

# HiScreen™ Capto™ SP ImpRes HiScreen Capto Q ImpRes

Capto SP ImpRes and Capto Q ImpRes are strong cation and strong anion exchange BioProcess™ media, respectively, for intermediate purification and polishing of a wide range of biomolecules. The combination of the high flow agarose technique used for all Capto products and the small particle size of Capto ImpRes results in good pressure-flow properties as well as impressive resolution.

Capto SP ImpRes and Capto Q ImpRes provides:

- High resolution chromatography media on the well established Capto platform with traditional ligands
- Flexibility of design – large operational window of flow rates and bed heights
- Efficient design of robust polishing processes using convenient and scalable tools and formats
- High productivity enabling improved process economy

HiScreen Capto SP ImpRes and HiScreen Capto Q ImpRes are prepacked 4.7 ml columns for optimization of methods and parameters, such as selectivity, binding and elution conditions, as well as small scale purifications. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.



# Table of Contents

1	Product description .....	3
2	General process development .....	6
3	Optimization .....	7
4	Operation .....	8
5	Cleaning-in-place (CIP) .....	12
6	Scaling up .....	14
7	Adjusting pressure limits in chromatography system software .....	14
8	Storage .....	16
9	Troubleshooting .....	17
10	Ordering information .....	18

Please read these instructions carefully before using the HiScreen columns.

## **Intended use**

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

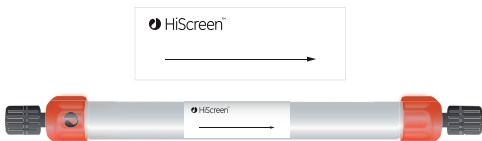
## **Safety**

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

# 1 Product description

## HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.



**Fig 1.** HiScreen column

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

**Note:** *HiScreen columns cannot be opened or refilled*

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

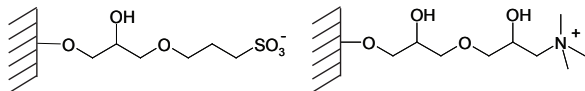
**Table 1.** Characteristics of HiScreen column

Column volume (CV)	4.7 ml
Column dimensions	0.77 × 10 cm
Column hardware pressure limit <sup>1</sup>	8 bar (0.8 MPa)

<sup>1</sup> The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

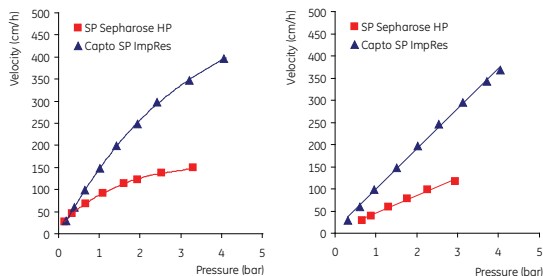
## Properties of Capto SP ImpRes and Capto Q ImpRes

The structures of the well established SP (sulfonate group) and Q (quaternary amine group) ligands used for Capto ImpRes are shown in Figure 2.



**Fig 2.** The strong ion exchange group of Capto SP ImpRes (left) and Capto Q ImpRes (right).

The media are based on the high flow agarose base matrix, which gives good pressure-flow properties (Fig 3).



**Fig 3.** Example of pressure flow curves for Capto SP ImpRes and SP Sepharose HP in open bed (left) and packed bed (right), respectively. Running conditions: AxiChrom™ 300 (30 cm i.d.), 20 cm bed height with Packing Factor 1.15 in water at 20°C. Pressure contribution from system, tubings and column is excluded.

The bead size is small (40  $\mu\text{m}$ ), which allows for high resolution. The combination of the well established SP and Q ligands with a small high flow agarose bead makes the media ideal for high throughput intermediate purification and polishing. Further characteristics of the media are found in the following table.

**Table 2.** Characteristics of Capto SP ImpRes and Capto Q ImpRes

	Capto SP ImpRes	Capto Q ImpRes
<b>Matrix</b>	High flow agarose	High flow agarose
<b>Average particle size (<math>d_{50v}</math>)<sup>1</sup></b>	36 to 44 $\mu\text{m}$	36 to 44 $\mu\text{m}$
<b>Functional group</b>	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$
<b>Total ionic capacity</b>	0.13 to 0.16 mmol $\text{H}^+$ /ml medium	0.15 to 0.18 mmol $\text{Cl}^-$ /ml medium
<b>Binding capacity<sup>2</sup></b>	>70 mg lysozyme/ml medium >95 mg BSA/ml medium	>55 mg BSA/ml medium >48 mg $\beta$ -Lactoglobulin/ml medium
<b>Recommended flow velocity<sup>3</sup></b>	100 to 300 cm/h	100 to 300 cm/h
<b>Maximum flow velocity<sup>3</sup></b>	400 cm/h	400 cm/h
<b>pH stability<sup>4</sup></b>		
<b>Working range</b>	4 to 12	2 to 12
<b>Cleaning-in-place</b>	3 to 14	2 to 14
<b>Chemical stability</b>	All commonly used aqueous buffers, 1 M NaOH <sup>5</sup> , 8 M urea, 6 M guanidine hydrochloride, 30% iso-propanol and 70% ethanol	
<b>Avoid</b>	Oxidizing agents, cationic detergents	Oxidizing agents, anionic detergents
<b>Working temperature</b>	4°C to 30°C	4°C to 30°C
<b>Storage</b>	20% ethanol, 0.2 M sodium acetate	20% ethanol

