MabSelect™ SuRe LX

Affinity chromatography

Instructions for Use

MabSelect™ SuRe LX is an affinity chromatography BioProcess™ resin for capturing high-titer monoclonal antibodies from large volumes of feed by packed bed chromatography.

- High dynamic binding capacity for high-titer cultures reduces process time and amount of resin used.
- Alkali-tolerant, protein A-derived ligand allows for the use of 0.1 to 0.5 M sodium hydroxide for cleaning-in-place (CIP). This eliminates the need for expensive and corrosive CIP reagents.
- High-flow agarose matrix allows for processing of large volumes of feed.
- Improved performance and reduction in overall costs.
- Straightforward scale-up to production-sized AxiChrom™ 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 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 Description

The protein A-derived MabSelect SuRe LX ligand is produced in *E. 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 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 SuRe LX has high dynamic binding capacities at extended residence times, and is developed for high titer antibody processes. Alkali tolerance, high capacity, and low ligand leakage plus the rigid base matrix, make MabSelect SuRe LX suitable for the purification of monoclonal antibodies for clinical applications.

Fig. 1, on page 4 shows the relation between dynamic binding capacity and residence time for MabSelect SuRe LX.

Fig. 2, on page 4 shows stability in alkaline conditions of MabSelect SuRe LX in terms of dynamic binding capacity.

Fig. 3, on page 5 shows dynamic binding capacity of MabSelect SuRe LX compared to MabSelect SuRe.

Resin properties are summarized in *Table 1, on page 6*.

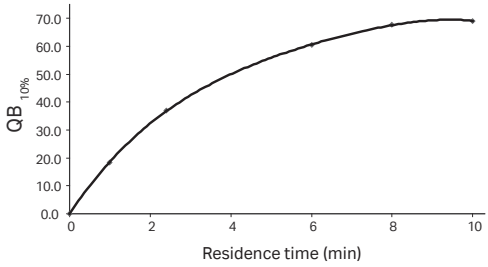


Fig 1. Relation between dynamic binding capacity and residence time for HiScreen™ MabSelect SuRe LX for polyclonal IgG.

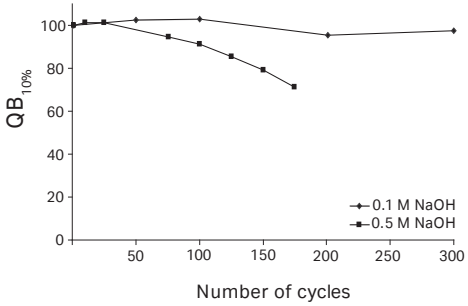


Fig 2. Dynamic binding capacity (residence time 6 min) for MabSelect SuRe LX after CIP with 0.1 and 0.5 M NaOH for 0 to 300 cycles.

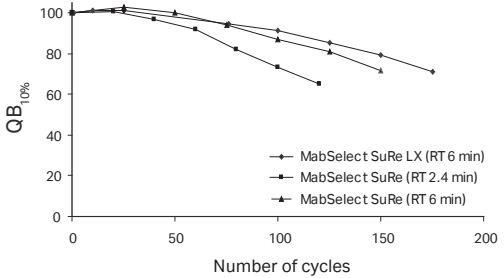


Fig 3. Dynamic binding capacity (residence time [RT] 2.4 and 6 min) for MabSelect SuRe LX and MabSelect SuRe after CIP with 0.5 M NaOH for 0 to 175 cycles.

Each cycle in Figure 2 and 3 consisted of:

- 5 column volumes binding buffer pH 7.4
- 5 column volumes 0.1 M acetic acid pH 3.0
- 0.1 or 0.5 M NaOH, 15 minutes contact time
- 5 column volumes binding buffer pH 7.4

The dynamic binding capacity for polyclonal IgG ($QB_{10\%}$) was measured regularly during the study.

Table 1. Resin properties

Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50v}[1]	85 µm
Ligand	MabSelect SuRe ligand (alkali-tolerant, protein A-derived (from *E. coli*))
Coupling chemistry	Epoxy
Dynamic binding capacity[2]**, $QB_{10\%}$**	~ 60 mg human IgG/mL resin
Chemical stability	Stable in aqueous buffers commonly used in protein A chromatography
pH stability, operational	3 to 12
pH stability, CIP[3]	2 to 13.7
CIP stability	0.1 to 0.5 M NaOH
Maximum operating flow velocity[4]	500 cm/h
Temperature stability[5]	2°C to 4°C
Storage	2°C to 8°C, 20% ethanol or 2% benzyl alcohol
Delivery conditions	20% ethanol

[1] Median particle size of the cumulative volume distribution.

