



MabSelect SuRe

Affinity chromatography

Instructions for Use

MabSelect SuRe™ is an alkali-tolerant protein A-derived BioProcess™ resin for capturing monoclonal antibodies from large volumes of feed by packed bed chromatography. BioProcess chromatography resins are developed and supported for production-scale chromatography. 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.

- Alkali-tolerant protein A-derived ligand allows the use of 0.1 to 0.5 M sodium hydroxide for cleaning-in-place (CIP).
- Improved product quality and reduction in overall costs.
- Eliminates the need for expensive and corrosive CIP reagents.
- High dynamic binding capacity (DBC) reduces process time and amount of resin used.
- High-flow agarose matrix allows processing of large volumes of feed.
- Simple scale-up to production-sized BPG, AxiChrom™ and Chromaflow™ columns.

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Read these instructions carefully before using the product.

Safety

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

1 Description

The protein A-derived MabSelect SuRe ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal products. The ligand has been specially engineered to create an affinity resin with enhanced alkali stability and high binding capacity for IgG. 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. Alkali tolerance, high capacity, and low ligand leakage plus the rigid base matrix, make MabSelect SuRe ideal for the purification of monoclonal antibodies for clinical applications.

Figure 1 shows stability in alkaline conditions of MabSelect SuRe in terms of dynamic binding capacity. A pressure/flow curve is shown in Figure 2.

The characteristics of the resin are summarized in Table 1.

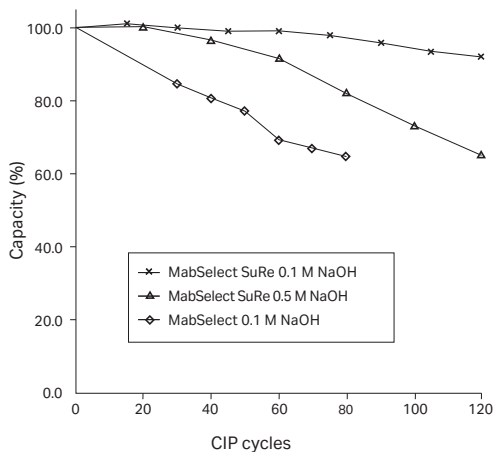


Fig 1. Dynamic binding capacity for MabSelect™ and MabSelect SuRe after CIP with 0.1 or 0.5 M NaOH, 15 min contact time, for 0 to 120 cycles

Each cycle in Figure 1 consists of:

- 10 column volumes binding buffer, pH 7.4
- 6 column volumes elution buffer, pH 3.0
- 3 column volumes of binding buffer, pH 7.4
- 0.1 or 0.5 M NaOH, 15 min contact time
- 6 column volumes binding buffer, pH 7.4

The dynamic binding capacity (DBC, 10% breakthrough) for MabSelect SuRe was measured every 20 cycles. The capacity of conventional Protein A resin (MabSelect) is also included in the figure for comparison.

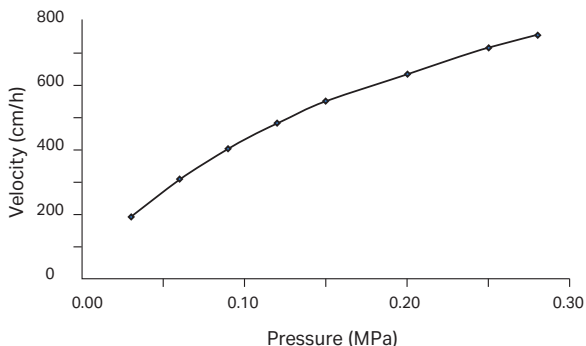


Fig 2. Typical pressure/flow profile for MabSelect SuRe in a packed bed (bed height 20 cm) in a BPG 300 column (i.d. 300 mm) in water

Table 1. Characteristics of MabSelect SuRe

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v}¹	~ 85 μm
Ligand	Allkali-tolerant, protein A-derived (<i>E. coli</i>)
Coupling chemistry	Epoxy
Dynamic binding capacity, Q_{B10}²	~ 35 mg hIG/mL resin
Chemical stability	Stable to commonly used aqueous buffers
pH stability, operational³	3 to 12
pH stability, CIP⁴	3 to 13.7
Cleaning-in-place stability⁵	0.1 to 0.5 M NaOH
Recommended maximum operating flow velocity	500 cm/h ⁶
Temperature stability	2°C to 40°C
Delivery conditions	20% ethanol
Storage	2°C to 8°C, 20% ethanol
Regulatory support	Regulatory support file is available. No material of animal origin is used in the manufacturing process.

