

Capto™ S

Capto Q

Capto DEAE

Capto S, Capto Q and Capto DEAE are, respectively, strong cation, strong anion and weak anion exchange BioProcess™ media for capture and intermediate purification of proteins from large feed volumes by packed bed chromatography.

All Capto media provide:

- Raised productivity with high dynamic binding capacity at high flow.
- Increased yield with rapid mass transfer.
- Reduced process time with high volume throughput.
- Cost-effective processing with smaller unit operations.
- High stability for effective and rigorous CIP procedures.



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Please read these instructions carefully before using the products.

Safety

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

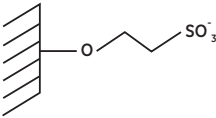
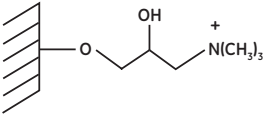
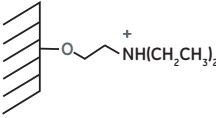
1 BioProcess chromatography media

BioProcess chromatography media are developed and supported for production scale chromatography. All BioProcess chromatography media are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of chromatography media for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess media cover all purification steps from capture to polishing.

2 Properties of Cpto S, Cpto Q and Cpto DEAE

For ion exchange chromatography, Cpto S uses a sulfonate group, Cpto Q uses a quaternary amine group and Cpto DEAE uses a diethylaminoethyl group, as shown in *Table 1*.

Table 1. The active groups of Cpto S, Cpto Q and Cpto DEAE.

Media type	Active group	Description
Cpto S		Strong cation exchange group.
Cpto Q		Strong anion exchange group.
Cpto DEAE		Weak anion exchange group.

Capto S, Capto Q and Capto DEAE are designed to increase speed, capacity, and throughput in the capture and intermediate purification of biomolecules. By offering high capacity at high flow velocities with low back pressure, process cycle times may be reduced and productivity increased.

Capto S, Capto Q and Capto DEAE are based on the same high flow agarose matrix, which is designed to give low back pressures at high flow velocities (700 cm/h in a 1 m diameter column with 20 cm bed height at < 3 bar in water). Note that the back pressure will vary with column type and medium bed height. The next figure shows the pressure/flow curve for Capto S packed in AxiChrom™ 1000.

All media have dextran surface extenders for increased capacity and fast mass transfer.

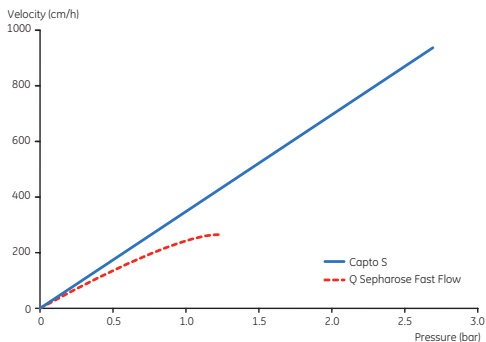


Fig 1. Pressure/flow curve for Capto S compared to Q Sepharose™ Fast Flow. *Running conditions:* AxiChrom 1000 for Capto S, Chromaflow™ 1000 for Q Sepharose Fast Flow, 20 cm packed bed, in water at 20°C. The pressure includes pressure drop from the bed and the column. System/tubing pressure is excluded.

The highly cross-linked agarose base matrix gives the media high chemical and physical stability. Characteristics such as capacity, elution behavior, and pressure/flow rate are unaffected by the solutions commonly used in process chromatography and cleaning procedures (*Table 2, Table 3 and Table 4*).

Table 2. Characteristics of Capto S.

