MabSelect™ SuRe 70 protein A resin

Affinity chromatography

Instructions for Use

MabSelect™ SuRe 70 protein A resin is a BioProcess™ affinity chromatography resin for capturing high-titer monoclonal antibodies from large volumes of feed by packed bed chromatography.

Key features of the resin include the following:

- cost-efficiency at low resin utilization, such as in clinical manufacturing
- high dynamic binding capacity; higher than 70 g/L in a well-packed column at 6 min residence time, for efficient resin use
- alkaline stability thanks to the established MabSelect SuRe™ protein A ligand that allows for 200 cleaning-in-place (CIP) cycles with 0.1 M NaOH and approximately 100 CIP cycles with 0.5 M NaOH
- convenience for use in short bed height formats and rapid resin cycling mode to further improve productivity in the protein A purification step

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1 Introduction

Important

Read these instructions carefully before using the product.

Safety

For safe use and handling of the product, refer to the *Safety Data Sheet*.

Intended use

The product is intended for research use and manufacturing use only. The product must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

2 Product description

BioProcess resin

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

All BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available for BioProcess resins to assist in process validation and submissions to regulatory authorities. The RSF contains additional product data such as characteristics, quality, and chemical stability.

Resin description

The protein A-derived MabSelect SuRe 70 ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal-derived products. The ligand is specifically engineered to create an affinity resin with enhanced alkaline stability and protease stability. The specificity of binding to the Fc region of IgG is similar to that of conventional protein A and provides efficient and reliable purification in one step. Cytiva™ MabSelect SuRe 70 protein A resin is designed for high-titer antibody processes and has very high dynamic binding capacities. The resin is suitable for rapid resin cycling, as it achieves high dynamic binding capacity at short residence times. Alkaline stability, high capacity, low ligand leakage, and a rigid base matrix make MabSelect SuRe 70 ideal for the purification of monoclonal antibodies for clinical applications.

The characteristics of the resin are summarized in *Resin properties, on page 5*.

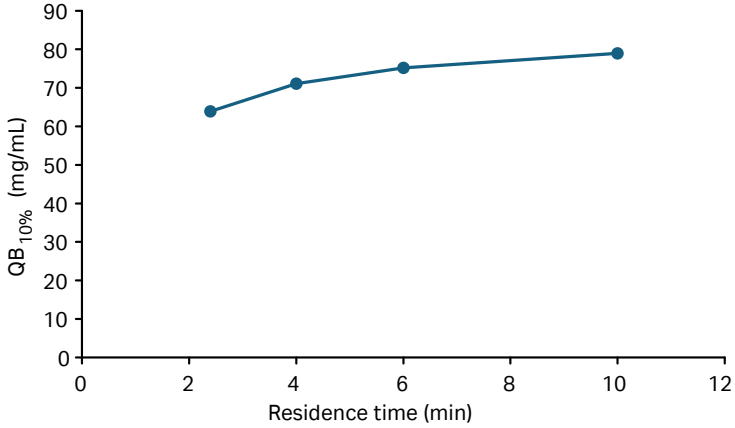
Resin properties

Property	MabSelect SuRe 70
Matrix	Highly cross-linked agarose
Particle size, d_{50v}[1]	50 µm
Ligand	Alkaline-stabilized, protein A-derived (from *E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity, $QB_{10\%}$[2]	~ 75 mg trastuzumab/mL resin, 6 minutes residence time ~ 71 mg trastuzumab/mL resin, 4 minutes residence time
Chemical stability	Stable in aqueous buffers commonly used in protein A chromatography
pH stability	
Operational[3]	3 to 12
CIP[4]	2 to 13.7
Operating flow velocity	
Maximum[5,6]	220 cm/h
Temperature stability[5]	2°C to 40°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 (6 min residence time) and 150 cm/h (4 min residence time) in a HiScreen™ column with a 10 cm bed height in PBS buffer, pH 7.4.
3 pH range where the resin can be operated without significant change in function.
4 pH range where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function.
5 In an AxiChrom™ 1000 column with 100 cm inner diameter and 20 cm bed height, operating pressure up to 3 bar, using buffers with the same viscosity as water at 20°C.
6 At 10 cm bed height the linear flow velocity can be increased to approximately 400 cm/h.