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 500 cm/h in an XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M NaH_2PO_4 , 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 See Figure 1 and Section 7 Cleaning-In-Place (CIP).

⁶ In a BPG 300 column with 30 cm diameter and 20 cm bed height using buffers with the same viscosity as water at 25°C.

2 Process development

For initial studies of MabSelect SuRe in small-scale columns, we recommend prepacked HiScreen™ columns or HiScale™ 10/40 columns with 10 cm bed height. Choose a residence time (see Table 1, footnote 2) that fulfills your demand on dynamic binding capacity and nominal flow velocity according to Figure 3. Make sure the chosen bed height and velocity do not conflict with the large-scale pressure/flow limitations.

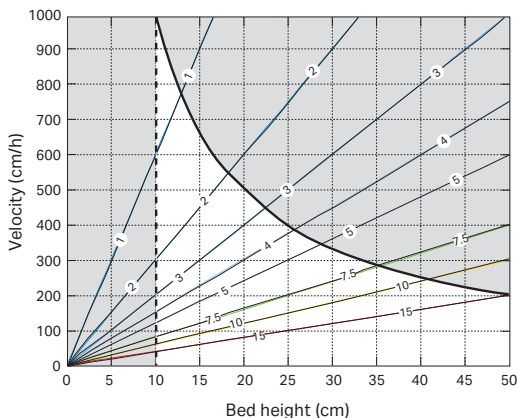


Fig 3. Recommended operating window for MabSelect SuRe (white area). Choosing bed height and operating velocity in terms of residence time, pressure restrictions, and large-scale column packing challenges

Figure 3 shows the possible combinations of bed height and operational nominal flow velocity for MabSelect SuRe. The figure also displays the residence time in the interval 1 to 15 minutes for any bed height and velocity. Included are also pressure drop limitations and packing limitations at large-scale. The solid curved line shows the calculated large-scale column pressure restriction which is 2 bar according to specification (500 cm/h at 2 bar and 20 cm bed height). The dashed vertical line indicates that operating at below 10 cm bed height 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 3 can be used as a guide when determining suitable bed height and operating velocity in terms of residence time and thus capacity and pressure drop.

Figure 4 shows the relation between dynamic binding capacity and residence time for MabSelect SuRe.

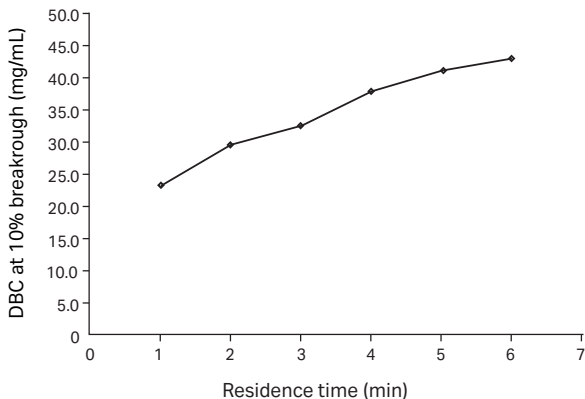


Fig 4. Relation between dynamic binding capacity and residence time for MabSelect SuRe for a purified monoclonal antibody

3 Recommended screening conditions

Note: *To save material, screening can also be performed using PreDictor™ plates.*

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:

- Equilibrate the column with 5 column volumes of buffer A.
- Apply a small sample of antibody.
- Wash the column with 5 column volumes of buffer A.
- Elute the column with a linear gradient of 10 column volumes to 100% buffer B.
- 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).
- Regenerate the column with 5 to 10 column volumes of 100% buffer B.
- Wash the column with 3 column volumes of buffer A.
- Perform CIP with 5 column volumes of 0.1 to 0.5 M NaOH.
- Re-equilibrate the column with buffer A.