[2] Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h in a column with a bed height of 20 cm, i.e. residence time is 6.0 min.

[3] See *Fig. 2, on page 4* and *Chapter 8 CIP, on page 28*.

[4] In AxiChrom 300 column, bed height 20 cm, operating pressure < 2 bar, water at 20°C.

[5] Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

3 Process development

For initial studies on MabSelect SuRe LX, PreDictor™ plates are recommended. PreDictor plates are 96-well plates prefilled with chromatography resins, that can be used for rapid screening of chromatographic conditions at a small scale. For further optimization in small-scale columns, we recommend prepacked HiScreen columns or HiScale™ columns.

Choose a residence time (see *Dynamic binding capacity,*) that fulfills your demand on dynamic binding capacity and flow velocity according to *Fig. 5, on page 8*. Ancillary cycle operations including wash, elution, and equilibration steps can be run at maximum operational velocities, see *Table 1, on page 6*. Example of a pressure-flow curve in water is seen in *Fig. 4, on page 7*.

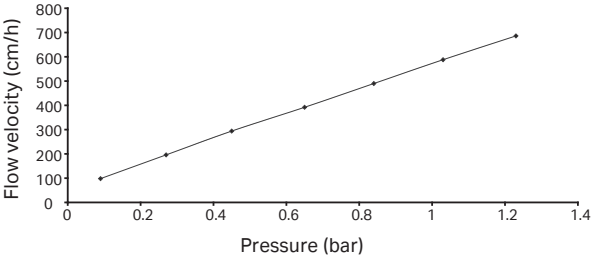


Fig 4. Example of a pressure-flow curve in water 20°C for 20 cm packed bed of MabSelect SuRe LX in AxiChrom 300, packing factor 1.15. The additional pressure from test system and tubing is subtracted.

Make sure that the selected bed height and flow velocity are compatible with the large-scale pressure-flow limitations (*Fig. 5, on page 8*).

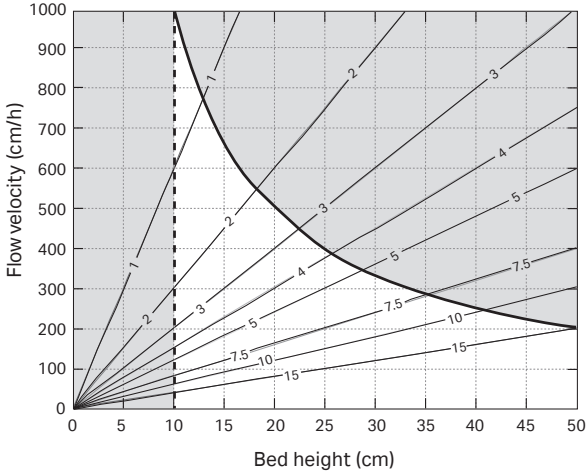


Fig 5. Operating window for MabSelect SuRe LX (white area). Choosing bed height and operating velocity in terms of residence time, pressure restrictions, and large-scale column packing challenges.

Fig. 5, on page 8 shows the possible combinations of bed height and operational nominal fluid velocity for MabSelect SuRe LX. The figure also displays the residence time in the interval 1 to 15 minutes for any bed height and velocity. Pressure drop limitations and packing limitations at large scale are also included. The solid curved line shows the calculated large-scale column pressure restriction that 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.

Fig. 5, on page 8 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.

4 Recommended screening conditions

Examples of suitable buffers:

- Equilibration buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2.
- Elution buffer: 0.1 M sodium citrate, pH 3.0 to 3.6.

Experimental conditions:

- Equilibrate the column with 5 column volumes (CV) of equilibration buffer.
- Apply a small sample of antibody at residence time ≥ 6 min.
- Wash the column with 5 CV of equilibration buffer.
- Elute the column with a 10 CV linear gradient from 0% to 100% elution buffer.
- 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 CV of 100% elution buffer.
- Wash the column with 3 CV of equilibration buffer.
- Perform CIP with 5 CV of 0.1 to 0.5 M NaOH.
- Re-equilibrate the column with equilibration buffer.

Washing

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

- residence time
- volumes
- pH
- conductivity

Elution

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 (*Fig. 6, on page 11*) after suitable elution conditions have been determined. 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.

Example

Fig. 6, on page 11 shows an example of purification of a monoclonal antibody from a clarified mammalian cell culture on MabSelect SuRe LX. The load was 21 mg antibody/mL CV, and the yield was 94% of highly purified antibody. A HiScale 16/20 column with a CV of 20 mL and a bed height of 10 cm was used.