<sup>1</sup>  $d_{50v}$  is the average particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough as measured at a residence time of 4 minutes (150 cm/h) in a Tricorn™ 5/100 column with 10 cm bed height.

<sup>3</sup> Water at room temperature.

For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow rate is recommended.

<sup>4</sup> Working range: pH interval where the medium can be handled without significant change in function.

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

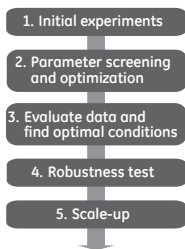
<sup>5</sup> No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

## 2 General process development

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 makes it 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 Section *Scaling up*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the medium and environmental constraints.



**Fig 4.** Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol.

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 variable ranges. DoE may be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see Section *Scaling up*.

## 3 Optimization

### 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 during optimization the method should be designed in laboratory scale.

### Optimizing binding conditions

Screen for optimal binding conditions by testing a range of pH values within which the target protein is known to be stable. If the isoelectric point (pI) of the target protein is known, then begin with a narrower pH range, for example, 0.5 to 1 pH unit away from pI. In some cases the sample conductivity is equally important as the pH when screening for optimal binding conditions.

For certain proteins, dynamic binding capacities increase at increased conductivity. Our recommendation is to screen for optimal ionic strength by varying the conductivity of the sample between 2 and 15 mS/cm as well as screening for optimal binding pH.

### Optimizing elution conditions

Linear ionic strength gradients should always be used for method development or when starting with an unknown sample. Linear ionic strength gradients are easy to prepare and very reproducible when generated by a suitable chromatography system. The results obtained can then serve as a basis from which to optimize the separation.

Step-wise elution allows the target protein to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. Due to the high concentrations of protein in the eluted pool it might be necessary to decrease the flow rate and thereby avoid exceeding the maximum back pressure for the column.

## Automated buffer preparation

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

# 4 Operation

## Prepare buffers

The elution buffer is usually of the same composition and pH as the start buffer, but it contains additional salt, most often sodium chloride. The pH of the start buffer should be at least 0.5 to 1 pH unit above the isoelectric point (pI) of the target molecule when using an anion exchanger (Q) and at least 0.5 to 1 pH unit below the pI when using a cation exchanger (SP).

The buffer species and buffer concentration are important for reproducible and robust methods. The buffer concentration depends partly on the buffer capacity at a given pH and should be at least 10 mM (only rarely above 100 mM). Where the conductivity of the buffers needs to be considered, it can be increased by increasing the buffer concentration or adding sodium chloride.

Try the following buffers for samples with unknown charge properties.

### **Anion exchange (Q)**

Start buffer: 20 mM Tris-HCl, pH 8.0

Elution buffer: 20 mM Tris-HCl, 1 M NaCl, pH 8.0



## Cation exchange (SP)

Start buffer: 50 mM sodium acetate, pH 5.0

Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0

or

Start buffer: 50 mM MES, pH 6.0

Elution buffer: 50 mM MES, 1 M NaCl, pH 6.0

**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.*

## Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of these methods: <ul style="list-style-type: none"><li>• Dilute the sample with start buffer.</li><li>• Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see Table below).</li></ul>
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the longevity of the column when loading large sample volumes.

**Table 3.** Prepacked columns for desalting

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts ( $M_r > 5000$ ).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml <sup>1</sup> 1.75 to 2.5 ml <sup>2</sup>	3.5 ml <sup>1</sup> up to 2.5 ml <sup>2</sup>	Prepacked with Sephadex G-25 Medium.	For desalting, buffer exchange, and cleanup of
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml <sup>1</sup> 0.2 to 0.5 ml <sup>2</sup>	1.0 ml <sup>1</sup> up to 0.5 ml <sup>2</sup>	Runs by gravity flow or centrifugation	proteins and other large biomolecules ( $M_r > 5000$ ).
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml <sup>1</sup> 0.75 to 1.0 ml <sup>2</sup>	1.5 ml <sup>1</sup> up to 1.0 ml <sup>2</sup>		

<sup>1</sup> Volumes with gravity elution

<sup>2</sup> Volumes with centrifugation

## Recommended flow rates

**Table 4.** Recommended flow rates for HiScreen Capto IEX ImpRes columns.

Flow velocity (cm/h)	Flow rate (ml/min)
100	0.8
200	1.6
300	2.3

## Purification

Collect fractions throughout the separation.