Washing

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

Elution

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 (Figure 5) is often preferred in large-scale applications since it allows the target monoclonal antibody to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. It might be necessary to decrease the flow rate due to the high concentrations of protein in the eluate.

Example

Figure 5 shows an example of purification of a monoclonal antibody from a clarified mammalian cell culture on MabSelect SuRe. The load was 21 mg antibody/mL column volume (CV), and the yield was 94% of highly purified antibody. An XK 16/20 column with a CV of 20 mL and a bed height of 10 cm was used.

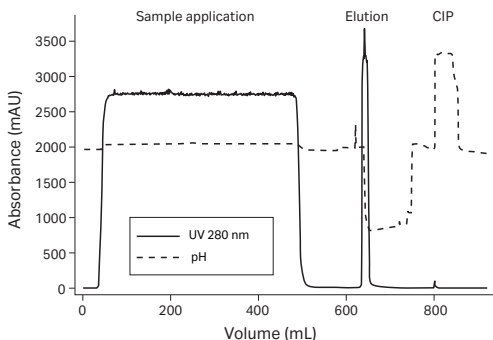


Fig 5. Purification of a monoclonal antibody from a mammalian cell culture on MabSelect SuRe

Dynamic binding capacity

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

4 Removal of leached ligand from final product

The ligand leakage from MabSelect SuRe is generally low. For example, the eluate from the purification run shown in Figure 5 contained 3 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 solutions, such as cation and anion exchange chromatography, or multimodal anion exchange chromatography, which can be used to remove leached ligand. For more details about removal of leached ligand and antibody aggregates, see the application note *Two step purification of monoclonal IgG1 from CHO cell culture supernatant* (28907892).

5 Packing columns

Recommended columns

Table 2. Recommended columns for MabSelect SuRe

Column	Inner diameter (mm)	Bed volume¹	Bed height (cm)
Lab-scale			
HiScale 10/40	10	8 to 20 mL ⁶	max 25 ⁶
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
Production-scale			
AxiChrom ²	50 to 200	0.2 to 12.5 L	max 30
AxiChrom ²	300 to 1000	7 to 314 L	max 30
BPG ³	100 to 300	1 to 28 L	max 40
Chromaflo standard ^{4,5}	400 to 800	12 to 151 L	max 30

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

² Intelligent Packing method according to MabSelect SuRe can be used.

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

⁴ See Application note: *Packing MabSelect and MabSelect SuRe resins using verified methods* (11000752).

⁵ Larger pack stations might be required at larger diameters.

⁶ Packing methods for bed heights up to 25 cm are provided

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

For more details about packing HiScale columns, see instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*. For information on packing of process-scale columns, contact your local Cytiva representative.

Packing Tricorn columns

Packing preparations

Materials needed

MabSelect SuRe

Plastic spoon or spatula

Glass filter G3

Vacuum suction equipment

Filter flask

Measuring cylinder

Tricorn column

Tricorn packing tube

20% ethanol with 0.2 M NaCl

Equipment

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

Equilibrate all materials to room temperature.

A pressure relief valve can be attached onto the outlet valve of the system to avoid drainage of the column at packing. A small back pressure of 0.02 MPa (0.2 bar, 2.9 psi) 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_{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}	Concentration of the slurry

Preparing the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder. To calculate the amount of resin to fill into the column, use the following equation:

$$V = (A_C \times L_{\text{packed}} \times (PF + 0.09)) / C_{\text{slurry}}$$

Equilibrating to packing solution

- 1 Put a glass filter funnel onto a filtering flask.
- 2 Suspend the resin by shaking the measuring cylinder and pour 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 the column

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)	700	700
Packing flow rate (mL/min)	2.29	9.16
Flow condition (cm/h)	700	700
Flow condition (mL/min)	2.29	9.16