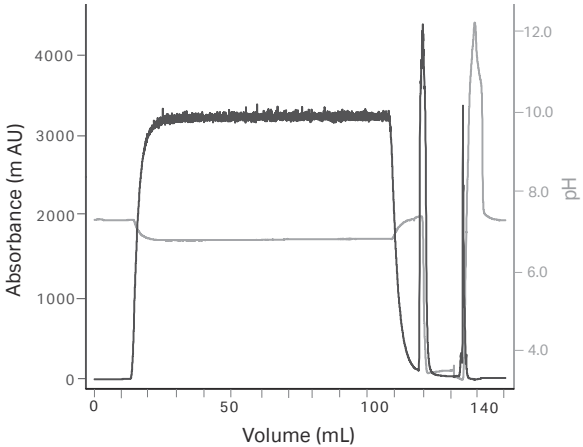


Fig 6. Purification of a monoclonal antibody from a mammalian cell culture on MabSelect SuRe LX.

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 determined over a range of different sample residence times.

5 Removal of leached ligand from final product

Ligand leakage from MabSelect SuRe LX is generally low. For example, the eluate from the purification run shown in *Fig. 6, on page 11* contained 11 ppm (ng ligand/mg antibody) of leached ligand. However, in many monoclonal antibody applications it is required to remove leached ligand from the final product. Techniques to remove leached ligand include:

- ion exchange chromatography (IEC)
- multimodal chromatography (MMC)

For an example of the removal of leached ligand and antibody aggregates, refer to application note *Two-step purification of monoclonal IgG_1 from CHO cell culture supernatant (CY13148)*. Methods used for removal of leached ligand from MabSelect SuRe are applicable also for removal of leached ligand from MabSelect SuRe LX.

6 Packing columns

Recommended columns

Table 2. Recommended columns for MabSelect SuRe LX

Column	Inner diameter (mm)	Bed volume[1]	Bed height (cm)
Laboratory-scale			
HiScale 10/40	10	8 to 20 mL[2]	Max 25[2]
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

Column	Inner diameter (mm)	Bed volume[1]	Bed height (cm)
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[3]	50 to 200	0.2 to 12.5 L	max 40
AxiChrom[3]	300 to 1000	7 to 314 L	max 40
BPG[4]	100 to 300	1 to 28 L	max 40
Chromaflow™ standard[5,6]	400 to 800	12 to 151 L	max 30

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

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

3 Intelligent packing method for MabSelect SuRe can be used.

4 The pressure rating of BPG 450 is too low for use with MabSelect resins.

5 Refer to Application note *Packing MabSelect and MabSelect SuRe LX resins using verified methods* (CY13950).

6 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 below 10 cm.

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 LX
- 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 to the outlet valve of the system to avoid drainage of the column during 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 = $L_{settled}/L_{packed}$
PF	Packing factor PF = L_{cons}/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

To measure the slurry concentration, pour the resin in a measuring cylinder and let the resin settle in 20% ethanol at least overnight or use the Slurry concentration kit (29096100). To calculate the amount of resin to fill into the column, use the following equation:

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

Equilibrate to packing solution

1. Put a glass filter funnel onto a filter flask.
2. Shake the measuring cylinder to suspend the resin and pour the slurry into the glass filter funnel.

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

Pack the column

Table 3. Main packing parameters for Tricorn columns

Parameter	Tricorn 5	Tricorn 10
Bed height (cm)	10	10
Slurry/packing solution	20% ethanol with 0.2 M NaCl	
Slurry concentration (%)	50	50
Packing flow velocity (cm/h)	700	700
Packing flow rate (mL/min)	2.29	9.16
Conditioning flow velocity (cm/h)	700	700
Conditioning flow rate (mL/min)	2.29	9.16

Packing procedure

Step	Action
1	Wet the filters with packing solution and assemble the column according to *Tricorn Empty High Performance Columns* (28409488) 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:*** *Make sure that no air is trapped below the filter.*
5	Connect the column top to the pump and start a downflow with packing solution. The flow velocity is shown in *Table 3, on page 16*.
6	Let the flow run for 5 min.
7	Turn off the flow and attach a stop plug to the column bottom.
8	Disassemble the packing tube and remove excess resin, using a pipette.
9	Top up the column with packing solution.
10	Attach the top adapter. ***Note:*** *Make sure that no air is trapped below 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 prevent air from entering the column.

