GE Healthcare
Life Sciences

Instructions 28-9339-62 AE **Prepacked columns**

HiScreen™ Capto™ Q
HiScreen Capto S
HiScreen Capto DEAE

Capto Q, Capto S and Capto DEAE are ion exchange BioProcess™ media for capture and intermediate purification of proteins. HiScreen Capto Q, HiScreen Capto S and HiScreen Capto DEAE 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.

HiScreen Capto Q, HiScreen Capto S and HiScreen Capto DEAE 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™.

Table of Contents

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.

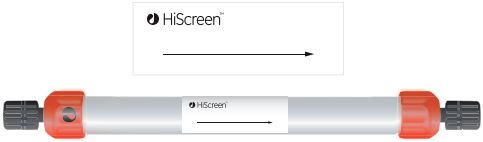

Fig 1. HiScreen column

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 [1]	8 bar (0.8 MPa)

[1] 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 S, Q and DEAE

- Capto Q: strong anion exchanger (quaternary amine group)
- Capto S: strong cation exchanger (sulfoethyl group)
- Capto DEAE: weak anion exchanger (diethylaminoethyl group)

The ligands, Q, S and DEAE, are coupled to a chemically modified, high flow agarose matrix, that provides particle rigidity without compromising the pore size. In addition, dextran surface extenders coat the agarose matrix.

These characteristics of the agarose matrix allow for fast mass transfer, resulting in high dynamic binding capacities of Capto Q, Capto S and Capto DEAE at high flow rates. This makes the media suitable for high volume process scale applications.

The characteristics of Capto Q, Capto S and Capto DEAE are listed in Table 2.

Table 2. Characteristics of Capto ion exchange media

	Capto Q	Capto S	Capto DEAE
Matrix	High flow agarose with a dextran surface extender		
Average particle size (d_{50v}) [1]	90 µm	90 µm	90 µm
Ion exchange type	Strong anion, Q	Strong cation, S	Weak anion, DEAE
Charged group	$-N^+(CH_3)_3$	$-SO_3^-$	$-N^+H(CH_2CH_3)_2$
Total ionic capacity	0.16 to 0.22 mmol Cl^-/ml medium	0.11 to 0.14 mmol Na^+/ml medium	0.29 to 0.35 mmol Cl^-/ml medium
Dynamic binding capacity	> 100 mg BSA/ml medium [2]	> 120 mg lysozyme/ml medium [3]	> 90 mg ovalbumin/ml medium[2]
Recommended flow velocity [4]	150 to 350 cm/h	150 to 350 cm/h	150 to 350 cm/h
Maximum flow velocity [4]	700 cm/h	700 cm/h	700 cm/h
pH stability [5]			
Working range	2 to 12	4 to 12	2 to 9 (See Fig. 2)
Cleaning-in-place	2 to 14	3 to 14	2 to 14
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH [6], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol		
Avoid	Oxidizing agents, anionic detergents	Oxidizing agents, cationic detergents	Oxidizing agents, anionic detergents
Working temperature [7]	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 0.2 M sodium acetate in 20% ethanol	4°C to 30°C in 20% ethanol

1 d_{50v} is the average particle size of the cumulative volume distribution.

2 Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 min, 600 cm/h in a Tricorn™ 5/100 column with 10 cm bed height in a 50 mM Tris-HCl buffer, pH 8.0.

3 Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 min, 600 cm/h in a Tricorn 5/100 column with 10 cm bed height, in a 30 mM sodium phosphate buffer, pH 6.8.

[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] 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.

[6] No significant change in dynamic binding capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

[7] Low temperature can decrease capacity of Capto S and Capto DEAE.

Capto DEAE titration curve

Capto DEAE has both weak and strong ion exchange properties, see Fig 2. At pH ~5 most of the weak ion exchange ligands are protonated and positively charged. As pH increases, the ligands gradually lose bound protons and thereby the positive charge. At a pH between 10 and 11, all weak ion exchange ligands are uncharged; only permanently positively charged quaternary ammonium groups remain.

