin bed to displace the air in the adapter tubing.
11	Connect the top adapter to the pump. Make a drop-to-drop connection to prevent air from entering the column.
12	Start a flow with packing solution. The packing flow velocity is shown in Main parameters for different column sizes, on page 20 . Let the flow run for 5 min.
13	Mark the bed height and pause the pump.
14	Turn the adapter down toward the mark, and give the adapter an extra $\frac{1}{8}$ turn.
15	Start a conditioning flow with packing solution. The conditioning flow velocity is shown in Main parameters for different column sizes, on page 20 . Let the flow run for 10 min.
16	Measure the bed height.

The column is ready for efficiency testing.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

7.2.2 Packing HiScale columns

Main parameters for different column sizes

The tables below list the main packing parameters for HiScale columns.

Column	HiScale 10/40		
Bed height (cm)	10	20	25
Slurry/packing solution	0.4 M NaCl in 20% ethanol		
Slurry concentration (%)	50	50	70
Slurry volume (mL)	17.3	34.6	30.9
Packing factor (PF)	1.10	1.10	1.10
Packing flow velocity (cm/h)	200	200	200
Packing flow rate (mL/min)	2.62	2.62	2.62
Conditioning flow velocity (cm/h)	700	400	320
Conditioning flow rate (mL/min)	9.16	5.24	4.19

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/packing solution	0.4 M NaCl in 20% ethanol		
Slurry concentration (%)	50	50	50
Slurry volume (mL)	51.0	97.4	163
Packing factor (PF)	1.12	1.08	1.00
Packing flow velocity (cm/h)	200	200	400
Packing flow rate (mL/min)	6.7	6.7	13.4
Conditioning flow velocity (cm/h)	500	400	N/A
Conditioning flow rate (mL/min)	16.8	13.4	N/A

Column	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/packing solution	0.4 M NaCl in 20% ethanol		
Slurry concentration (%)	50	50	50
Slurry volume (mL)	126	252	440
Packing factor (PF)	1.15	1.10	1.10
Packing flow velocity (cm/h)	200	200	200
Packing flow rate (mL/min)	17.7	17.7	17.7
Conditioning flow velocity (cm/h)	500	400	230
Conditioning flow rate (mL/min)	44.2	35.4	20.4

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/packing solution	0.4 M NaCl in 20% ethanol		
Slurry concentration (%)	50	50	50
Slurry volume (mL)	520	1022	1725
Packing factor (PF)	1.16	1.14	1.08
Packing flow velocity (cm/h)	200	200	200
Packing flow rate (mL/min)	65.4	65.4	65.4
Conditioning flow velocity (cm/h)	350	300	230
Conditioning flow rate (mL/min)	114.5	98.2	75.3

Pack a HiScale column

Step	Action
1	Assemble the column according to the column instructions <i>HiScale columns (10, 16, 26, 50)</i> and accessories available on cytiva.com .
2	Place the support nets and the nets on the adapters.
3	Fasten the column tube in a stand. Attach a packing connector and a packing tube on top of the column tube if needed to achieve the requested bed height.
4	Connect the bottom adapter to the system and prime the bottom net with a slow upflow (30 cm/h) of packing solution. Make sure that the net is thoroughly wetted and that no air bubbles are trapped under the net.

Step	Action
5	Fill the column with slurry suspended in packing solution. The volume required for the respective column size and final bed height is shown in Main parameters for different column sizes, on page 22 . If needed, top up the slurry with additional packing solution so the top adapter dips into the slurry to avoid air under the net.
6	Connect the top adapter to the pump and prime with a slow downflow. Hold the adapter with the net facing upwards.
7	Attach the top adapter on top of the packing tube or the column tube. Tighten all O-rings firmly.
8	Start a downflow with packing solution. The flow velocity for different column sizes is shown in Main parameters for different column sizes, on page 22 .
9	Let the flow run until the bed consolidates.
	<p>Note:</p> <p><i>Some fines can still swirl around in the packing solution after the bed is consolidated.</i></p>
10	Turn off the flow. Remove the top adapter from the packing tube and disassemble the packing tube over a beaker or a sink.
11	Reattach the top adapter to the column tube. Make sure that no air is trapped under the net.
12	Turn the adapter down until it is 10 mm above the resin bed to displace the air in the adapter tubing. Make sure that the bed surface is not disturbed.
13	Restart a downflow with packing solution. Let the flow run until the bed has consolidated.
14	Measure the consolidated bed height using the graduation markings on the column. The use of a light source can facilitate the measurement of the bed height.
15	Turn the end cap of the top adapter until the net is at the bed height that was measured in the previous step.
16	Calculate the final bed height by dividing the consolidated bed height by the desired packing factor. Packing factors for the respective column sizes and bed heights are shown in Main parameters for different column sizes, on page 22 .

Step	Action
17	Turn the end cap clockwise, smoothly and slowly, until the target bed height is reached. The pressure peaks caused by turning the end cap must not exceed the maximum pressure values that are specified for the resin.
18	Start a downflow with packing solution. The flow velocity for different column sizes is shown in Main parameters for different column sizes, on page 22 . Let the flow run for 10 CV.

The column is ready for efficiency testing.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

7.3 Packing large-scale columns

Introduction

MabSelect VH3 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). The methods for packing MabSelect PrismaA in large-scale columns apply to MabSelect VH3 as well.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

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 column control unit, 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 production-scale columns

The following table lists the properties of the recommended production-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
Chromaflow™ 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 packing stations might be required for 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

Typical packing solutions for MabSelect VH3 are:

- water
- 20% ethanol
- NaCl solution

Packing factors for MabSelect VH3

When packing columns, the packing factor (PF) is used to calculate the target bed height after the consolidation step. MabSelect VH3 resin settles differently depending on the solution. Adding NaCl to the packing solution slows the settling of the resin beads and allows them to settle less tightly. A solution of 0.15 M NaCl changes the consolidated bed height by 2% to 3% and the gravity settled bed height by 16%, compared to water. The table below shows typical packing factors for MabSelect VH3 resin in different solutions for optimal bed performance, for a 20 cm bed that has consolidated at 60 cm/h. The packing factor usually is higher for lower bed heights and lower for higher bed heights.