Step	Action
13	Start a downflow with packing solution. The conditioning flow velocity is shown in *Table 3, on page 16*. Let the flow run for 5 minutes.
14	Mark the bed height and pause the pump.
15	Turn the adapter down toward the mark, and give the adapter an extra 1/3 turn.
16	Start a conditiong downflow with packing solution. The conditioning flow velocity is shown in *Table 3, on page 16*. Let the flow run for 5 min. ***Note:*** *If a gap is formed between the bed and the adapter during flow conditioning, turn the adapter down toward the bed without stopping the flow.*

The column is ready to be tested.

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

Packing HiScale columns

Materials needed

- MabSelect SuRe LX
- HiScale column
- HiScale packing tube (depending on bed height)
- Plastic spoon or spatula
- Glass filter funnel G3
- Vacuum suction equipment

- Filter flask
- Measuring cylinder
- 20% ethanol with 0.4 M NaCl

Equipment

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

Equilibrate all materials to room temperature.

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). The parameters for calculating the bed height are described below:

Parameter	Description
$L_{settled}$	Settled bed height (cm) Bed height measured after settling by gravity
L_{cons}	Consolidated bed height (cm) Bed height measured after settling at a given flow velocity
L_{packed}	Packed bed height (cm)
CF	Compression factor, $CF = L_{settled} / L_{packed}$
PF	Packing factor, $PF = L_{cons} / L_{packed}$
A_C	Cross-sectional area of the column (cm^2)
V_C	Column volume, $V_C = L_{packed} \times A_C$ (mL)
C_{slurry}	Slurry concentration (%)

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 Slurry Concentration Kit (29096100).

For calculating the amount of resin to fill into the column, use the following equation: $V = (A_C \times L_{packed} \times CF) / C_{slurry}$

CF for MabSelect SuRe LX is 1.1 in 20% ethanol.

Washing the resin

Attach a glass filter funnel to a filter flask. Shake the resin to suspend it, pour it into the glass filter funnel, and wash it as follows:

- 5 times with 5 mL 20% ethanol with 0.4 M NaCl/mL resin.
- Gently stir with a spatula between additions.
- Transfer the washed resin to a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.

Packing the column

Table 4. Main packing parameters for HiScale 10/40

Parameter	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 flow velocity (cm/h)	1222	1222	1070
Packing flow rate (mL/min)	16	16	14
Conditioning flow velocity (cm/h)	1222	1222	1070

Parameter	HiScale 10/40		
Conditioning flow rate (mL/min)	16	16	14

[1] The packing factor stated refers to mechanical compression of the bed.

Table 5. Main packing parameters for HiScale 16/20 and HiScale 16/40

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

Table 6. Main packing parameters for HiScale 26/20 and HiScale 26/40

Parameter	HiScale 26/20	HiScale 26/40
Bed height (cm)	10	20
Slurry/packing solution	20% ethanol with 0.4 M NaCl	
Slurry concentration (%)	50	50
Packing factor (PF)	1.15	1.13
Packing flow velocity (cm/h)	300	300
Packing flow rate (mL/min)	27	27
Conditioning flow velocity (cm/h)	750	450
Conditioning flow rate (mL/min)	66	40

Procedure

Step	Action
1	Assemble the column according to the column instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*.
2	Attach the column tube to a laboratory 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, the air can be removed by light suction with a syringe.
4	Attach the bottom adapter unit to the bottom of the column tube and tighten the O-ring.
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	Put the packing tube into position on top of the column tube.
7	Connect the top adapter to the pump and prime it with a slow downflow. The net needs to be facing up as this is done.
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.

Step	Action
9	Attach the top adapter unit to the top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.
10	Start a downflow, see *Table 5, on page 21* and *Table 6, on page 21*.
11	Let the flow run until the bed has consolidated.
12	Use the graduation marks on the column to measure the bed height. There might be a buildup of resin at the column wall after the bed has consolidated. 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 bed height with the target packing factor. $L_{packed} = L_{cons}/PF$
14	Turn off the flow and put a stop plug in the bottom.
15	Disconnect the top adapter from the packing tube.
16	Over a beaker or a sink, disconnect the packing tube from the column.
17	Reattach the top adapter to the column tube. Make sure that no air is trapped under the net and lower the adapter to 1 to 2 cm above the bed, making sure that the surface is not disturbed.

Step	Action
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 bed height is reached.
19	Make sure that the pressure peaks that occur when turning the end knob down do not exceed the pressure specifications of the resin.
20	Start a downflow to flow condition the bed. The flow rate is shown in *Table 5, on page 21* and *Table 6, on page 21*.
21	Let the flow run for about 10 CV.

The column is ready to be tested.

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

7 Evaluation of packed column

Introduction

The quality of the packed bed and the column performance must be evaluated initially and monitored throughout the lifetime. The method for measuring the efficiency of a packed column is 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 sodium chloride solution to the column.

