

# HiScreen™ Capto™ MMC

## HiTrap™ Capto MMC

Capto MMC is a multimodal salt-tolerant BioProcess™ resin for capture and intermediate purification of proteins from large feed volumes by packed bed chromatography.

Capto MMC increases productivity and reduces cost with:

- high dynamic binding capacity at high conductivity
- high volume throughput
- smaller unit operations

HiScreen (4.7 mL) and HiTrap (1 and 5 mL) Capto MMC prepacked columns are used for optimization of methods and parameters such as selectivity, binding, and elution conditions, as well as small-scale purifications.

These prepacked columns provide fast, reproducible and easy separations in a convenient format. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.



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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.

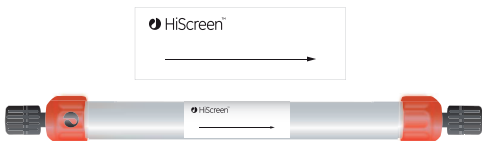
## **Safety**

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 arrow on the column label shows the recommended flow direction.



**Fig 1.** HiScreen column

For scale-up, when a higher bed height is required, two columns can be connected in series using a union to give a 20 cm bed height (see Section *Scaling up*).

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

**Note:** Check that the connector is tightened to prevent leakage.

**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, 116 psi)

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

## HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 2 lists the characteristics of HiTrap columns.



Fig 2. HiTrap, 1 mL column.



Fig 3. HiTrap, 5 mL column

**Note:** *HiTrap columns cannot be opened or refilled.*

**Note:** *Make sure that the connector is tight to prevent leakage.*

**Table 2.** Characteristics of HiTrap columns

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	0.5 MPa (5 bar, 72 psi)	0.5 MPa (5 bar, 72 psi)

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

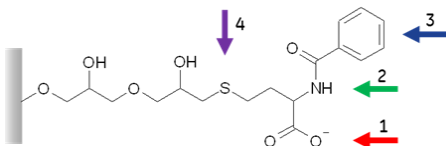
### Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

## Properties of Capto MMC

### Multimodal functionality

Capto MMC has a ligand with multimodal functionality (see figure below). The multimodal functionality gives a different selectivity compared to traditional ion exchangers and also provides the possibility of operating in different regions with respect to pH and conductivity.



**Fig 4.** The Capto MMC ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interactions (1), hydrogen bonding (2), hydrophobic interactions (3), and thiophilic interactions (4). The chromatography resin is designed for polishing, and is based on the high flow agarose base matrix with a small bead size, which gives good pressure-flow properties and high resolution.

### High flow agarose matrix

The ligand is coupled to a chemically modified, high flow agarose matrix. The agarose matrix provides particle rigidity without compromising the pore size. These properties of the agarose matrix allow for fast mass transfer, resulting in high dynamic binding capacities of Capto MMC at high flow rates. This makes the resin suitable for high volume process scale applications.

The characteristics of Capto MMC are listed in Table 3.

**Table 3.** Characteristics of Capto MMC resin.

<b>Matrix</b>	Highly cross-linked agarose, spherical
<b>Ion exchange type</b>	Multimodal weak cation exchanger
<b>Ionic capacity</b>	0.07 to 0.09 mmol H <sup>+</sup> /mL resin
<b>Particle size, <math>d_{50v}</math><sup>1</sup></b>	~ 75 $\mu$ m
<b>Recommended operating flow rate</b>	Recommended operating flow rate: <sup>2</sup> 2 to 4.7 mL/min (HiScreen) 1 to 4 mL/min (HiTrap 1 mL) 5 to 20 mL/min (HiTrap 5 mL)
<b>Pressure/flow characteristics (large scale)<sup>2</sup></b>	At least 600 cm/h at $\leq$ 0.3 MPa in a 1 m diameter column and 20 cm bed height (at 20°C using process buffers with the same viscosity as water) <sup>3</sup>
<b>Dynamic binding capacity, <math>Q_{B10}</math><sup>4</sup></b>	$\geq$ 45 mg BSA/mL resin at 30 mS/cm
<b>pH stability, operational<sup>5</sup></b>	3 to 12
<b>pH stability, CIP<sup>6</sup></b>	3 to 14
<b>Working temperature<sup>7</sup></b>	4°C to 30°C
<b>Chemical stability</b>	Stable to commonly used aqueous buffers, 1M acetic acid, 1.0 M NaOH <sup>8</sup> , 8 M Urea, 6 M guanidine hydrochloride, 70% ethanol, 30% isopropanol.
<b>Avoid</b>	Oxidizing agents, anionic detergents
<b>Storage</b>	20% ethanol, 4°C to 30°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> At room temperature, using buffers with the same viscosity as water. See also table 6-8.

