cytiva

# **HiScreen** MabSelect PrismA
# **HiScreen** MabSelect SuRe LX
# **HiScreen** MabSelect SuRe
# **HiScreen** MabSelect Xtra
# **HiScreen** MabSelect

## Instructions for Use

HiScreen™ MabSelect™ PrismA, HiScreen MabSelect SuRe™ LX, HiScreen MabSelect SuRe, HiScreen MabSelect Xtra™, and HiScreen MabSelect are ready to use columns, prepacked with affinity BioProcess™ chromatography resin for capturing monoclonal antibodies and Fc-containing recombinant proteins. HiScreen columns provide fast and reproducible separations in a convenient format. These prepacked 4.7 mL columns are ideal for method optimization, as well as for small-scale purifications. The columns are optimally used with liquid chromatography systems like ÄKTA™.

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## Important

Read these instructions carefully before using the products.

## Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

## Safety

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

# 1 Product description

## HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with the biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label shows the recommended flow direction, see image below.

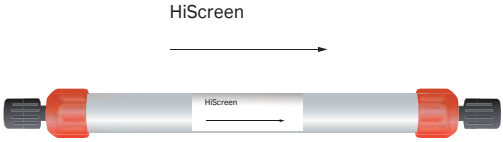


**Fig 1.** HiScreen column

HiScreen column format is ideal to use for method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 mL, and the 10 cm bed height make it possible to perform scalable experiments at relevant process flow rates. Reuse of the column with 10 cycles of feed has been tested but it is very much dependent of the nature of the sample. When needed, two columns can easily be connected in series with a union to give 20 cm bed height (see *Chapter 7 Scaling up, on page 28*.)

***Note:*** *Do not open or refill HiScreen columns.*

**Table 1.** Characteristics of HiScreen column

| | |
|---|---|
| Column volume (CV) | 4.7 mL |
| Column dimensions | 0.77 × 10 cm |
| Column hardware pressure limit | 0.8 MPa (8 bar) |

***Note:*** *The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/ liquid viscosity, and the column tubing used.*

## Properties of MabSelect resins

MabSelect chromatography resins are designed to tolerate high flow rates and high pressure. This, in combination with low ligand leakage, makes MabSelect resins well suited for purification of monoclonal antibodies,both for laboratory use and manufacturing.

The protein A-derived ligands are produced in *Escherichia coli*. Fermentation and subsequent purification of the ligand are performed in the absence of animal products.

The characteristics of the MabSelect resins are summarized in *Table 2, on page 6*, *Table 3, on page 8*, and *Table 4, on page 10*.

## MabSelect PrismA

The ligand has been engineered to create an affinity resin with enhanced alkali and protease stability. The resin is stable in concentrations up to 1.0 M NaOH. The specificity of binding to the Fc region of IgG is similar to that of conventional Protein A and provides excellent purification in one step.
MabSelect PrismA has very high dynamic binding capacity at most commonly used residence times.

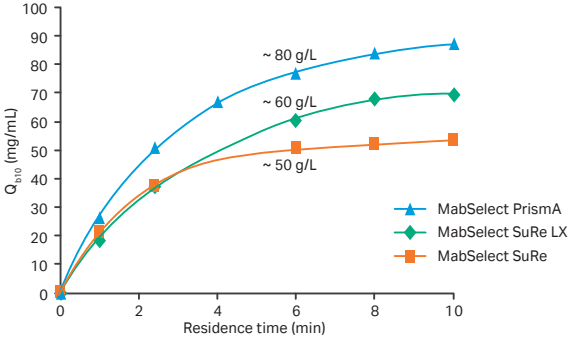


**Fig 2.** DBC of MabSelect PrismA compared with its predecessor MabSelect SuRe and MabSelect SuRe LX resins at 10% breakthrough ($Q_{B10}$), determined in HiScreen columns.

