MabSelect™ PrismA

MabSelect PrismA is an affinity BioProcess™ chromatography resin for capturing monoclonal antibodies and Fc-containing recombinant proteins. The high binding capacity and the high flow base matrix are combined to give exceptional productivity in protein A capture chromatography.

MabSelect PrismA provides:

- Enhanced dynamic binding capacity at commonly used residence times
- Highly concentrated elution pools for operating flexibility and small unit operations
- Excellent alkaline stability due to a protein A-derived ligand which allows the use of 0.5 to 1.0 M sodium hydroxide for Cleaning-In-Place (CIP)
- Simple scale-up to production-sized AxiChrom™ columns

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Read these instructions carefully before using the products.

Safety

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

1 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.

2 Product description

The protein A-derived ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal derived products. The ligand has been specially engineered to create an affinity resin with enhanced alkali stability and protease stability. The specificity of binding to the Fc region of IgG is similar to that of conventional Protein A and provides excellent purification in one step. MabSelect PrismA has very high dynamic binding capacities at most commonly used residence times, and is developed for modern antibody processes, see *Figure 1*.

Alkali tolerance, high capacity, and low ligand leakage plus the rigid base matrix, make MabSelect PrismA ideal for the purification of monoclonal antibodies for clinical applications. The characteristics of the resin are summarized in *Table 1*.

Table 1. Characteristics of MabSelect PrismA

Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50V} [1]	~ 60 µm
Ligand	MabSelect PrismA ligand (alkali-tolerant, protein A-derived from *E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10\%}$ [2]	~ 65 mg polyclonal IgG/mL resin, 4 minutes residence time ~ 80 mg polyclonal IgG/mL resin, 6 minutes residence time
Chemical stability	Stable to commonly used aqueous buffers for Protein A chromatography
pH stability,	
Operational [3]	3 to 12
CIP [4]	2 to 14
Recommended 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 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 minutes residence time) and 150 cm/h (4 minutes residence time) in a lab scale column with a 10 cm bed height in PBS buffer, pH 7.4.

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

4 pH range where 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 buffers with the same viscosity as water at 20°C.

Figure 1 shows the relation between dynamic binding capacity and residence time for MabSelect PrismA. *Figure 2* shows stability in alkaline conditions of MabSelect PrismA in terms of dynamic binding capacity. *Figure 3* shows a pressure/flow curve in water, 20°C, for 10 and 20 cm packed bed of MabSelect PrismA in 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.

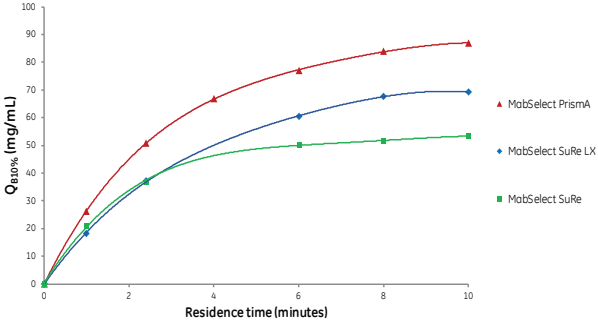

Fig 1. Relation between dynamic binding capacity ($Q_{B10\%}$) and residence time for MabSelect PrismA, HiScreen™ column, for polyclonal IgG. MabSelect SuRe™ LX and MabSelect SuRe have been included for comparison.

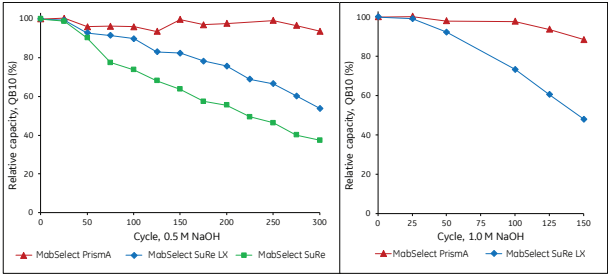

Fig 2. Relative dynamic binding capacity, $Q_{B10\%}$, for MabSelect PrismA after CIP with 0.5 M NaOH for 300 cycles, and 1.0 M NaOH for 150 cycles, 15 min contact time. MabSelect SuRe LX and MabSelect SuRe have been included for comparison.