<sup>3</sup> The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

<sup>4</sup> Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a Tricorn™ 5/100 column at 10 cm bed height (2 min residence time) for BSA in 50 mM sodium acetate, pH 4.75, 250 mM NaCl.

<sup>5</sup> pH range where resin can be operated without significant change in function.

<sup>6</sup> pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

<sup>7</sup> Capto MMC can be used under cold-room conditions, but for some proteins the capacity may decrease.

<sup>8</sup> 1.0 M NaOH should only be used for cleaning purposes.

## 2 Optimization

### Aim

The aim of designing and optimizing a method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. For optimization of binding conditions, pH and conductivity (salt concentration) should be screened.

### Screening

When using HiScreen Capto MMC, protein binding can be expected at pH values lower than those used with traditional ion exchangers due to the contribution from non-electrostatic interactions. The multimodal nature of the ligand results in different interaction modes, electrostatic versus hydrophobic, depending on buffer conditions and target protein characteristics. A thorough screening of conditions is therefore strongly recommended on HiScreen Capto MMC in order to optimize conditions for binding and elution.

## Workflow

The recommended workflow is described in the figure below. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.



Fig 5. Recommended workflow during process development.

## Optimizing the process

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery. To reduce time, sample, and buffer consumption, the method should be designed in laboratory-scale.

## Optimizing binding conditions

During binding, Capto MMC behaves predominantly like a weak cation exchanger. Since it allows binding at high conductivity, it may not be necessary to screen for optimal loading conductivity with respect to binding capacity. However, binding selectivity may still be affected by the loading conductivity.

For Capto MMC the dynamic binding capacities decrease for some proteins at lower temperatures. Screening for buffer concentration at the temperature where the process is intended to be run will give the optimal dynamic binding capacity.



## Optimizing elution conditions

The fact that Capto MMC allows efficient capture of proteins at high conductivity in many cases limits the use of increasing salt concentrations as an efficient way of eluting proteins. Optimal elution is often achieved by a combination of changes in pH, buffer concentration, and eluting salt.

Always use *Linear gradient elution* for method development, or when starting with an unknown sample. The results obtained can then serve as a basis from which to optimize the separation.

*Step elution* allows the target protein to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. It can be necessary to decrease the flow rate due to the high concentrations of protein in the eluted pool.

- Start to screen for optimal elution pH and use buffers with a pH of 0.5, 1.5, and 2.5 above the isoelectric point (pI) of the target protein. Use step elution, or a pH gradient. Use the pH where the target molecule starts to elute as a basis if further optimization is needed in order to increase recovery.
- If required, continue with screening for eluting salt concentration. Use 0.5, 1.0, and 1.5 M salt concentration at the earlier determined pH.
- If further optimization is needed, increase the concentration of buffering salt in the elution buffer (e.g., from 50 mM to 250 mM).
- If the target protein still elutes in an asymmetrical peak over a number of fractions, the eluting salt can be changed (e.g., from NaCl to NH<sub>4</sub>Cl).
- Additives such as urea and organic modifiers can further increase the recovery of some proteins.

More information on how to optimize the process can be found in the Instructions *Capto MMC* (11003505) and the Application Note *Optimizing elution conditions on Capto MMC using Design of Experiments* (11003548).

# 3 Operation

## Prepare buffers

The buffer species and buffer concentration are important for reproducible and robust methods. Table 4 shows suitable buffers for cation exchangers and suggested starting concentrations. The buffer concentration should be at least 25 mM, but could sometimes be over 100 mM. Since elution from Capto MMC might require changes in both pH and salt concentration, take extra care in optimizing the elution buffer, see Section *Optimization*.