**Table 2.** Characteristics of MabSelect PrismA

| | |
|---|---|
| **Matrix** | Rigid, highly cross-linked agarose |
| **Particle size, $d_{50V}$** [1] | ~ 60 µm |
| **Ligand** | MabSelect PrismA ligand (alkali-tolerant, protein A-derived from *E. coli*) |
| **Coupling chemistry** | Epoxy |
| **Dynamic binding capacity, $Q_{B10\%}$** [2] | ~ 65 mg polyclonal IgG/mL resin, 4 minutes residence time<br>~ 80 mg polyclonal IgG/mL resin, 6 minutes residence time |
| **Recommended flow velocity** | Flow values for HiScreen MabSelect PrismA columns are shown in *Table 7, on page 17* |
| **Maximum operating flow velocity** [3] | 300 cm/h |
| **pH stability**, | |
| **Operational** [4] | 3 to 12 |
| **CIP** [5] | 2 to 14 |
| **Chemical stability** | Stable to commonly used aqueous buffers for Protein A chromatography |
| **Temperature stability** | 2°C to 40°C |
| **Storage** | 2°C to 8°C in 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 minutes residence time) and 150 cm/h (4 minutes residence time) in a lab scale column with a 10 cm bed height in PBS buffer, pH 7.4.

3 In an AxiChrom™ column with 30 cm diameter with a 20 cm bed height, using buffers with the same viscosity as water at 20°C.

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

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

## MabSelect SuRe

The protein A-derived ligand has been engineered to create an affinity resin with high alkali stability and high binding capacity for IgG. The resin is stable in concentrations up to 0.5 M NaOH. 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

This chromatography resin is optimized for high dynamic binding capacity for high titer cultures of antibodies. The resin is designed with the same alkali-tolerant, protein A-derived ligand as in MabSelect SuRe. This makes also this chromatography resin stable in concentrations up to 0.5 M NaOH.

**Table 3.** Characteristics of MabSelect SuRe and MabSelect SuRe LX

| | MabSelect SuRe | MabSelect SuRe LX |
|---|---|---|
| **Matrix** | Rigid, highly cross-linked agarose | |
| **Median particle size ($d_{50V}$)** [1] | ~85 µm | ~85 µm |
| **Ligand** | Alkali-stabilized protein A-derived (*E. coli*) | |
| **Coupling chemistry** | Epoxy activation | Epoxy activation |
| **Dynamic binding capacity, $Q_{B10\%}$** | ~30 mg human IgG/mL resin [2] | ~60 mg human IgG/mL resin [3] |
| **Recommended flow velocity** [4] | Flow values for HiScreen MabSelect SuRe and MabSelect SuRe LX columns are shown in *Table 8, on page 18* | |
| **Maximum flow velocity** [4] | 500 cm/h | 500 cm/h |
| **pH stability** | | |
| **Operational** [5] | 3 to 10 | 3 to 10 |
| **CIP** [6] | 3 to 13.7 | 3 to 13.7 |
| **Chemical stability** | No significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M guanidine hydrochloride, or 20% ethanol | |
| **Temperature stability** | 2°C to 40°C | 2°C to 40°C |
| **Storage** | 2°C to 8°C in 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 500 cm/h in a column with a bed height of 20 cm, i.e. residence time is 2.4 min.

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

4 Water at room temperature. For viscous buffers and samples, the flow velocity must be optimized. Starting with a low flow rate is recommended.

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

6 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function. pH 13.7 corresponds to 0.5 M NaOH.

## MabSelect

The recombinant protein A has been engineered to favor an oriented coupling that gives an affinity chromatography resin with high binding capacity for IgG. The specificity of binding to the Fc region of IgG is similar to that of native protein A, and provides excellent purification in one step. The epoxy-based coupling chemistry ensures low ligand leakage.

## MabSelect Xtra

MabSelect Xtra addresses high levels of expression found in monoclonal antibody feedstocks. The chromatography resin is engineered to give high dynamic binding capacity. The recombinant protein A has been specially engineered to favor an oriented coupling that results in an enhanced binding capacity for IgG.

The specificity of binding to the Fc region of IgG is similar to that of native protein A and provides excellent purification in one step.