Dynamic binding capacity

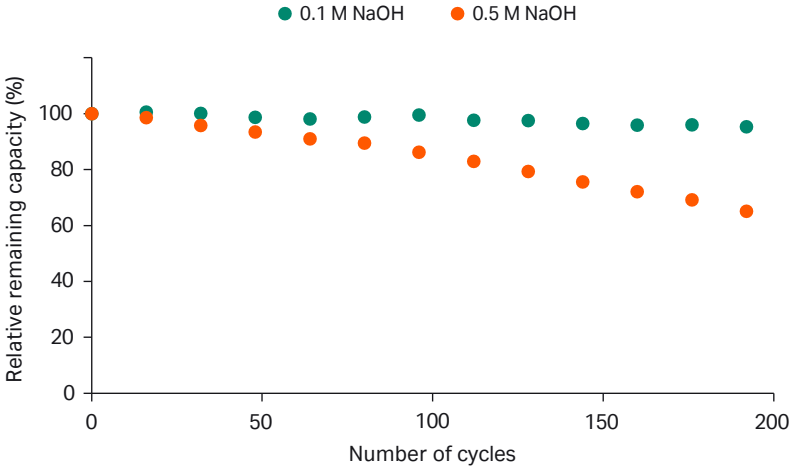
MabSelect SuRe 70 has a high dynamic binding capacity at most commonly used residence times. The figure below shows the dynamic binding capacity of the resin at 10% breakthrough ($QB_{10\%}$) for trastuzumab (with a bed height of 10 cm).



Alkaline stability

Alkaline stability of MabSelect SuRe 70 resin was evaluated in an accelerated study where the resin was exposed to 0.1 M and 0.5 M in intervals of 4 hours. Each interval corresponded to 16 CIP cycles with 15 minutes contact time. After each 4 hour CIP exposure, the resin was washed with 7 column volumes (CV) of PBS, pH 7.4 before measuring the dynamic binding capacity (6 minutes residence time) at 10% breakthrough ($QB_{10\%}$).

The graph below shows the relative remaining dynamic binding capacity. The resin remains stable after cleaning with 0.1 M NaOH for 200 cycles and approximately 100 cycles using 0.5 M NaOH.



3 Process development

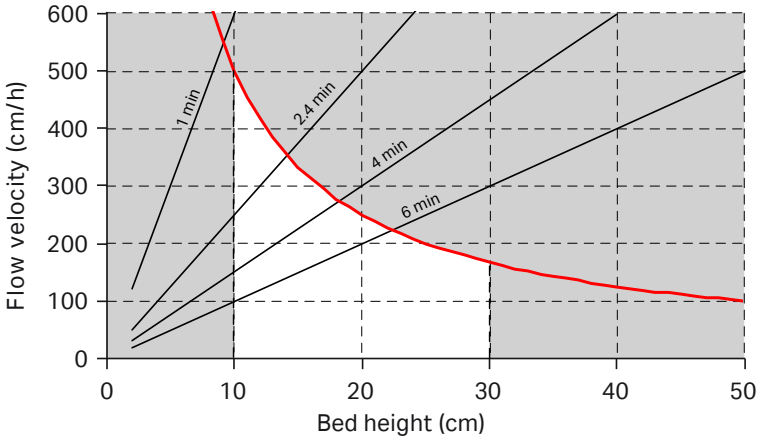
Recommended formats

For optimization of MabSelect SuRe 70 in small-scale columns, we recommend prepacked HiScreen or HiTrap™ columns. The resin can also be packed in laboratory-scale columns, such as HiScale™ or Tricorn™ columns.

Operating window

The figure below shows the recommended combinations of bed height and operational flow velocity for the resin at large scale and the resulting residence time in the interval 1 to 6 minutes.

- The red curved line shows the calculated large-scale column pressure restriction which is 3 bar according to specification (220 cm/h at 3 bar and 20 cm bed height).
- The white area represents the recommended operating window.
- At 10 cm bed height, the linear velocity can be increased to approximately 400 cm/h.



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

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

4 Operation

Recommended buffers

With MabSelect SuRe 70 resin, we recommend using phosphate and acetate buffers for typical monoclonal antibody (mAb) processes. However, 20 mM sodium citrate can be used for screening purposes to determine a suitable elution pH, see the *Screening protocol (for elution pH)* further down.

Outline of a typical mAb capture process

The protocol below lists steps and conditions used in a typical large-scale purification.