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

**Table 4.** Buffers for cation exchange chromatography

pH interval	Substance and concentration	Counter-ion	pKa (2°C) <sup>1</sup>
1.4 to 2.4	Maleic acid, 20 mM	Na <sup>+</sup>	1.92
2.6 to 3.6	Methyl malonic acid, 20 mM	Na or Li <sup>+</sup>	3.07
2.6 to 3.6	Citric acid, 20 mM	Na <sup>+</sup>	3.13
3.3 to 4.3	Lactic acid, 50 mM	Na <sup>+</sup>	3.86
3.3 to 4.3	Formic acid, 50 mM	Na <sup>+</sup> or Li <sup>+</sup>	3.75
3.7 to 4.7	Succinic acid, 50 mM	Na <sup>+</sup>	4.21
4.3 to 5.3	Acetic acid, 50 mM	Na <sup>+</sup> or Li <sup>+</sup>	4.75
5.1 to 6.1	Succinic acid, 50 mM	Na <sup>+</sup>	5.64
5.2 to 6.2	Methyl malonic acid, 50 mM	Na <sup>+</sup> or Li <sup>+</sup>	5.76
5.6 to 6.6	MES, 50 mM	Na <sup>+</sup> or Li <sup>+</sup>	6.27
6.7 to 7.7	Phosphate, 50 mM	Na <sup>+</sup>	7.20
7.0 to 8.0	HEPES, 50 mM	Na <sup>+</sup> or Li <sup>+</sup>	7.56
7.8 to 8.8	BICINE, 50 mM	Na <sup>+</sup>	8.33

<sup>1</sup> Handbook of Chemistry and Physics, 83rd edition, CRC, 2002–2003.

For samples with unknown charge properties, try the following:

### Start buffer

25 mM sodium acetate, pH 4.5

### Elution buffer

25 mM phosphate buffer, 1 M NaCl, pH 7.5

If pH gradient elution is desired, try the following:

**Start buffer** (1 L, 100 mM sodium phosphate/citrate, pH 3)

Step	Action
1	Dissolve 29.4 g tri-sodium-citrate $\times$ 2 H <sub>2</sub> O ( $M_r = 294$ ) and 13.8 g NaH <sub>2</sub> PO <sub>4</sub> $\times$ H <sub>2</sub> O ( $M_r = 138$ ) in 800 mL ultra pure water.
2	Adjust the pH to 3 with 1 M HCl and the volume to 1 L.

**Elution buffer** (1 L, 100 mM sodium phosphate/citrate, pH 7.5)

Step	Action
1	Dissolve 29.4 g tri-sodium-citrate $\times$ 2 H <sub>2</sub> O ( $M_r = 294$ ) and 17.8 g NaH <sub>2</sub> PO <sub>4</sub> $\times$ 2 H <sub>2</sub> O ( $M_r = 178$ ) in 800 mL ultra pure water.
2	Adjust the pH to 7.5 with 1 M HCl and the volume to 1 L.

**Note:** *Water and chemicals used for buffer preparation must be of high purity. Filter buffers through a 0.22  $\mu$ m or a 0.45  $\mu$ m filter before use.*

## Prepare the sample

Adjust the sample to the composition of the start buffer, using one of the following methods:

- 1 Dilute the sample with the start buffer.  
Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap Desalting or PD-10 Desalting column (see Table 5).
- 2 Filter the sample through a 0.45  $\mu$ m filter or centrifuge immediately before loading the sample to the column. This prevents clogging and increases the lifetime of the column when loading large sample volumes.
- 3 For larger feed volumes, the preparation is preferably performed by diafiltration or by adjustment of pH and the conductivity.

## 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 5.** Prepacked columns for desalting.

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

<sup>1</sup> Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

<sup>2</sup> Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

<sup>3</sup> Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

<sup>4</sup> Volumes with gravity elution

<sup>5</sup> Volumes with centrifugation

## Recommended flow rates

The pressure-flow specification for the Capto MMC resin in large columns is  $\geq 600$  cm/h in a 20 cm bed height at 3 bar and a buffer viscosity comparable to water. The residence time should be the same as the time intended for the final-scale of the process. The tables below show the recommended flow rates for different operations.

**Table 6.** Recommended flow rates using HiScreen Capto MMC.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration <sup>1</sup>	$\leq 4.7$	$\leq 600$	$\geq 1$
Wash <sup>1</sup>	$\leq 4.7$	$\leq 600$	$\geq 1$
Sample load	1.2 to 2.3	150 to 300	2 to 4
Cleaning-in-place <sup>2</sup>	$\leq 0.78$	$\leq 100$	$\geq 6$

**Table 7.** Recommended flow rates using HiTrap Capto MMC, 1 mL.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration <sup>1</sup>	≤ 4.0	≤ 600	≥ 0.25
Wash <sup>1</sup>	≤ 4.0	≤ 600	≥ 0.25
Sample load	0.25 to 0.5	35.5 to 75	2 to 4
Cleaning-in-place <sup>2</sup>	≤ 0.16	≤ 25	≥ 6

**Table 8.** Recommended flow rates using HiTrap Capto MMC, 5 mL.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration <sup>1</sup>	≤ 20	≤ 600	≥ 0.25
Wash <sup>1</sup>	≤ 20	≤ 600	≥ 0.25
Sample load	1.2 to 2.5	35.5 to 75	2 to 4
Cleaning-in-place <sup>2</sup>	≤ 0.84	≤ 25	≥ 6

<sup>1</sup> The flow rates are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, e.g. 20% ethanol, lower flow rates should be used.