**Table 4.** Characteristics of MabSelect and MabSelect Xtra

| | MabSelect | MabSelect Xtra |
|---|---|---|
| **Matrix** | Rigid, highly cross-linked agarose | |
| **Median particle size ($d_{50v}$)** [1] | ~85 µm | ~75 µm |
| **Ligand** | Recombinant protein A (*E. coli*) | |
| **Ligand coupling method** | Epoxy activation | Epoxy activation |
| **Binding capacity** | ~30 mg human IgG/mL medium[2] | ~40 mg human IgG/mL medium[3] |
| **Recommended flow velocity**[4] | Flow values for HiScreen MabSelect columns and MabSelect Xtra columns are shown in *Table 8, on page 18* and *Table 9, on page 18* | |
| **Maximum flow velocity**[3] | 500 cm/h | 300 cm/h |
| **pH stability** | | |
| **Operational**[5] | 3 to 10 | 3 to 10 |
| **CIP**[6] | 2 to 12 | 2 to 12 |
| **Chemical stability** | No significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M guanidine hydrochloride, or 20% ethanol | |
| **Working temperature** | 2°C to 40°C | 2°C to 40°C |
| **Storage** | 2°C to 8°C in 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 500 cm/h in a column with a bed height of 20 cm, i.e. residence time is 2.4 min.

3 Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 250 cm/h in a column with a bed height of 10 cm, i.e. residence time is 2.4 min.

4 Water at room temperature. For viscous buffers and samples, the flow velocity must be optimized. Starting with a low flow rate is recommended.

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

pH below 3 is sometimes required to elute strongly bound antibody species. However, protein ligands may hydrolyze at very low pH.

6 pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

# 2 Process development

## General description

It is important to consider process cost, cleaning of the resin, and environmental constraints early in the development of a purification process. HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 ml, and the 10 cm bed height make possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height, see *Chapter 7 Scaling up, on page 28*.

Design of Experiments (DoE) is an effective tool for method parameter screening, optimization, and robustness testing of a purification process, refer to handbook *Design of Experiments in Protein Production and Purification* (*cytiva.com/handbooks*).

A common approach in DoE is to define a reference experiment (center point), and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the ranges for the variables. DoE can be used for parameter screening and optimization, as well as for robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by variations, and shows the process reliability during normal usage. A robustness test evaluates factors potentially causing variability in the process, detected by responses of methods, for example purity or yield. For this purpose, small deliberate variations in the process parameters are introduced.

For scale-up, see *Chapter 7 Scaling up, on page 28*.

*Fig. 3, on page 12* shows typical steps during a general process development.

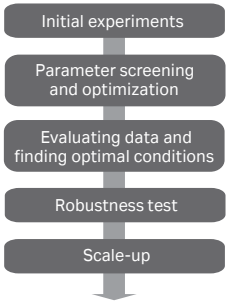


**Fig 3.** Typical steps during a process development.

# 3 Optimization

## Preferred ligands

In general, most IgGs can be purified using protein A, but for some IgG, protein G is the preferred ligand. See *Table 5, on page 13* for relative binding strengths for protein A and protein G.

**Table 5.** Relative binding strengths for protein A and protein G

| Species | Subclass | Protein A binding* | Protein G binding |
|---|---|---|---|
| Human | IgA | variable | - |
| | IgD | - | - |
| | IgE | - | - |
| | $IgG_1$ | ++++ | ++++ |
| | $IgG_2$ | ++++ | ++++ |
| | $IgG_3$ | - | ++++ |
| | $IgG_4$ | ++++ | ++++ |
| | IgM† | variable | - |
| Avian egg yolk | IgY† | - | - |
| Cow | | ++ | ++++ |
| Dog | | ++ | + |
| Goat | | - | ++ |
| Guinea pig | $IgG_1$ | ++++ | ++ |
| | $IgG_2$ | ++++ | ++ |
| Hamster | | + | ++ |
| Horse | | ++ | ++++ |
| Koala | | - | + |
| Llama | | - | + |
| Monkey (rhesus) | | ++++ | ++++ |
| Mouse | $IgG_1$ | + | ++++ |
| | $IgG_{2a}$ | ++++ | ++++ |
| | $IgG_{2b}$ | +++ | +++ |
| | $IgG_3$ | ++ | +++ |
| | IgM† | variable | - |
| Pig | | +++ | +++ |
| Rabbit | No distinction | ++++ | +++ |
| rat | $IgG_1$ | - | + |
| | $IgG_{2a}$ | - | ++++ |
| | $IgG_{2b}$ | - | ++ |
| | $IgG_3$ | + | ++ |
| Sheep | | +/- | ++ |