Solution	Packing factor
Water	1.18
20% ethanol	1.18
NaCl solution	1.20

For MabSelect VH3 resin with the recommended packing factor, the compression factor would be 1.10.

7.4 Evaluation of packed column

Introduction

The quality of the packed bed and the column performance must be evaluated initially, and then monitored throughout the lifetime. The method for measuring the efficiency of a packed column are in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s).

Frequency

Test the column efficiency to evaluate the packing quality in the following conditions:

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

Column efficiency testing

Packed column efficiency is best expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a test sample such as 1% acetone solution or 0.8 M NaCl to the column.

Note: Use 0.4 M NaCl in water as eluent for a test sample of 0.8 M NaCl in water.

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 used remain the same so that the results are comparable.

Test results are affected by changes in the following parameters:

- solute
- solvent
- eluent
- sample volume
- flow velocity
- liquid pathway
- temperature
- chromatography system

For more information about column efficiency testing, refer to the application note *Column efficiency testing* available on [cytiva.com](https://www.cytiva.com).

Sample volume and flow velocity

For optimal column efficiency testing results, the sample volume must be approximately 1% of the CV and the flow velocity must be 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 column use.

Method for measuring HETP and A_s

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

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

L = bed height (cm)

N = number of theoretical plates

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

V_R = retention volume; the 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 must be 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.

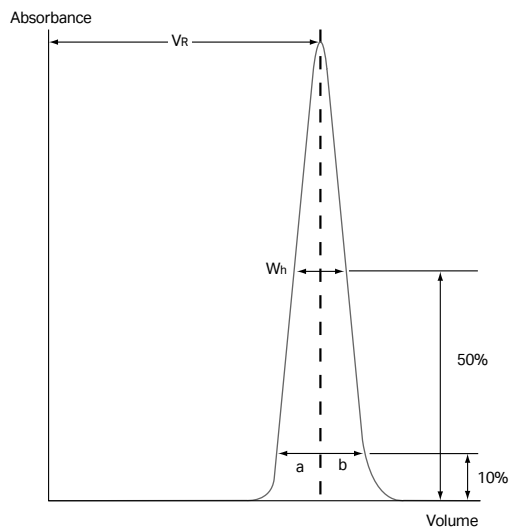
The peak asymmetry factor is calculated as follows:

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

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

a = ascending part of the peak width at 10% of the 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.



8 CIP

Introduction

CIP removes very tightly bound, precipitated, or denatured substances from the resin. The accumulated impurities or 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 0.5 M NaOH for CIP.

CIP must be performed regularly to prevent the enrichment of the impurities or contaminants and to maintain the capacity, flow properties, and general performance of the packed columns.

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

- before first-time use after packing or repacking a column and after long-term storage
- after each cycle with real feed
- when a deterioration of column performance is observed, such as a gradual 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, see the protocol in [Optimizing elution and wash conditions, on page 11](#).*

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.

CIP recommendation

CIP is usually performed immediately after regeneration/strip. 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. Always perform CIP in reverse-flow mode.

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) in reverse flow, with a contact time of 15 minutes.
3	Wash immediately with at least 5 CV binding buffer at pH 7 to 8.

9 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. For more information, refer to application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*.

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) in reverse flow, with a contact time of 15 minutes.
3	Wash immediately with at least 5 CV binding buffer at pH 7 to 8.

10 Storage

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

Equilibrate packed columns with a solution 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.

11 Scale-up

Introduction

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 a bed volume according to required binding capacity.
- Select a column diameter according to the volume throughput requirements. Then determine the bed height that gives the target residence time. Bed heights of 10 to 25 cm are generally appropriate.

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

- Keep the sample concentration and the elution conditions constant.
- Verify the purification step.

See [Process development, on page 9](#) for the appropriate window of operation for MabSelect VH3.

12 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Decrease the flow rate.
	The in-line filter is clogged.	Replace the in-line filter.
	The column is clogged.	Perform CIP.
	The adapter net/filter is clogged.	Clean or replace the adapter net/filter.
Unstable pressure curve during sample loading	Air bubbles are trapped in the 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 impurities or 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	The 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	The 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](https://www.cytiva.com).

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

Related products

Product	Pack size	Product code
HiTrap MabSelect VH3	1 × 1 mL	17549351
	5 × 1 mL	17549352
	1 × 5 mL	17549353
	5 × 5 mL	17549354
HiScreen MabSelect VH3	1 × 4.7 mL	17549315
PreDicator RoboColumn MabSelect VH3, 200 µL	1 × 8 columns	17549333
PreDicator RoboColumn MabSelect VH3, 600 µL	1 × 8 columns	17549334
PreDicator MabSelect VH3, 2 µL	4 × 96-well filter plates	17549330
PreDicator MabSelect VH3, 20 µL	4 × 96-well filter plates	17549331
PreDicator MabSelect VH3, 50 µL	4 × 96-well filter plates	17549332
MabSelect VH3 Validation column (10/200)	15.7 mL	17549370
Tricorn 5 Medium Filter Kit	1 × 5 units	29258132
Tricorn 10 Medium Filter Kit	1 × 5 units	29258131
Slurry Concentration Kit	1 unit	29096100
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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