Each cycle in *Figure 2* consisted of:

- 5 column volumes (CV) of binding buffer, pH 7.4
- 5 CV 0.1 M acetic acid, pH 3.0
- 3 CV NaOH (0.5 M to 1.0 M), 15 minutes contact time
- 5 CV binding buffer, pH 7.4

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

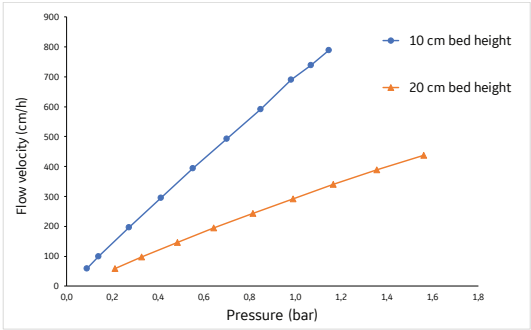

Fig 3. Example of a pressure/flow curve in water 20°C for 10 and 20 cm bed height of MabSelect PrismA in AxiChrom 300, packing factor 1.18. The additional pressure from test system and tubing has been subtracted.

3 Process development

For initial studies on MabSelect PrismA, PreDictor™ plates, or PreDictor RoboColumn™ units are preferably used. PreDictor plates are 96-well plates prefilled with chromatography resin, for rapid screening of chromatographic conditions at small-scale. PreDictor 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 HiScreen™ or HiTrap™ columns.

Choose a residence time (see *Figure 1*) that meets your needs for dynamic binding capacity and nominal fluid velocity according to *Figure 4*. Ancillary cycle operations including wash, elute and equilibration steps can be run at maximum operational flow velocities, see *Table 1*. An example of a pressure/flow curve in water is seen in *Figure 3*.

Figure 4 shows the recommended combinations of bed height and operational nominal flow velocity for MabSelect PrismA. The Figure also shows 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 height below 10 cm is not favorable. The reason for this is that large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.

Figure 4 can be used as a guide when determining suitable bed height and operating flow velocity in terms of residence time and thus capacity and pressure drop.

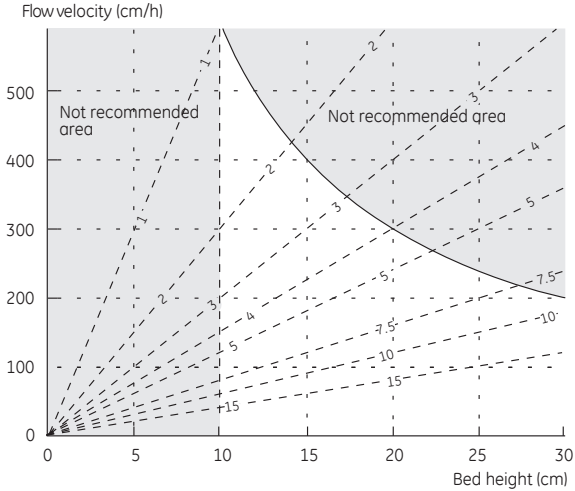

Fig 4. Operating window for MabSelect PrismA (white area). Choosing bed height and operating flow velocity in terms of residence time, pressure restrictions, and large-scale column packing challenges.

4 Recommended screening conditions

Examples of suitable buffers:

- Buffer A: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2
- Buffer B: 0.1 M sodium citrate, pH 3.0 to 3.6

Experimental conditions:

Step	Action
1	Equilibrate the column with 5 column volumes (CV) of buffer A.
2	Apply a small sample of antibody at residence time > 6 minutes.
3	Wash the column with 5 CV buffer A.
4	Elute the column with a 10 CV linear gradient from 0% to 100% buffer B.
5	Collect fractions into titrating diluent (e.g., 1.0 M Tris-HCl, pH 8.0 so that the diluent volume equals 5% of the programmed fraction volume).
6	Regenerate the column with 5 to 10 CV 100% buffer B.
7	Wash the column with 3 CV buffer A.
8	Perform CIP with at least 3 CV NaOH (0.5 to 1 M), 15 min contact time.
9	Re-equilibrate the column with buffer A.