* ++++ = strong binding; ++ = medium binding; - = weak or no binding

† Purify using HiTrap™ IgM and HiTrap IgY Purification HP columns, respectively.

## Optimizing elution conditions

Determine the highest pH that allows efficient elution of antibody when optimizing the elution conditions. This prevents denaturation of sensitive antibodies caused by exposure to low pH. Use a basic solution to neutralize the eluted fractions.

Step-wise elution allows the target 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 eluted pool.

## Removal of leached protein A from final product

All Protein A ligands can be analyzed using commercial available protein A immunoassays. The ligand leakage from the MabSelect resins is generally low. For example, MabSelect SuRe gives 5 to 20 ppm (ng ligand/mg antibody) of leached ligand in eluate. However, in some monoclonal antibody applications it is necessary to eliminate leached ligand from the final product.

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

For more details about removal of leached ligand and antibody aggregates, refer to application note *Two step purification of monoclonal IgG1 from CHO cell culture supernatant* (28907892).

### Automated buffer preparation

Users of ÄKTA chromatography systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to conveniently screen resins over a range of pH values and elution conditions.

# 4 Operation

Optimal conditions must be evaluated for each sample. Flow rate, buffer composition, pH, gradient elution, CIP conditions, and length of each step are examples of factors that may affect the purification.

### Preparation of buffers

The following buffers are recommended:

**Start buffer :** 20 mM sodium phosphate, 0.15 M NaCl, pH 7.2

**Elution buffer :** 0.1 M sodium citrate, pH 3.0 to 3.6

***Note:*** *When purifying mouse $IgG_1$ on protein A resin, an increased binding capacity will be achieved by including 2.5 M NaCl in the binding buffer.*

***Note:*** *Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.*

## Preparation of the sample

| Step | Action |
|---|---|
| 1 | Adjust the sample to the composition of the start buffer using one of these two methods (optional):<br>• Dilute the sample with start buffer.<br>• Exchange buffer using a prepacked column for desalting, see *Table 6, on page 17*. |
| 2 | To prepare the sample:<br>• Filter the sample through a 0.45 µm filter.<br>or<br>• Centrifuge immediately before loading it to the column.<br>***Note:***<br>*This prevents clogging and increases the life time of the column when loading large sample volumes.* |

## Prepacked columns for desalting

The prepacked columns described in the table below are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules ($M_r$ > 5000).

**Table 6.** Prepacked columns for desalting

| Column | Loading volume | Elution volume |
|---|---|---|
| HiPrep™ 26/10 Desalting[1] | 2.5 to 15 mL | 7.5 to 20 mL |
| HiTrap Desalting[2] | 0.25 to 1.5 mL | 1.0 to 2.0 mL |
| PD-10 Desalting[3] | 1.0 to 2.5 mL[4] | 3.5 mL |
| | 1.75 to 2.5 mL[5] | Up to 2.5 mL |
| PD MiniTrap™ G-25 | 0.1 to 2.5 mL[4] | 1.0 mL |
| | 0.2 to 0.5 mL[5] | Up to 0.5 mL |
| PD MidiTrap™ G-25 | 0.5 to 1 mL[4] | 1.5 mL |
| | 0.75 to 1 mL[5] | Up to 1.0 mL |

1 Prepacked with Sephadex™ G-25 Fine, requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine, requires a syringe, a pump or a chromatography system to run.