<sup>2</sup> For cleaning-in-Place use at least 3 column volumes with a total contact time of at least 15 minutes. See also *Chapter 4 Cleaning-in-place (CIP)*.

## Purification

Collect fractions throughout the separation.

**Flow rate:** See Tables 6, 7 and 8 for recommended flow rates for different operations.

**Column tubing:** Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 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.

Step	Action
------	--------

- |   |                                                                                                  |
|---|--------------------------------------------------------------------------------------------------|
| 1 | Remove the stoppers and connect the column to the system. Avoid introducing air into the column. |
|---|--------------------------------------------------------------------------------------------------|

**Note:**

*To prevent air from entering the column, make a drop-to-drop connection.*

**Note:**

*To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28401081).*

- |   |                                                                                                                                                                                                                                                         |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 | Wash with 5 column volumes (CV) of ultra pure water to remove ethanol. This step makes sure that buffer salts do not precipitate, which they do if they are exposed to ethanol. The step can be omitted if precipitation is not likely to be a problem. |
| 3 | Equilibrate the column with at least 5 CV start buffer or until the UV baseline, eluent pH, and conductivity are stable.                                                                                                                                |
| 4 | Adjust the sample to the chosen starting pH and conductivity and load on the column.                                                                                                                                                                    |
| 5 | Wash with 5 to 10 CV start buffer until the UV trace of the effluent returns to near baseline.                                                                                                                                                          |

Step	Action
6	<p>Elute, either by linear gradient elution or a step elution, see below. If required, the collected eluted fractions can be buffer exchanged or desalted using columns listed in Table 5.</p> <ul style="list-style-type: none"><li>• <i>Linear gradient elution</i> Elute with 0% to 100% elution buffer in 10 to 20 CV.</li><li>• <i>Step elution</i> Elute with 5 CV elution buffer at the pH chosen and include NaCl at the chosen concentration. Repeat at higher NaCl concentrations until the target protein has been eluted.</li></ul>
7	<p>Wash with 5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.</p>
8	<p>If required, perform a CIP to clean the column.</p>
9	<p>Re-equilibrate with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.</p>

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To save time, higher flow rates during the high salt wash and re-equilibration steps can be used.

**Note:** *Do not exceed the maximum recommended flow and/or back pressure for the column.*

## 4 Cleaning-in-place (CIP)

### General description

Correct preparation of samples and buffers maintains columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

CIP removes very tightly bound, precipitated, or denatured substances from the resin. If such contaminants are allowed to accumulate, they can affect the chromatographic properties of the prepacked column, reduce the capacity of the resin and, potentially, come off in subsequent runs. If the fouling is severe, it can block the column, increase back pressure, and affect the flow properties.

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

It is recommended to perform a CIP:

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



## CIP protocol

The nature of the sample will ultimately determine the final CIP protocol so the CIP procedure below may require optimization. NaOH concentration, contact time, and frequency are typically the main parameters to vary during the optimization of the CIP.

The CIP procedure below removes common contaminants.

**Flow rate:** For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during purifications (see Tables 6, 7 and 8).

Step	Action
1	Wash with at least 2 column volumes (CV) of 2 M NaCl.
2	Wash with at least 4 CV 1.0 M NaOH.
3	Wash with at least 2 CV 2 M NaCl.
4	Wash with at least 2 CV ultra pure water.
5	Wash with 5 CV start buffer or until eluent pH and conductivity have reached the required values.

## 5 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up. For quick small scale-up of purification, either two HiTrap columns (one in the top of the other) or two HiScreen columns can conveniently be connected in series with a union (18112093) to give increased bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors such as clearance of critical impurities may change when column bed height is modified and should be validated using the final bed height.

Scaling up is otherwise typically performed by keeping bed height and linear flow velocity (cm/h) constant while increasing bed diameter and volumetric flow rate (mL/min or L/h).

Bulk BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing. Resin is available for further scaling-up, see *Ordering information*.

A general description of the scaling up procedure is described below.