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

Optimizing elution conditions

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column. This will prevent denaturing sensitive antibodies due to exposure to low pH. Stepwise elution, see *Figure 5*, is often preferred in large-scale applications since it allows the target monoclonal antibody 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 the high concentrations of protein in the eluate.

Figure 5 shows an example of purification of a monoclonal antibody from a clarified mammalian cell culture on MabSelect PrismA. The load was 63 mg antibody/mL CV, and the yield was 97% of highly purified antibody. A Tricorn™ 5/100 column with a CV of 2 mL and a bed height of 10 cm was used.

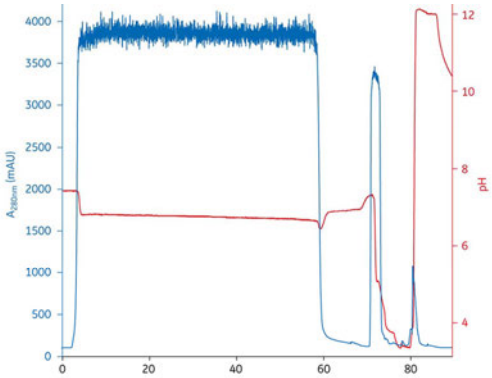

Fig 5. Purification of a monoclonal antibody from a mammalian cell culture on MabSelect PrismA.

Optimizing dynamic binding capacity

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 should therefore be defined over a range of different sample residence times.

5 Removal of leached ligand from final product

The PrismA protein A ligand can be analyzed using commercial available protein A immunoassays. The ligand leakage from MabSelect PrismA is generally low. For example, the eluate from the purification run shown in *Figure 5* contained 19 ppm (ng ligand/mg antibody) of leached ligand. However, in many monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product.

There are a number of chromatographic techniques to remove leached ligand, such as ion exchange chromatography, multimodal exchange chromatography, and size exclusion chromatography.

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* (28907892). Methods used for removal of leached ligand from MabSelect SuRe is applicable also for removal of leached ligand from MabSelect PrismA.

6 Packing lab-scale columns

Recommended lab-scale columns

Table 2. Recommended columns

Column	Inner diameter (mm)	Bed volume [1]	Bed height
HiScale™ 16/20	16	20 to 40 mL	max. 20
HiScale 16/40	16	20 to 70 mL	max. 35
HiScale 26/20	26	53 to 106 mL	max. 20
HiScale 26/40	26	53 to 186 mL	max. 35
HiScale 50/20	50	196 to 393 mL	max. 20
HiScale 50/40	50	196 to 687 mL	max. 35
Tricorn 5/100	5	2 mL	10
Tricorn 5/150	5	2 to 3 mL	max. 15
Tricorn 5/200	5	2 to 4 mL	max. 20
Tricorn 10/100	10	8 mL	10
Tricorn 10/150	10	8 to 12 mL	max. 15
Tricorn 10/200	10	8 to 16 mL	max. 20

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

Materials needed

- MabSelect PrismA
- Plastic spoon or spatula
- Glass filter G3
- Vacuum suction equipment
- Filter flask
- Measuring cylinder

For Tricorn columns:

- Tricorn column
- Tricorn packing tube
- 20% (v/v) ethanol with 0.2 M NaCl
- 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)
- 20% (v/v) ethanol with 0.4 M NaCl

Note:

A special support net is available for packing MabSelect PrismA in Tricorn columns:

- *Tricorn 5 Medium Filter Kit*
- *Tricorn 10 Medium Filter Kit*

These nets can be ordered as a Tricorn accessory, see Ordering information.

Equipment

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

Equilibrate all materials to room temperature.

A pressure relief valve can be attached to the outlet valve of the system to avoid drainage of the column during packing. A small back pressure of 0.2 bar is sufficient.

Definitions

The bed height of a gravity settled bed 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).