3 Prepacked with Sephadex G-25, can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 Volumes with centrifugation.

## Recommended flow rates

**Table 7.** HiScreen MabSelect PrismA

| Operation | Flow rate (mL/min) | Flow velocity (cm/h) | Residence time (min) |
|---|---|---|---|
| Equilibration | ≤ 2.3 | ≤ 300 | ≥ 2 |
| Wash | ≤ 2.3 | ≤ 300 | ≥ 2 |
| Sample load | 0.6 to 2.3 | 75 to 300 | 8 to 2 |
| CIP | ≤ 0.78 | ≤ 100 | ≥ 6 |

**Table 8.** HiScreen MabSelect SuRe, HiScreen MabSelect SuRe LX, HiScreen MabSelect

| Operation | Flow rate (mL/min) | Flow velocity (cm/h) | Residence time (min) |
|---|---|---|---|
| Equilibration | ≤ 3.8 | ≤ 500 | ≥ 1.2 |
| Wash | ≤ 3.8 | ≤ 500 | ≥ 1.2 |
| Sample load | 0.6 to 2.3 | 75 to 300 | 8 to 2 |
| CIP | ≤ 0.78 | ≤ 100 | ≥ 6 |

**Table 9.** HiScreen MabSelect Xtra

| Operation | Flow rate (mL/min) | Flow velocity (cm/h) | Residence time (min) |
|---|---|---|---|
| Equilibration | ≤ 2.3 | ≤ 300 | ≥ 2 |
| Wash | ≤ 2.3 | ≤ 300 | ≥ 2 |
| Sample load | 0.6 to 2.3 | 75 to 300 | 8 to 2 |
| CIP | ≤ 0.78 | ≤ 100 | ≥ 6 |

## Column tubing

Choose the optimal tubing kit for the column and the application you intend to run, inner diameter 0.25, 0.50, or 0.75 mm. A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

## Purification

Follow the steps below to perform a purification.

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

| Step | Action |
|---|---|
| 1 | If the eluted sample needs to be neutralized, add an alkaline buffer as 1 M Tris-HCl, pH 9.0, to the collection tubes. |
| 2 | Remove the stoppers and connect the column to the system.<br>***Note:***<br>*Make a drop-to-drop connection to prevent air from entering the column.*<br>***Note:***<br>*Use fingertight 1/16" connector (28401081).* |
| 3 | Wash with 5 column volumes (CV) of distilled water to remove the ethanol. This prevents precipitation of buffer salts at exposure to ethanol.<br>***Note:***<br>*The viscosity for 20% ethanol is higher than for water. For this step, do not use a higher flow rate than 1.2 mL/min (150 cm/h).* |
| 4 | Equilibrate the column with 5 CV start buffer. |
| 5 | Load the sample onto the column. |
| 6 | Wash with 5 to 10 CV start buffer or until the UV trace of the effluent returns to near baseline. |

| Step | Action |
|---|---|
| 7 | Elute by linear gradient elution or a step elution:<br>• *Step elution*<br>Elute with 2 to 5 CV elution buffer.<br>• *Linear gradient elution*<br>Elute with 0-100% elution buffer in 10 to 20 CV. |
| 8 | Wash the column with 5 CV elution buffer. |
| 9 | Wash the column with 3 CV start buffer. |
| 10 | Re-equilibrate the column with 5 to 10 CV start buffer, or until the UV signal, eluent pH, and conductivity reach the required values.<br>***Note:***<br>*Do not exceed the maximum recommended flow rate or back pressure for the column.* |
| 11 | For cleaning the column, see *Chapter 5 Cleaning-in-place (CIP), on page 21*. |
| 12 | If required, perform a buffer exchange or a desalting of the collected eluted fractions. See *Table 6, on page 17* for recommended columns. |

# 5 Cleaning-in-place (CIP)

## General description

CIP removes very tightly bound, precipitated, or denatured substances/proteins from the resin. The accumulated contaminants can affect the chromatographic properties of the prepacked column, reduce the capacity, or contaminate the subsequent runs.

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

It is recommended to perform a CIP:

- After every run with feed.
- When an increase in the back pressure is noticed.
- If a reduced column performance is observed.
- When the same column is used for purification of different proteins to prevent possible cross-contamination.
- Before first time use or after long term storage.