Phase	Buffer	Volume	Residence time
Equilibration	20 mM sodium phosphate, 150 mM NaCl, pH 7.4	3 CV	6 min
Sample application	As required	70%–80% of $QB_{10\%}$	6 min
Wash 1	20 mM sodium phosphate, 500 mM NaCl, pH 7.0	5 CV	6 min
Wash 2	50 mM sodium acetate, pH 6.0	1 CV	6 min
Elution	50 mM sodium acetate, pH 3.5	3 CV	6 min
Acidic strip	100 mM acetic acid, pH 2.9	2 CV	6 min
CIP	0.1 to 0.5 M NaOH	3 CV*	5 min
Re-equilibration	20 mM sodium phosphate, 150 mM NaCl, pH 7.4	3–5 CV†	5 min

* 15 min contact time in reverse-flow mode.
† In reverse-flow mode.

Screening protocol (for elution pH)

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.

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

Note: *A blank run, including CIP, is recommended before the first run with antibody feed. This decreases the ligand leakage during the chromatography step.*

Phase	Solution	Volume
Equilibration	20 mM sodium phosphate, 150 mM NaCl, pH 7.4	5 CV
Sample application	Small amount of antibody sample, 6 min residence time	As required
Wash	20 mM sodium phosphate, 150 mM NaCl, pH 7.4	5 CV
Elution	20 mM sodium citrate, linear gradient, pH 7.4 to pH 3.0	10 CV
Acidic strip	20 mM sodium citrate, pH 3.0	5 CV
CIP	0.1 to 0.5 M NaOH, 15 min residence time	At least 3 CV
Re-equilibration	20 mM sodium phosphate, 150 mM NaCl, pH 7.4	5 CV

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

Optimizing sample load

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 must therefore be defined for different sample residence times, see *Dynamic binding capacity, on page 6*. Set the load during a mAb capture process to 70% to 80% of the dynamic binding capacity determined.

Optimizing the wash conditions

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

- conductivity
- pH
- volumes
- flow rate

Step purification

After suitable elution conditions have been determined, it is recommended to set up a step elution protocol, particularly for large-scale purifications. 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 as high protein concentrations during elution can result in high column pressure.

5 Removal of leached ligand from the final product

Leakage of the MabSelect SuRe 70 protein A ligand can be measured using a commercially available ELISA kit developed for the detection of this specific SuRe ligand. MabSelect SuRe 70 protein A free ligand in solution is available for leakage determination and other analytical purposes.

Ligand leakage from the resin is generally low. However, in many applications it is a requirement to remove leached ligand from the final product.

Techniques to remove leached ligand include:

- ion exchange chromatography (IEX)
- multimodal chromatography (MMC)
- size exclusion chromatography (SEC)

For an example of the removal of leached ligand and antibody aggregates, refer to the application note *Two-step purification of monoclonal IgG1 from CHO cell culture supernatant (CY13148)*, available on *cytiva.com*. Methods used for the removal of leached ligand from MabSelect SuRe™ and MabSelect SuRe LX are also applicable to the removal of leached ligand from MabSelect SuRe 70.

6 Packing columns

In this chapter

6.1 General packing information

Definitions

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

$L_{settled}$	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 (%)

Slurry preparation

Slurry preparation can be performed manually, or mechanically, for example by using a Media Wand™ or a Media Handling Unit. Media Wand is suitable for large-scale packing, suspending the resin directly in the container and transferring it to the slurry tank in a single operation. For small-scale packing, shaking gives good results. Alternatively, use soft stirrers without sharp edges for stirring. Measure the slurry concentration accurately to get the correct amount of resin for packing to target bed height or compression. Let the resin settle overnight in 20% ethanol in a measuring cylinder to determine the slurry concentration or use the Slurry Concentration Kit, see *Related products, on page 27*.

6.2 Packing laboratory-scale columns

Recommended columns

The following table lists the properties of laboratory-scale Tricorn 5 columns.

Column	Inner diameter (mm)	Maximum bed volume (mL)	Maximum bed height (cm)
Tricorn 5/100	5	2	10
Tricorn 5/150	5	3	15
Tricorn 5/200	5	4	20

Materials

- MabSelect SuRe 70 protein A resin
- plastic spoon or spatula
- P4 glass filter funnel
- vacuum suction equipment
- filter flask
- measuring cylinder
- packing solution
- Tricorn column
- Tricorn packing tube
- Tricorn 5 medium filter kit

Make sure that all materials and equipment are at room temperature before packing the column.