$L_{settled}$	Settled bed height/Gravity settled bed height. Bed height measured after settling by gravity.
L_{conc}	Consolidated bed height. Bed height measured after settling the resin at a given flow velocity.
L_{packed}	Packed bed height.
CF	Compression factor, $CF = L_{settled} / L_{packed}$
PF	Packing factor, $PF = L_{conc} / L_{packed}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry

Slurry preparation

Let the resin settle in 20% ethanol at least overnight in a measuring cylinder to determine the slurry concentration.

Calculate the volume of resin required with the following equation:

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

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

Equilibration to packing solution

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 funnel.
3	Wash 5 times with 2 column volumes (CV) of packing solution. Gently stir with a spatula between additions.
4	Pour the washed resin from the funnel into a beaker.
5	Add packing solution to obtain a 50% slurry concentration.

Packing Tricorn columns

Table 3. Main features of the packing method for Tricorn columns

Column	Tricorn 5	Tricorn 10
Bed height (cm)	10	10
Slurry/packing solution	20% ethanol with 0.2 M NaCl	
Slurry concentration (%)	50	50
Packing velocity (cm/h)	600	600
Packing flow rate (mL/min)	1.96	7.85
Flow condition (cm/h)	600	600
Flow condition (mL/min)	1.96	7.85

Step	Action
1	Wet the filters with ethanol and assemble the column according to *Tricorn Empty High Performance Columns* (28409488).
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.
4	Assemble a bottom piece to the top of the packing tube. Make sure no air is trapped under the filter.

Step	Action
5	Connect the column top to the pump and start a downflow with packing solution. The packing flow velocity is shown in *Main features of the packing method for Tricorn columns, on page 15*.
6	Pack the column for 10 column volumes (CV).
7	Turn the flow off and attach a stop plug to the column bottom.
8	Disassemble the packing tube and remove access resin 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 down until it is 1 to 2 mm above the resin bed to displace the air in the adapter tubing.
12	Connect the top adapter to the pump. Make a drop to drop connection to not introduce air to prevent air from entering the column.
13	Start a downflow with packing solution. The packing flow velocity is shown in *Main features of the packing method for Tricorn columns, on page 15*. Let the flow run for 5 minutes.
14	Mark the bed height and pause the pump.
15	Turn the adapter down to the mark, and then give the adapter an extra 1/3 turn.
16	Start a conditioning downflow with packing solution. The conditioning flow velocity is shown in *Main features of the packing method for Tricorn columns, on page 15*. Let the flow run for 10 CV. ***Note:*** *If a gap is formed between the bed and adapter during flow conditioning, turn the adapter down to the bed without stopping the flow.*

The column is ready for efficiency test, see *Evaluation of packed column*.

Packing HiScale columns

Table 4. Main features of the packing method for HiScale 16

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.12	1.10	1.10
Packing velocity (cm/h)	200	200	200
Packing flow rate (mL/min)	6.7	6.7	6.7
Flow condition (cm/h)	500	400	230
Flow condition (mL/min)	16.8	13.4	7.7

Table 5. Main features of the packing method for HiScale 26

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

Table 6. Main features of the packing method for HiScale 50

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.16	1.14	1.10
Packing velocity (cm/h)	200	200	200
Packing flow rate (mL/min)	65.4	65.4	65.4
Flow condition (cm/h)	350	300	230
Flow condition (mL/min)	114.5	98.2	75.3

Step	Action
1	Assemble the column according to the column instructions, *HiScale columns (16, 26, 50) and accessories* (28967470).
2	Attach a packing connector and a packing tube on top of the column tube. 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 fluid. Make sure the net is thoroughly wetted. If air bubbles are trapped under the net they can be removed by light suction with a syringe.
4	Fill the column with slurry suspended in packing solution. If needed, top the slurry up with extra packing fluid 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 in the adapter, hold the adapter with the net facing upwards.
6	Attach the top adapter on top of the packing column tube. Tighten all sealing firmly.
7	Start a downflow with packing solution. The flow velocity is shown in *Table 4* to *Table 6*.
8	Let the flow run until the bed consolidates. Do not let the bed consolidate for too long as that will make reading of the bed height more inaccurate.
9	Use the scale on the column to measure the bed height. The use of a light source can facilitate the measurement of the bed height.
10	Turn the flow off. Disassemble 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 the 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.