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

CIP is usually performed immediately after the purification. Before applying the alkaline NaOH CIP solution, it is recommended to equilibrate 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.

## CIP protocols

The nature of the sample ultimately determines the final CIP protocol. Optimize the following parameters for CIP:

- contact time
- frequency
- concentration of the NaOH solution

If the column back pressure level is not restored after CIP, it is recommended to proceed with additional CIP treatment.

***Note:*** *High concentrations of NaOH and/or longer contact time clean the columns more efficiently. However, these conditions might also lead to a decrease in the dynamic binding capacity. The conditions for CIP must be designed for efficient CIP and minimized loss of capacity.*

The CIP procedure below describes removal of the most common contaminants. Use a lower flow rate during the entire CIP procedure, see *Recommended flow rates, on page 17*. This decreases the effect of increased pressure caused by the higher viscosity of the CIP solutions.

## HiScreen MabSelect PrismA

MabSelect PrismA is a highly alkali-tolerant chromatography resin that allows the use of 0.5 M to 1.0 M NaOH for CIP. Depending on the nature of the contaminants, it might be necessary to combine different protocols, for example 0.5 M NaOH every cycle and 1.0 M NaOH every 10 cycles.

| Step | Action |
|---|---|
| 1 | Wash the column with 3 column volumes (CV) of start buffer. |
| 2 | Wash with 3 CV 0.5 to 1.0 M NaOH with a contact time of 15 minutes. |
| 3 | Wash immediately with at least 5 CV sterile and filtered start buffer at pH 7 to 8. |

## HiScreen MabSelect SuRe and HiScreen MabSelect SuRe LX

These are alkali-tolerant chromatography resins allowing the use of up to 0.5 M NaOH as CIP agent.

| Step | Action |
|---|---|
| 1 | Wash the column with 3 column volumes (CV) of start buffer. |
| 2 | Wash with at least 2 CV 0.1 to 0.5 M NaOH with a contact time of 10 to 15 minutes. |
| 3 | Wash immediately with at least 5 CV start buffer at pH 7 to 8. |

## HiScreen MabSelect and HiScreen MabSelect Xtra

These resins can withstand up to 50 mM NaOH and it is therefore recommended to use other cleaning agents as well.

**CAUTION**
70% ethanol requires the use of explosion-proof areas and equipment.

| To remove... | Then... |
|---|---|
| Precipitated or denatured substances | 1. Wash with 2 column volumes (CV) of 6 M guanidine hydrochloride, contact time at least 10 min.<br>2. Wash immediately with at least 5 CV filtered start buffer.<br>or<br>1. Wash with 2 CV 50 mM NaOH of 1.0 M NaCl or 50 mM NaOH in 0.5 M $Na_2SO_4$, contact time ~ 10 min.<br>2. Wash immediately with at least 5 CV filtered start buffer. |
| Hydrophobically bound substances | 1. Wash with 2 CV 50 mM NaOH 1.0 M NaCl or 50 mM NaOH in 0.5 M $Na_2SO_4$, contact time ~ 10 min.<br>Wash with 2 CV nonionic detergent (e.g., conc. 0.1%), contact time ~ 10 min.<br>2. Wash immediately with at least 5 CV start buffer.<br>or<br>1. Wash with 3 to 4 CV 70% ethanol or 30% isopropanol, contact time ~ 10 min. Increasing gradients may be applied to avoid air bubble formation when using high concentrations of organic solvents.<br>2. Wash immediately with at least 5 CV start buffer. |

# 6 Sanitization

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

## HiScreen MabSelect PrismA

MabSelect PrismA is a highly alkali-tolerant chromatography resin that allows the use of NaOH as sanitizing agent. NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins.