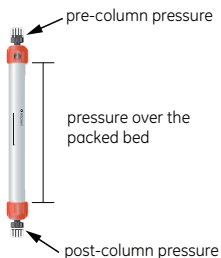
Step	Action
1	Select bed volume according to required sample load. Keep sample concentration constant.
2	Select column diameter to obtain the desired bed height. The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.
3	The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes.

## 6 Adjusting pressure limits

The pressure in chromatography system software is generated by the flow through a column. The pressure affects the packed bed and the column hardware, see the figure below. The pressure is increased during running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

**Note:** *Exceeding the flow limit (see recommended flow rates in Table 3) can damage the column.*



**Fig 6.** Pre-column and post-column measurements.

### ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed,  $\Delta p$ ). The pre-column pressure limit is the column hardware pressure limit (see Table 1 and Table 2).

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	<ul style="list-style-type: none"><li>• Replace the column with a piece of tubing.</li><li>• Run the pump at the maximum intended flow rate.</li><li>• Record the pressure as total system pressure, P1.</li></ul>
2	<ul style="list-style-type: none"><li>• Disconnect the tubing and run the pump at the same flow rate used in step 1.</li><li>• Note that there will be a drip from the column valve.</li><li>• Record the pressure as P2.</li></ul>
3	<ul style="list-style-type: none"><li>• Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1).</li><li>• Replace the pressure limit in the software with the calculated value.</li></ul> <p><i>Result:</i> The actual pressure over the packed bed (<math>\Delta p</math>) during the run is equal to the actual measured pressure which is the total system pressure (P1).</p>

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

## 7 Storage

Wash with 2 column volumes (CV) of ultra pure water followed by 2 CV 20% ethanol.

Store at 4°C to 30°C. Do not freeze.

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

## 8 Troubleshooting

<b>Problem</b>	<b>Possible cause</b>	<b>Corrective action</b>
High back pressure during the run	Solutions with high viscosity are used.	Use a lower flow rate.
	The column is clogged	Clean the column, see section Cleaning-in-place (CIP)
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	If possible, de-gas the sample using a vacuum de-gasser.
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 high salt wash before CIP or use lower flow rate.
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.

## 9 Ordering information

Product	Quantity	Product code
HiScreen Capto MMC	1 × 4.7 mL	28926980
HiTrap capto MMC	5 × 1 mL	11003273
	5 × 5 mL	11003275

Related products	Quantity	Product code
Capto MMC	25 mL	17531710
	100 mL <sup>1</sup>	17531701

<sup>1</sup> Process-scale quantities are available. Please contact your local representative.

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

<sup>1</sup> One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Accessories HiTrap	Quantity	Code No.
1/16" male/luer female <i>(For connection of syringe to top of HiTrap column)</i>	2	18111251
Tubing connector flangeless/M6 female <i>(For connection of tubing to bottom of HiTrap column<sup>1</sup>)</i>	2	18100368
Tubing connector flangeless/M6 male <i>(For connection of tubing to top of HiTrap column)</i>	2	18101798
Union 1/16" female/M6 male <i>(For connection to original FPLC System through bottom of HiTrap column)</i>	6	18111257
Union M6 female /1/16" male <i>(For connection to original FPLC System through top of HiTrap column)</i>	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28401081

<b>Accessories HiTrap</b>	<b>Quantity</b>	<b>Code No.</b>
Stop plug female, 1/16" <i>(For sealing bottom of HiTrap column)</i>	5	11000464
Fingertight stop plug, 1/16"	5	11000355

- <sup>1</sup> Union 1/16" female/M6 male is also needed.  
Two, five, or seven stop plugs female included in HiTrap packages depending on the product.  
One fingertight stop plug is connected to the inlet and the outlet of each HiTrap column at delivery.

<b>Related literature</b>	<b>Product code</b>
Ion Exchange Chromatography and Handbook, Principles and Methods	11000421
Ion Exchange Columns and Media, Selection guide	18112731
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778
Optimizing elution conditions on Capto MMC using Design of Experiment, Application note	11003548
Capto MMC, Data file	11003545

For local office contact information, visit  
[www.gelifesciences.com/contact](http://www.gelifesciences.com/contact)

GE Healthcare Bio-Sciences AB  
Björkgatan 30  
751 84 Uppsala  
Sweden

[www.gelifesciences.com/protein-purification](http://www.gelifesciences.com/protein-purification)

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GE Healthcare Europe GmbH  
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited  
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.  
100 Results Way, Marlborough, MA 01752, USA

HyClone Laboratories, Inc.  
925 W 1800 S, Logan, UT 84321, USA

GE Healthcare Japan Corporation  
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