Equipment

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

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

Equilibrate to packing solution

Follow the steps below to equilibrate and to suspend the resin in packing solution to the slurry concentration recommended. The recommendation is to use 0.2 M NaCl, 20% ethanol as packing solution and a slurry concentration of ~ 50% when packing the resin in a Tricorn 5 column.

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

Pack Tricorn 5 columns

Use 0.2 M NaCl, 20% ethanol as packing solution.

Step	Action
1	Wet the filters with ethanol 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.
4	Assemble a bottom piece with a pre-wet filter to the top of the packing tube. ***Note:*** *Make sure that no air is trapped under the filter.*
5	Remove the stop plug from the column bottom and connect the column top to the pump. Start a downflow with packing solution at a flow rate of 3.5 mL/min. Let the flow run for 10 minutes.
6	Turn off the flow and attach a stop plug to the column bottom.
7	Disassemble the packing tube and remove excess resin, using a pipette.

Step	Action
8	Top up the column with packing solution.
9	Place a pre-wet top filter on top of the fluid in the column.
10	Attach the top adapter.
	Note: *Make sure that 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	Remove the stop plug from the column bottom and connect the top adapter to the pump. Make a drop-to-drop connection to prevent air from entering the column.
13	Start a downflow with packing solution at a flow rate of 3.5 mL/min. Let the flow run for 5 minutes.
14	Pause the pump and turn the adapter down toward the resin bed.
15	Start a conditioning flow with packing solution at 0.5 mL/min. Let the flow run for 10 CV.
16	Measure the bed height.

The column is ready for efficiency testing.

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

Note: *If a packed Tricorn column is used to determine the dynamic binding capacity, results are lower than those using a prepacked HiScreen or a packed AxiChrom column, due to differences in packing methods.*

6.3 Evaluation of packed column

Introduction

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

Frequency

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

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

Column efficiency testing

Packed column efficiency is best expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a test sample such as 1% acetone solution or 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 guideline, an acceptance value of < 3 for (h) can be used.

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

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

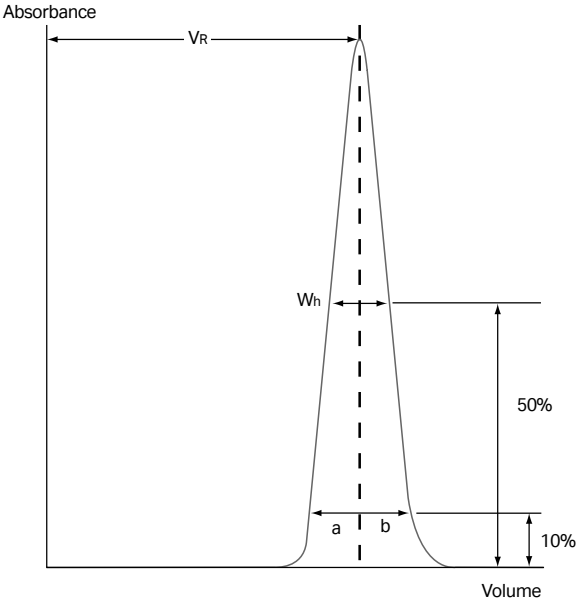
The peak asymmetry factor is calculated as follows:

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

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.



7 Cleaning-in-place (CIP)

Introduction

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 packed bed and helps to maintain capacity, flow properties, and general performance. MabSelect SuRe 70 is an alkaline-stabilized chromatography resin that allows for the use of 0.1 to 0.5 M NaOH for CIP.

Note: *It is important to perform an acidic strip (pH 3) before CIP in order to remove impurities and target molecules that were not completely eluted, see the protocol in* *Outline of a typical mAb capture process, on page 9*.

It is recommended to perform CIP in the following situations:

- before first-time use–especially after packing the column–or after long-term storage
- after each cycle with real feed
- when a reduction in column performance such as an increase in back pressure 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

CIP optimization

NaOH concentration, contact time, and frequency are typically the main parameters to vary during CIP optimization. Longer contact times increase CIP efficiency. However, this can also lead to a decrease in dynamic binding capacity.

CIP conditions must be designed for efficiency and minimal loss of capacity. The characteristics of the feed material determine the final CIP. However, the general recommendation is to clean the column every cycle during normal use. Depending on the type of impurities or contaminants, a combination of protocols might be required, for example, 0.1 M NaOH every cycle and 0.5 M NaOH every 10 cycles.