***Note:*** *High 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 be designed for efficient sanitization and minimized loss of capacity.*

| Step | Action |
|---|---|
| **1** | Wash the column with 3 column volumes (CV) of start buffer. |
| **2** | Wash with at least 3 CV 0.5 to 1.0 M NaOH with a contact time of 15 minutes, see the note above. |
| **3** | Wash immediately with at least 5 CV sterile and filtered start buffer at pH 7 to 8. |

## HiScreen MabSelect SuRe and HiScreen MabSelect SuRe LX

HiScreen MabSelect SuRe and HiScreen MabSelect SuRe LX are alkali-tolerant, allowing the use of up to 0.5 M NaOH as sanitizing agent. NaOH is very effective for inactivating viruses, bacteria, yeasts, and endotoxins.

***Note:*** *High 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 be designed for efficient sanitization and minimized loss of capacity.*

| Step | Action |
|---|---|
| **1** | Wash the column with 3 column volumes (CV) of start buffer. |
| **2** | Wash the column with at least 3 CV 0.1 to 0.5 M NaOH. Use a contact time of at least 15 minutes, see the note above. |
| **3** | Wash immediately with at least 5 CV sterile start buffer at pH 7 to 8. |

## HiScreen MabSelect and HiScreen MabSelect Xtra

These resins can withstand up to 50 mM NaOH and it is therefore recommended to use other cleaning agents.

| Step | Action |
|---|---|
| 1 | Wash the column with 0.1 M acetic acid in 20% ethanol. |
| 2 | Leave the column in contact with the solution for 1 hour. |
| 3 | Wash with at least 5 CV sterile start buffer at pH 7 to 8. |

or

| Step | Action |
|---|---|
| 1 | Wash the column with 70% ethanol. |
| 2 | Leave the column in contact with the solution for 12 hours. |
| 3 | Wash with at least 5 CV sterile start buffer at pH 7 to 8. |

# 7 Scaling up

After optimizing the method at laboratory-scale, the process is ready for scaling up. Scale-up to a larger column is typically performed by keeping the bed height and flow velocity (cm/h) constant while increasing the bed diameter and the flow rate (mL/min or L/h). For quick small scale-up of purification, two HiScreen columns can be connected in series with a union (Product code 18112093) to give a 20 cm bed height. Bulk resin is available for further scaling up, see *Chapter 11 Ordering information, on page 33*.

***Note:*** *The back pressure is increased with an increased bed height. Decrease the flow rate to adjust the back pressure.*

# 8 Adjusting pressure limits

The pressure generated by the flow through a column affects the packed bed and the column hardware, see *Fig. 4, on page 29*. Increased pressure is generated when running/using one or a combination of the following conditions:

- high flow rate
- high viscosity for buffers or sample
- low temperature
- a flow restrictor

***Note:*** *Exceeding the flow limit can damage the column, see Table 2, on page 6, Table 3, on page 8, and Table 4, on page 10.*

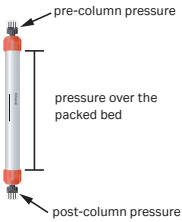


**Fig 4.** Pre-column and post-column measurements.

## ÄKTA avant and ÄKTA pure

The system monitors automatically the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit, see *Table 1, on page 3*.

The maximum pressure the packed bed can withstand depends on resin characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

## ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump

To obtain the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

| Step | Action |
|---|---|
| 1 | Replace the column with a piece of tubing. |
| 2 | Run the pump at the maximum intended flow rate. |
| 3 | Record the pressure as total system pressure, P1. |
| 4 | Disconnect the tubing and run the pump at the same flow rate used in *step 2* . |
| 5 | Note that there will be a drip from the column valve. |
| 6 | Record the pressure as P2. |
| 7 | Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit, *Table 1, on page 3*. |

| Step | Action |
|---|---|
| 8 | Replace the pressure limit in the software with the calculated value.<br><br>*Result:*<br>The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure minus the total system pressure (P1). |

***Note:*** *Repeat the procedure each time the parameters are changed.*

# 9 Storage

Store HiScreen MabSelect PrismA, HiScreen MabSelect SuRe LX, HiScreen MabSelect SuRe, HiScreen MabSelect Xtra, and HiScreen MabSelect in 20% ethanol at 2°C to 8°C.

Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

# 10 Troubleshooting

**Table 10.** Troubleshooting

| Problem | Possible cause | Corrective action |
|---|---|---|
| High back pressure during the run | Solutions with high viscosity are used. | Use lower flow rate. |
| Unstable pressure curve during sample loading | Air bubbles trapped in the sample pump. | Remove air bubbles that might have been trapped in the sample pump. If possible, degas the sample using a vacuum degasser. |
| Gradual broadening of the eluate peak | Insufficient elution and CIP, caused by contaminants accumulating in the column. | Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| Gradual decrease in yield | Insufficient elution and CIP. | Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| Precipitation during elution | Suboptimal elution conditions and CIP. | Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| Gradual increase in CIP peaks | Suboptimal elution conditions and CIP. | Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| High back pressure during CIP | Proteins are precipitated in the column. | Optimize elution conditions and/or run acid regeneration (pH 3 or less) before CIP. Use lower flow rate. |
| High ligand leakage during the first purifications | New column | Perform a blank run, including CIP, before the first purification cycle on a new column. |
| Reduced column performance despite optimized elution and CIP | Column longevity, which depends mainly on the sample type and sample preparation. | Change to a new column. |

# 11 Ordering information

| Product | Quantity | Product code |
|---|---|---|
| HiScreen MabSelect PrismA | 1 × 4.7 mL | 17549815 |
| HiScreen MabSelect | 1 × 4.7 mL | 28926973 |
| HiScreen MabSelect Xtra | 1 × 4.7 mL | 28926976 |
| HiScreen MabSelect SuRe | 1 × 4.7 mL | 28926977 |
| HiScreen MabSelect SuRe LX | 1 × 4.7 mL | 17547415 |

| Related products | Quantity | Product code |
|---|---|---|
| HiTrap MabSelect PrismA | 1 x 1 mL | 17549851 |
| | 5 x 1 mL | 17549852 |
| | 1 x 5 mL | 17549853 |
| | 5 x 5 mL | 17549854 |
| HiTrap MabSelect SuRe | 1 x 1 mL | 29049104 |
| | 5 x 1 mL | 11003493 |
| | 1 x 5 mL | 11003494 |
| | 5 x 5 mL | 11003495 |
| HiTrap MabSelect Xtra | 5 x 1 mL | 28408258 |
| | 1 x 5 mL | 28408260 |
| | 5 x 5 mL | 28408261 |
| HiTrap MabSelect | 5 x 1 mL | 28408253 |
| | 1 x 5 mL | 28408255 |
| | 5 x 5 mL | 28408256 |
| MabSelect PrismA | 25 mL | 17549801 |
| | 200 mL [1] | 17549802 |
| MabSelect SuRe LX | 25 mL | 17547401 |
| | 200 mL [1] | 17547402 |
| MabSelect SuRe | 25 mL | 17543801 |
| | 200 mL [1] | 17543802 |

| Related products | Quantity | Product code |
|---|---|---|
| MabSelect Xtra | 25 mL | 17526907 |
| | 200 mL[1] | 17526902 |
| MabSelect | 25 mL | 17519901 |
| | 200 mL[1] | 17519902 |

[1] Process-scale quantities are available. Contact your local representative.

| Related products | Quantity | Product code |
|---|---|---|
| HiTrap Desalting | 1 × 5 mL | 29048684 |
| | 5 × 5 mL | 17140801 |
| HiPrep 26/10 Desalting | 1 × 53 mL | 17508701 |
| | 4 × 53 mL | 17508702 |
| PD-10 Desalting | 30 | 17085101 |

| Accessories HiScreen | Quantity | Product code |
|---|---|---|
| HiTrap/HiPrep, 1/16" male connector for ÄKTA systems *(For connection of columns with 1/16" fittings to ÄKTA systems)* | 8 | 28401081 |
| Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)* | 2 | 18112093 |
| Fingertight stop plug, 1/16"[1] *(For sealing a HiScreen column)* | 5 | 11000355 |

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

| Related literature | Product code |
|---|---|
| Antibody Purification Handbook | 18103746 |
| Affinity Chromatography Handbook, Principles and Methods | 18102229 |
| Selection Guide Columns and resins for antibody purification and immunoprecipitation | 28935197 |

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