Packing procedure

- 1 Wet the filters with ethanol and assemble the column according to *Tricorn Empty High Performance Columns (28409488)*.
- 2 Attach a packing tube on top of the column tube.
- 3 Fill the column with slurry suspended in packing solution and top up with packing solution.
- 4 Attach a bottom piece, making sure no air is trapped under the filter.
- 5 Connect the column top to the pump and start a packing flow running downwards the column with packing solution. The flow velocity is shown in Table 3.
- 6 Pack the column for 10 column volumes.
- 7 Turn the flow off and attach a stop plug to the column bottom.
- 8 Dismount the packing tube and remove access resin using a pipette.
- 9 Top up the column with packing solution.
- 10 Attach the adapter. Air in the adapter tubing is displaced by turning the adapter down until it is 1 to 2 mm above the resin bed.
- 11 Start a packing flow running downwards the column with packing solution. The flow velocity is shown in Table 3. Let the flow run for 5 minutes.

- 12** Mark the bed height and pause the pump.
- 13** Turn the adapter down to the mark, and then give the adapter an extra 1/3 turn.
- 14** Start a conditioning flow running downwards the column with the packing solution. The flow velocity is shown in Table 3. Let the flow run for 10 column volumes.

Note: *If a gap is formed between the bed and the adapter during flow conditioning, turn the adapter down to the bed without stopping the flow.*

The column is ready to be tested.

Packing HiScale columns

Packing preparations

Materials needed

MabSelect SuRe

HiScale column

HiScale packing tube (depending on bed height)

Plastic spoon or spatula

Glass filter G3

Vacuum suction equipment

Filter flask

Measuring cylinder

20% ethanol with 0.4 M NaCl

Equipment

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

Equilibrate all materials to room temperature.

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_{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}	Concentration of the slurry

Preparing the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28925932. This method can also be used with HiScale columns.

Washing the resin

Put a glass filter funnel onto a filtering flask. Suspend the resin by shaking and pour into the funnel and wash according to the following instructions:

- 5 times with 5 mL 20% ethanol with 0.4 M NaCl/mL resin
- Gently stir with a spatula between additions.
- Move the washed resin from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration. For HiScale 10/40, a slurry concentration of 58% is recommended.

Packing the column

Table 4. Main features of the packing method for HiScale 10/40

Column	HiScale 10/40		
Bed height (cm)	10	20	25
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	58	58	58
Packing factor (PF)	1.00 ¹	1.00 ¹	1.00 ¹
Packing velocity (cm/h)	1222	1222	1070
Packing flow rate (mL/min)	16	16	14
Flow condition (cm/h)	1222	1222	1070
Flow condition (mL/min)	16	16	14

¹) When packing HiScale10/40 columns, the compression of the resin to desired bed height is done either with a combination of flow and mechanical compression or by using flow compression only. The latter is applicable for MabSelect Sure. Since the packing factor in Table 4 refers only to mechanical compression it is consequently 1.00.

Table 5. Main features of the packing method for HiScale 16/20 and HiScale 16/40

Column	HiScale 16/	HiScale 16/40	
	20		
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.10	1.10	1.06
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	10	10	10
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	25	15	8.6

Table 6. Main features of the packing method for HiScale 26/20 and HiScale 26/40

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	1.13	1.10
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	27	27	27
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	66	40	23

Table 7. Main features of the packing method for HiScale 50/20 and HiScale 50/40

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)	1.15	1.10	1.06
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	100	100	100
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	250	150	86

Packing procedure

- 1** Assemble the column according to the column instructions (*HiScale columns (10, 16, 26, 50) and accessories, 28967470*).
- 2** Put the column tube in a stand.
- 3** Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry but if air is trapped under the net it can be removed by a light suction with a syringe.
- 4** Attach the bottom adapter unit in the bottom of the column tube and tighten the O-ring firmly.
- 5** Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet.
- 6** Attach the packing tube on top of the column tube if needed to achieve the requested bed height.
- 7** Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing the roof as this is done. If air is trapped under the net it can be removed by a light suction with a syringe.
- 8** Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
- 9** Put the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.
- 10** Start a downward flow with .packing velocity according to Table 4, 5, 6, or 7.
- 11** Let the flow run until the bed has consolidated.
- 12** Use the scale on the column to measure the bed height. There might be a buildup of resin at the column wall after the bed is consolidated and to easier see where the top of the bed is, a light source can be used.