Step	Action
14	Calculate the final bed height by dividing the consolidated bed height with the desired packing factor. Packing factors for the different column sizes and bed heights are shown in *Table 4* to *Table 6*.
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 flow velocity is shown in *Table 4* to *Table 6*. Let the flow run for 10 column volumes.

The column is ready for efficiency test, see *Evaluation of packed column*.

7 Packing large-scale columns

MabSelect PrismA 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 *Application note 11000752* (*Packing MabSelect and MabSelect SuRe resins using verified methods*) that describes packing methods for similar resins in large-scale chromatography columns.

Intelligent Packing in AxiChrom columns

When packing AxiChrom 50 to 200 columns with ÄKTA systems, 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 ÄKTAprocess™ system.

In the Intelligent Packing wizard, packing methods are created by entering values for the packing variables, such as column, resin, packing factor, slurry concentration, and target bed height.

Recommended columns

Table 7. Recommended large-scale columns

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

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).

$L_{settled}$	Settled bed height/Gravity settled bed height. Bed height measured after settling by gravity.
L_{conc}	Consolidated bed height. Bed height measured after settling the resin at a given flow velocity.
L_{packed}	Packed bed height.
CF	Compression factor, $CF = L_{settled} / L_{packed}$
PF	Packing factor, $PF = L_{conc} / L_{packed}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry

Compression factor for MabSelect PrismA

The compression factor (CF) is used to calculate the resin volume needed to pack a desired bed height. CF for gravity settled MabSelect PrismA in 20% ethanol is 1.10.

Packing factors for MabSelect PrismA

When packing BPG and AxiChrom columns, the packing factor (PF) is used to calculate the target bed height after the consolidation step. MabSelect PrismA 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 2% and the gravity settled bed height 16%, compared to water. *Table 8* shows typical packing factors for MabSelect PrismA in different solutions for optimal bed performance, where the bed is consolidated at 60 cm/h.

Table 8. Typical packing factors for MabSelect PrismA

Solution	Packing factor
Water	1.18
20% (v/v) ethanol	1.18
0.4 M NaCl	1.20

Packing solution

Recommended packing solutions for MabSelect PrismA are water, 20% ethanol, and sodium chloride solution.

Slurry preparation

MabSelect PrismA is supplied as a suspension in 20% ethanol. When preparing the slurry, start by calculating the the volume (V) of resin needed to pack to the desired bed height, see *Measuring slurry concentration*.

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

Before packing, equilibrate the resin to the packing solution. The column can be used for wash and equilibration, refer to the instructions for the relevant column.

Measuring slurry concentration

It is important to measure the slurry concentration accurately so that the amount of resin for packing to target bed height or compression is correct.

MabSelect PrismA is supplied as a suspension in 20% ethanol. To calculate the amount of gravity settled resin needed, use the following equation:

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

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

Let the resin settle in 20% ethanol at least overnight in a measuring cylinder to determine the slurry concentration.

The slurry concentration can also be determined in a Tricorn 10/100 column. A Slurry Concentration Kit with all the material required for determination of slurry concentration is available, see *Ordering information*.

8 Evaluation of packed column

Introduction

The packing quality needs to be checked by column efficiency testing. The test must be done after the packing, and at regular intervals during the working life of the column, and also when the separation performance is deteriorated.

Column efficiency testing

The best 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, chromatography system, etc., influence the results.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (Product code: 28937207).

Sample volume and flow velocity

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:

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

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 units.

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

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

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

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be $0.8 < A_S < 1.8$.

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.

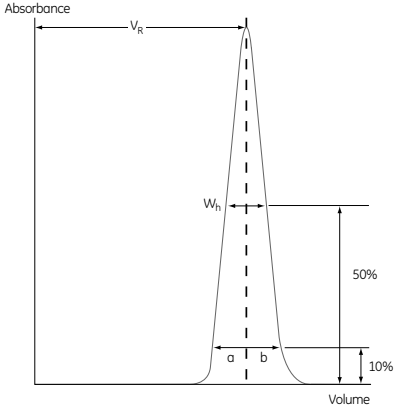

Fig 6. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

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 prepacked column, reduce the capacity, or contaminate the subsequent runs. MabSelect PrismA is a highly alkali-tolerant chromatography resin that allows the use of up to 1.0 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 prepacked columns.