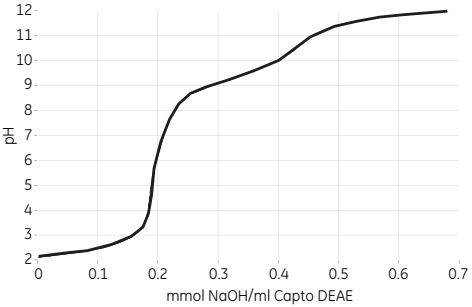

Fig 2. Titration curve for Capto DEAE

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.

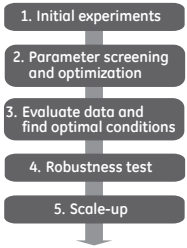

Fig 3. Typical steps during process development.

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 of the target protein is known, then begin with a narrower pH range, for example, 0.5 to 1 pH unit away from the isoelectric point. 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.

For Capto S and Capto DEAE, 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

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.

Since Capto S, Capto Q and Capto DEAE allow efficient capture at high flow rate, pay special attention to optimizing elution conditions to avoid tailing peaks when eluting the protein of interest.

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 DEAE) and at least 0.5 to 1 pH unit below the pI when using a cation exchanger (S).

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 and DEAE)

Start buffer: 20 mM Tris-HCl, pH 8.0

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

Cation exchange (S)

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: • Dilute the sample with start buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see Table below).
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[1] 1.75 to 2.5 ml[2]	3.5 ml[1] up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml[1] 0.2 to 0.5 ml[2]	1.0 ml[1] up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml[1] 0.75 to 1.0 ml[2]	1.5 ml[1] up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

Recommended flow rates

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

Flow velocity (cm/h)	Flow rate (ml/min)
150	1.2
300	2.3
400	3.1
600	4.7

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. ***Note:*** *To prevent leakage, ensure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).*
3	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.
4	Equilibrate the column with at least 5 CV start buffer for Capto Q and Capto S and at least 10 CV start buffer for Capto DEAE or until the UV baseline, eluent pH and conductivity are stable.
5	Adjust the sample to the chosen starting pH and conductivity and load on 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, 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. • *Linear gradient elution* Elute with 0 to 100% elution buffer (up to 1 M NaCl) in 10 to 20 CV. • *Step elution* Elute with 5 CV elution buffer including NaCl at chosen concentration. Repeat at higher NaCl concentrations until the target protein has been eluted.
8	Wash with 5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.
9	If required, perform a CIP to clean the column.
10	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.

Note: *A more rigorous CIP procedure can be required for Capto DEAE than for Capto Q and Capto S. For more details, see instructions "Capto S, Capto Q, Capto DEAE", code number 28-4074-52.*

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: *Capto Q and Capto S* 5 CV start buffer or until eluent pH and conductivity have reached the required values. *Capto DEAE* At least 10 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.*

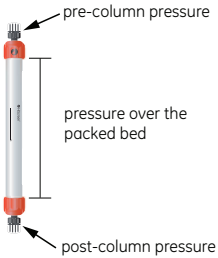

Fig 4. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor 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).

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™, ÄKTAFPLC™ 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 *total system pressure*, 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 (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

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

8 Storage

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

Capto S: Wash with 2 CV of 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

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Clean the column, see Section Cleaning-in-place (CIP).* High viscosity of solutions. *Use lower flow rate.*
Unstable pressure curve during sample loading	Air bubbles 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	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
High back pressure during CIP	Proteins precipitated in 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	*Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.*

10 Ordering information

Product	Quantity	Code No.
HiScreen Capto Q	1 × 4.7 ml	28-9269-78
HiScreen Capto S	1 × 4.7 ml	28-9269-79
HiScreen Capto DEAE	1 × 4.7 ml	28-9269-82

Related products	Quantity	Code No
HiTrap Capto Q	5 × 1 ml	11-0013-02
	5 × 5 ml	11-0013-03
HiTrap Capto S	5 × 1 ml	17-5441-22
	5 × 5 ml	17-5441-23
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
	5 × 5 ml	28-9165-40
Capto Q	25 ml	17-5316-10
	100 ml [1]	17-5316-02
Capto S	25 ml	17-5441-10
	100 ml [1]	17-5441-01
Capto DEAE	25 ml	17-5443-10
	100 ml [1]	17-5443-01
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

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

Accessories HiScreen	Quantity	Code No
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems *(For connection of columns with 1/16" fittings to ÄKTA systems)*	8	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18-1120-93
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11-0003-55

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

Related literature	Code No.
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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