- 13 Calculate the final bed height by dividing the consolidated bed height with the desired packing factor.

$$L_{\text{packed}} = L_{\text{cons}} / \text{PF}$$

Note: For HiScale 10/40 columns, the compression of the packed bed is done either with a combination of flow and mechanical compression or by using flow compression only. The latter is applicable for MabSelect Sure. Since the packing factor in Table 4 refers only to mechanical compression it is consequently 1.00.

For HiScale 10/40, proceed to Step 22

- 14 Turn off the flow and put a stop plug in the bottom.
- 15 Dismount the top adapter from the packing tube.
- 16 Over a beaker or a sink, detach the packing tube from the column.
- 17 Remount the top adapter in the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.
- 18 Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.
- 19 Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the resin.
- 20 Start a downward flow to flow condition the bed. The flow rate is shown in Table 4, 5, 6, or 7.
- 21 Let the flow run for about 10 column volumes.
The column is ready to be tested.

HiScale 10/40

- 22 For HiScale 10/40, increase the flow to compress and condition the bed. The flow is shown in Table 4. Continue this phase for 5 column volumes (CV). At the end of this phase, mark the bed height while keeping up the flow.

- 23** Turn off the flow and untighten the top adapter slightly (release the pressure on the O-ring) which facilitates adjustment of the adapter without "twisting" the O-ring. This procedure must be performed with the bottom tubing open which makes it possible to monitor the pressure. The pressure that is generated from turning the adapter down must not exceed the pressure during the conditioning phase. Continue to turn the adapter down until reaching the bed height mark.
- 24** Before starting the conditioning flow, the O-ring must be firmly tightened.
- 25** Continue the conditioning phase with 10 CV with the conditioning flow according to Table 4.
- 26** The HiScale 10/40 column is now ready to be tested.

6 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing must be done after packing, at regular intervals during the working life of the column, and when separation performance is seen to deteriorate.

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). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

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

Note: *The calculated plate number will vary according to the test conditions and it must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc., will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum 2.5% of the column volume and the liquid 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 column use.

Method for measuring HETP and A_s

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

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

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

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

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

Figure 6 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

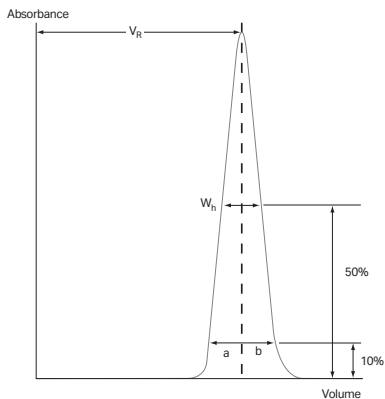


Fig 6. A typical test chromatogram showing the parameters used for HETP and A_s calculations

7 Cleaning-In-Place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the purification system. If such contaminants are allowed to accumulate, they can affect the chromatographic properties of the column, reduce the capacity of the column and, potentially, come off in subsequent runs. If the fouling is severe, it can block the column, increase back pressure, and reduce flow rate.

Regular CIP prevents the buildup of contaminants in the packed bed, and helps to maintain the capacity, flow properties, and general performance of MabSelect SuRe. We recommend performing a blank run, including CIP, before the first run with antibody feed.

MabSelect SuRe is an alkali-tolerant resin allowing the use of NaOH as CIP agent. NaOH is widely accepted for cleaning due to the low cost and the ability to dissolve proteins and saponify fats.

For difficult cases where CIP with NaOH is not sufficient to restore the column performance an extended protocol including wash with 100 mM thioglycerol pH 8.5 followed by CIP with 0.1 to 0.5 M NaOH is recommended. For more details, see the application note *High-throughput process development for design of cleaning-in-place protocols (28984564)*.

CIP protocol

- 1 Wash the column with 3 column volumes of binding buffer.
- 2 Wash with at least 2 column volumes of 0.1 to 0.5 M NaOH. Contact time 10 to 15 minutes.
- 3 Wash immediately with at least 5 column volumes of sterile and filtered binding buffer at pH 7 to 8.

CIP is usually performed immediately after the elution. Before applying the alkaline NaOH CIP solution, we recommend equilibrating the column with a solution of neutral pH in order 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.