It is recommended to perform a CIP:

- After every cycle with real feed
- When an increase in the 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 time storage

Note:

An acid regeneration (pH 3) before CIP is recommended if the antibodies were not completely eluted.

CIP protocol

Step	Action
1	Wash the column with 3 column volumes (CV) of binding buffer.
2	Wash with at least 3 CV NaOH (0.5 to 1.0 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.

NaOH concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP. High concentrations of NaOH and/or longer contact time increases the 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 will ultimately determine 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 may have to be combined, for example 0.5 M NaOH every cycle and 1.0 M NaOH every 10^{th} cycle.

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

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. MabSelect PrismA is alkali-tolerant allowing the use of NaOH as sanitizing agent. NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins. In addition, NaOH is inexpensive compared with other sanitizing agents.

Sanitization protocol

Step	Action
1	Wash the column with 3 column volumes (CV) of binding buffer.
2	Wash the column with at least 3 CV NaOH (0.5 to 1.0 M).
3	Use a contact time of at least 15 minutes for 0.5 to 1.0 M NaOH (see also the note below).
4	Wash immediately with at least 5 CV sterile and filtered binding buffer at pH 7 to 8.

Note:

Higher concentrations of NaOH and/or longer contact time inactivates 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 starting buffer and perform a blank run, including CIP, before use.

12 Scaling up

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

- Keep the residence time constant to maintain the dynamic binding capacity.
- Select bed volume according to required binding capacity. Verify the purification step with the new bed height, if it has changed.
- Select column diameter according to your 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 that the back pressure increases proportionally with increasing bed height at constant nominal velocity.
- Keep sample concentration and elution conditions constant.

See *Figure 4* for appropriate windows of operation for MabSelect PrismA.

13 Troubleshooting

The list describes faults observed from the monitor curves.

Fault	Possible cause/corrective action
High back pressure during the run	• Change the in-line filter. • The column is clogged. Perform CIP. • The adapter net/filter is clogged. Clean or replace the net/filter.
Unstable pressure curve during sample application	• Remove air bubbles that might have been trapped in 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, and/or perform CIP more frequently.
Gradual decrease in yield	• Too high sample load. Decrease the sample load. • Precipitation during elution. Optimize the elution conditions. • Insufficient elution and CIP. Optimize the elution conditions, the CIP protocol, and/or perform CIP more frequently.
Gradual increase in CIP peaks	• Insufficient elution and CIP. Optimize the elution conditions, the CIP protocol, and/or perform CIP more frequently.
High ligand leakage during the first purification cycle	• Perform a blank run, including CIP, before the first purification cycle on a new column.

14 Ordering information

Product	Quantity	Product code
MabSelect PrismA	25 mL	17549801
	200 mL	17549802
	1 L	17549803
	5 L	17549804
	10 L	17549805
MabSelect PrismA in benzyl alcohol	1 L [1]	17549823
	5 L [1]	17549824
	10 L [1]	17549825
HiTrap MabSelect PrismA	1 × 1 mL	17549851
	5 × 1 mL	17549852
	1 × 5 mL	17549853
	5 × 5 mL	17549854
HiScreen MabSelect PrismA	1 × 4.7 mL	17549815
PreDictor MabSelect PrismA, 6 µL	4 × 96-well filter plates	17549830
PreDictor MabSelect PrismA, 20 µL	4 × 96-well filter plates	17549831
PreDictor MabSelect PrismA, 50 µL	4 × 96-well filter plates	17549832
PreDictor RoboColumn MabSelect PrismA, 200 µL	8 × 200 µL	17549833
PreDictor RoboColumn MabSelect PrismA, 600 µL	8 × 600 µL	17549834
MabSelect PrismA, RTP column	1 L	17549861
	2.5 L	17549862
Tricorn 5 Medium Filter Kit	1 × 5 units	29258132
Tricorn 10 Medium Filter Kit	1 × 5 units	29258131
Slurry Concentration Kit	1 unit	29096100

[1] On request

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