For challenging cases where CIP with NaOH is not sufficient to restore the column performance, the recommendation is to use an extended protocol including wash with 100 mM thioglycerol, pH 8.5 followed by CIP with 0.1 to 0.5 M NaOH. For more details, refer to the application note *High-throughput process development for design of cleaning-in-place protocols (CY14702)*, available on cytiva.com.

CIP recommendation

CIP is usually performed immediately after regeneration/strip. Before applying the alkaline NaOH CIP solution, it is recommended to equilibrate the column with a neutral pH solution. This is to prevent direct contact between low pH elution buffer and high pH NaOH solution inside the column. Mixing acid and alkaline solutions might cause a temperature rise in the column. It is recommended to perform CIP in reverse-flow mode.

CIP protocol

Follow the steps below to perform a CIP.

Step	Action
1	Wash the column with 3 CV binding buffer at pH 7 to 8.
2	Wash with at least 3 CV 0.1 to 0.5 M NaOH in reverse-flow mode, with a contact time of 15 minutes.
3	Wash immediately with binding buffer at pH 7 to 8 in reverse-flow mode for at least 5 CV, or until pH and conductivity are stable.

8 Sanitization

Introduction

Sanitization reduces microbial contamination of the chromatographic bed to a minimum. The resin is alkaline-stabilized allowing for the use of NaOH as sanitizing agent. Depending on concentration, NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins. For more information, refer to the application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*, available on cytiva.com.

Note: *Microorganisms are inactivated more effectively by using higher NaOH concentrations and longer contact times. However, these conditions can also lead to a decrease in dynamic binding capacity. Therefore, sanitization conditions must be evaluated to maximize microbial killing and to minimize loss of dynamic binding capacity.*

Sanitization protocol

The steps below are a starting point for sanitizing the column.

Step	Action
1	Wash the column with 3 CV binding buffer at pH 7 to 8.
2	Wash the column with at least 3 CV of 0.1 M or 0.5 M NaOH in reverse-flow mode. Use a contact time of at least 1 h for either 0.1 M or 0.5 M NaOH.
3	Wash immediately with sterile binding buffer at pH 7 to 8 in reverse-flow mode for at least 5 CV, or until pH and conductivity are stable.

For challenging microbial contamination, a mixture of 30% to 40% 1- or 2-propanol in 0.5 M NaOH can be used. For bacterial endospore contamination, the resin can be cleaned with 20 mM peracetic acid (PAA) for 30 min or with 30 mM PAA for 15 min. It is recommended to use PAA sanitization only two or three times during the resin's lifetime. For more information, refer to the application note *Impact of sporicidal agent on MabSelect SuRe protein A resin lifetime (CY13949)*, available on cytiva.com.

9 Storage

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

Equilibrate packed columns with a solution containing 20% ethanol or 2% benzyl alcohol to prevent microbial growth.

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

10 Scale-up

Introduction

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

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

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

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

See *Operating window, on page 8* for the appropriate operating window for MabSelect SuRe 70.

11 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run.	Solutions with high viscosity are used.	Decrease the flow rate.
	The in-line filter is clogged.	Replace the in-line filter.
	The column is clogged.	Perform CIP.
	The adapter net/filter is clogged.	Clean or replace the adapter net/filter.
Unstable pressure curve during sample loading.	Air bubbles are trapped in the sample pump.	Remove any air bubbles from the sample pump.
		Degas the sample using a vacuum degasser or an air trap.
Gradual broadening of the eluate peak.	Insufficient elution and CIP caused by impurities or contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
Gradual decrease in yield.	The sample load is too high.	Decrease the sample load.
	Precipitation during elution.	Optimize the elution conditions.
	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
Gradual increase in CIP peaks.	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, include acid regeneration after the elution step, or perform CIP more frequently.
High ligand leakage during the first purification cycle.	The column is new.	Perform a blank run, including CIP, before the first purification cycle on a new column.

12 Ordering information

For additional information, refer to *cytiva.com*.

Products

Product	Pack size	Product code
MabSelect SuRe 70	25 mL	17542401
	200 mL	17542402
	1 L	17542403
	5 L	17542404
	10 L	17542405

Related products

Product	Pack size	Product code
HiTrap MabSelect SuRe 70	1 × 1 mL	17542411
	5 × 1 mL	17542412
	1 × 5 mL	17542413
	5 × 5 mL	17542414
HiScreen MabSelect SuRe 70	1 × 4.7 mL	17542410
Tricorn 5 Medium Filter Kit	1 × 5 units	29258132
Slurry Concentration Kit	1 unit	29096100

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