NaOH concentration, contact time, and frequency are typically the main parameters to vary during the optimization of the CIP. The

nature of the feed material will ultimately determine the final CIP. However, the general recommendation is to clean the column at least every 5 cycles during normal use. Depending on the nature of the contaminants, different protocols may have to be combined, for example 0.1 M NaOH every cycle and 0.5 M NaOH every 10 cycles.

8 Sanitization

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

- 1 Wash the column with 3 column volumes of binding buffer.
- 2 Equilibrate the column with 0.1 to 0.5 M NaOH.
- 3 Use a contact time of at least 15 minutes for 0.5 M NaOH or 30 minutes for 0.1 M NaOH (see also the note below).
- 4 Wash immediately with at least 5 column volumes of sterile and filtered binding buffer at pH 7 to 8.

For more challenging microbial contamination, a mixture of 30% to 40% 1- or 2-propanol in 0.5 M NaOH could be used for sanitization.

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 must therefore be evaluated to maximize microbial killing and to minimize loss of capacity.*

9 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.

10 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 in order 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 is 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 backpressure increases proportionally with increasing bed height at constant nominal velocity.
- Keep sample concentration and elution conditions constant.

See also Figure 3 for appropriate windows of operation for MabSelect SuRe.

11 Troubleshooting

The list describes faults observed from the monitor curves.

Fault	Possible cause/corrective action
High backpressure during the run	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• Might be due to insufficient elution and CIP caused by contaminants accumulating in the column. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.• Perform wash with thioglycerol followed by CIP with NaOH, according to Section 7.
Gradual decrease in yield	<ul style="list-style-type: none">• Too high sample load. Decrease the sample load.• Precipitation during elution. Optimize the elution conditions.• Might be due to insufficient elution and CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks	<ul style="list-style-type: none">• Might be due to insufficient elution or CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.• Perform wash with thioglycerol followed by CIP with NaOH, according to Section 7.
High ligand leakage during the first purification cycle	Perform a blank run, including CIP, before the first purification cycle on a new column.

12 Ordering information

Product	Quantity	Product code
MabSelect SuRe	25 mL	17543801
	200 mL	17543802
	1 L	17543803
	5 L	17543804
	10 L	17543805

Related product	Quantity	Product code
HiTrap™ MabSelect SuRe	1 × 1 mL	29049104
	5 × 1 mL	11003493
	1 × 5 mL	11003494
	5 × 5 mL	11003495
HiScreen MabSelect SuRe	1 × 4.7 mL	28926977
PreDictor MabSelect Sure, 6 µL	4 × 96-well filter plates	28925823
PreDictor MabSelect Sure, 20 µL	4 × 96-well filter plates	28925824
PreDictor MabSelect Sure, 50 µL	4 × 96-well filter plates	28925825
HiScale 10/40	1	29360550
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444
Tricorn 5/100	1	28406410
Tricorn 5/150	1	28406411
Tricorn 5/200	1	28406412
Tricorn 10/100	1	28406415
Tricorn 10/150	1	28406416
Tricorn 10/200	1	28406417

Accessories	Quantity	Product code
Tricorn Glass Tube 5/100	1	18115306
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
Packing tube 20 (HiScale 10)	1	29360551
Packing tube 20 (HiScale 16)	1	28986816
Packing tube 40 (HiScale 16)	1	28986815
Packing tube 20 (HiScale 26)	1	28980383
Packing tube 40 (HiScale 26)	1	28964505
Packing tube 20 (HiScale 50)	1	28980251
Packing tube 40 (HiScale 50)	1	28964506

Related literature	Product code
Data Files	MabSelect SuRe 11001165
	AxiChrom Columns 28929041
	BPG columns 18111523
	Chromaflow columns 18113892
	HiScale columns 28975523
	Tricorn empty High Performance columns 18114736
Application notes	MabSelect SuRe – Leakage and Toxicity 11001164
	MabSelect – Column packing 11000752
	Two step purification of monoclonal IgG1 from CHO cell culture supernatant 28907892
	High-throughput process development for design of cleaning-in-place protocols 28984564

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