**Flow rate:** See Table 4.

**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. <b>Note:</b> <i>To prevent leakage, ensure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).</i>
2	Wash with 1 column volume (CV) distilled water. This step removes the ethanol and avoids the precipitation of buffer salts upon exposure 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 or 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 3.</p> <ul style="list-style-type: none"> <li>• <i>Linear gradient elution</i> Elute with 0 to 100% elution buffer (up to 1 M NaCl) in 10 to 20 CV.</li> <li>• <i>Step elution</i> Elute with 5 CV elution buffer including NaCl at chosen concentration. Repeat at higher NaCl concentrations until the target protein has been eluted.</li> </ul>
7	Wash with 5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.
8	If required, perform a CIP to clean the column.
9	Re-equilibrate with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

To save time, higher flow rates during regeneration and re-equilibration steps can be used.

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

## 5 Cleaning-in-place (CIP)

### General description

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

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

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns. CIP is usually performed after the elution.

It is recommended to perform a CIP:

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

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

## 6 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up.

For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union (18-1120-93) to give 20 cm 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.

Scale-up is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (ml/min or l/h).

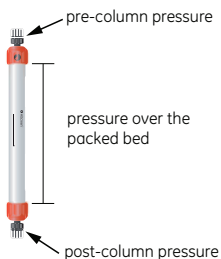
Bulk media is available for further scale-up, see Section *Ordering information*.

## 7 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when 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 Table 2) may damage the column.*



**Fig 5.** Pre-column and post-column measurements.

## ÄKTA avant

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).

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

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

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as <i>total system pressure</i> , P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed ( $\Delta p$ ) will during run be equal to actual measured pressure - <i>total system pressure</i> (P1).

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

## 8 Storage

**Capto Q ImpRes:** Wash with 2 column volumes (CV) distilled water followed by 2 CV of 20% ethanol.

**Capto SP ImpRes:** Wash with 2 CV distilled water followed by 2 CV of 20% ethanol containing 0.2 M sodium acetate.

Store at 4°C to 30°C. Do not freeze.

Ensure that the column is tightly sealed to avoid drying out.



## 9 Troubleshooting

<b>Problem</b>	<b>Possible cause/corrective action</b>
High back pressure during the run	The column is clogged. <i>Clean the column, see Section Cleaning-in-place (CIP).</i>  High viscosity of solutions. <i>Use lower flow rate.</i>
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump. <i>If possible, degas the sample using a vacuum degasser.</i>
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
Gradual decrease in yield	Insufficient elution and CIP. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
Precipitation during elution	Sub-optimal elution conditions and CIP. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
High back pressure during CIP	Proteins precipitated in column. <i>Optimize elution conditions and/or run high salt wash before CIP or use lower flow rate.</i>
Reduced column performance despite optimized elution and CIP	<i>Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.</i>

## 10 Ordering information

<b>Product</b>	<b>Quantity</b>	<b>Code No.</b>
HiScreen Capto SP ImpRes	1 × 4.7 ml	17-5468-15
HiScreen Capto Q ImpRes	1 × 4.7 ml	17-5470-15

<b>Related products</b>	<b>Quantity</b>	<b>Code No</b>
HiTrap Capto Q ImpRes	5 × 1 ml	17-5470-51
	5 × 5 ml	17-5470-55
HiTrap Capto SP ImpRes	5 × 1 ml	17-5468-51
	5 × 5 ml	17-5468-55
Capto Q ImpRes	25 ml	17-5470-10
	100 ml	17-5470-02
	1 L <sup>1</sup>	17-5470-03
Capto SP ImpRes	25 ml	17-5468-10
	100 ml	17-5468-02
	1 L <sup>1</sup>	17-5468-03
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

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

<b>Accessories HiScreen</b>	<b>Quantity</b>	<b>Code No</b>
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems <i>(For connection of columns with 1/16" fittings to ÄKTA systems)</i>	8	28-4010-81
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	18-1120-93
Fingertight stop plug, 1/16" <sup>1</sup> <i>(For sealing a HiScreen column)</i>	5	11-0003-55

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

<b>Related literature</b>	<b>Code No.</b>
Ion Exchange Chromatography and Chromatofocusing Handbook, Principles and Methods	11-0004-21
Ion Exchange Columns and Media, Selection Guide	18-1127-31
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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