Note: *If sodium chloride solution is chosen, 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 used remain the same so that the results are comparable.

Test results are affected by changes in:

- 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 (CY13149)*, available on *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:

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

L = bed height (cm)

N = number of theoretical plates

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

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same unit

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

The reduced plate height (h) is calculated as follows:

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

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

As a guidance, $h < 3$ is acceptable.

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

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

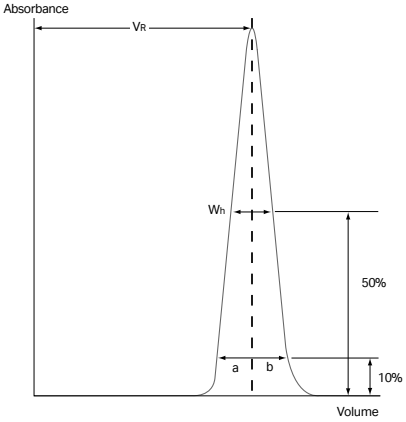
The peak asymmetry factor is calculated as follows:

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

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

CIP is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration.

Regular CIP prevents the buildup of impurities or contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of the resins.

A specific CIP protocol must be designed for each process according to the type of impurities or contaminants present.

We recommend performing a blank run, including CIP, before the first run with antibody feed.

It is recommended to perform a CIP:

- before first-time use - especially after packing the column – or after long-term storage
- between cycles
- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches

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

MabSelect SuRe LX 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 challenging cases were CIP with NaOH is not sufficient to restore 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 (CY14702)*.

CIP protocol

Perform all steps below with reversed flow.

Step	Action
1	Wash the column with 3 CV of binding buffer.
2	Wash with at least 3 CV of 0.1 to 0.5 M NaOH. Contact time at least 15 minutes.
3	Wash immediately with at least 5 CV of binding buffer at pH 7 to 8.

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

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

The type of feed material determines the final CIP. Depending on the nature of the impurities or contaminants, a combination of protocols might be required, for example 0.1 M NaOH every cycle and 0.5 M NaOH every 10 cycles.

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

9 Sanitization

Sanitization minimizes microbial contamination of the chromatographic bed. MabSelect SuRe LX 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 to other sanitizing agents.

Sanitization protocol

1. Wash the column with 3 CV of binding buffer.
2. Equilibrate the column with at least 2 CV of 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 CV of 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 can be used for sanitization.

Note: *Higher concentrations of NaOH and/or longer contact times inactivate microorganisms more effectively. However, these conditions might also lead to a decrease in dynamic binding capacity. Evaluate the conditions for sanitization to maximize microbial killing and to minimize loss of capacity.*

10 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.

11 Scale-up

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

- Keep the residence time constant in order to maintain the dynamic binding capacity.
- Select a bed volume according to the 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.*
- Verify the purification step.
- Keep the sample concentration and the elution conditions constant.

See *Fig. 5, on page 8* for the appropriate window of operation for MabSelect SuRe LX.

12 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	• Might be due to insufficient elution and CIP caused by impurities or 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. • 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	• Might be due to insufficient elution or 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.

13 Ordering information

For additional information, refer to *cytiva.com*

Product	Pack size	Product code
MabSelect SuRe LX	25 mL	17547401
	200 mL	17547402
	1 L	17547403
	5 L	17547404
	10 L	17547405

Related product	Pack size	Product code
HiScreen MabSelect SuRe LX	1 × 4.7 mL	17547415
PreDictor MabSelect SuRe LX, 6 µL	4 x 96-well plates	17547430
PreDictor MabSelect SuRe LX, 20 µL	4 x 96-well plates	17547431
PreDictor MabSelect SuRe LX, 50 µL	4 x 96-well plates	17547432
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

Related literature	Reference
Data files	
AxiChrom columns	CY10003
BPG columns	CY978
Chromaflow columns	CY13377
MabSelect SuRe LX	CY12583
Application notes	
Dynamic binding capacity study on MabSelect SuRe LX for capturing high-titer monoclonal antibodies	CY13341
High-throughput process development for design of cleaning-in-place protocols	CY14702
MabSelect SuRe – studies on ligand toxicity, leakage, removal of leached ligand, and sanitization	CY17448
Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place	CY13340
Packing MabSelect and MabSelect SuRe resins using verified methods	CY13950
Two-step purification of monoclonal IgG_1 from CHO cell culture supernatant	CY13148
Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951
Instructions	
HiScale columns (10, 16, 26, 50) and accessories	28967470

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