Matrix	High flow agarose with a dextran surface extender
Ion exchange type	Strong cation, S
Charged group	-SO ₃ ⁻
Total ionic capacity	0.11 to 0.14 mmol Na ⁺ /ml medium
Particle size ¹	90 μm (d _{50v})
Flow velocity	700 cm/h in columns up to 1 m in diameter with 20 cm bed height at 20°C using process buffers with the same viscosity as water at <3 bar (0.3 MPa)
Dynamic binding capacity ²	>120 mg lysozyme/ml medium
pH stability ³	
Working range	pH 4 to 12
Cleaning-in-place	pH 3 to 14
Working temperature ⁴	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M NaOH ⁵ , 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol
Avoid	Oxidizing agents, cationic detergents, long exposure (1 week, 40°C) to pH < 3
Storage	0.2 M sodium acetate in 20% ethanol

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

² Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 minute, 600 cm/h in a Tricorn™ 5/100 column with 10 cm bed height, in 30 mM sodium phosphate buffer, pH 6.8.

³ Working range pH: pH interval where the medium can be operated without significant change in function.

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

⁴ Low temperatures can decrease the capacity of Capto S.

⁵ No significant change in dynamic binding capacity and carbon content after 1 week of storage in 1 M NaOH at 40°C.

Table 3. Characteristics of Capto Q.

Matrix	High flow agarose with a dextran surface extender
Ion exchange type	Strong anion, Q
Charged group	-N ⁺ (CH ₃) ₃
Total ionic capacity	0.16 to 0.22 mmol Cl ⁻ /ml medium
Particle size ¹	90 µm (d _{50v})
Flow velocity	700 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
Dynamic binding capacity ²	> 100 mg BSA/ml medium
pH stability ³	
Working range	pH 2 to 12
Cleaning-in-place	pH 2 to 14
Working temperature	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH ⁴ , 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol
Avoid	Oxidizing agents, anionic detergents
Storage	20% ethanol

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

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

³ Working range pH: pH interval where the medium can be operated without significant change in function.

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

⁴ No significant change in dynamic binding capacity and carbon content after 1 week of storage in 1 M NaOH at 40°C.

Table 4. Characteristics of Capto DEAE.

Matrix	High flow agarose with a dextran surface extender
Ion exchange type	Weak anion, DEAE
Charged group	$-N^+H(CH_2CH_3)_2$
Total ionic capacity	0.29 to 0.35 mmol Cl ⁻ /ml medium
Particle size ¹	90 μm (d_{50v})
Flow velocity	700 cm/h in columns up to 1 m in diameter with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa).
Dynamic binding capacity ²	> 90 mg ovalbumin/ml medium
pH stability ³	
Working range ⁴	pH 2 to 9, see titration curve in <i>Figure 2</i> .
Cleaning-in-place	pH 2 to 14
Working temperature ⁵	4°C to 30°C
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
Storage	20% ethanol

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

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

³ Working range pH: pH interval where the medium can be operated without significant change in function.

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

⁴ The recommended working pH range for DEAE ion exchangers is for most protein separations the optimal. DEAE media, although predominantly weak anion exchangers, are not possible to fully discharge by raising the pH due to a minor content of quaternized amine groups. It is therefore possible to use DEAE media at higher pH values for separations of e.g., highly charged species as nucleotides.

⁵ Low temperatures can decrease the capacity of Capto DEAE.

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

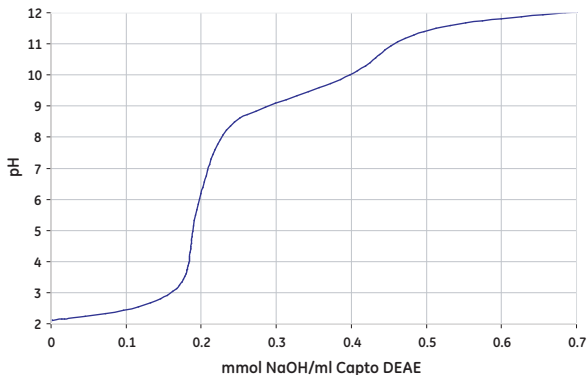


Fig 2. Titration curve for Capto DEAE. Capto DEAE has both weak and strong ion exchange properties. At pH approx. 5 most of the weak ion exchange ligands are protonated and positively charged. With increasing pH the ligands will gradually lose bound protons, and thus also charge. At a pH between 10 and 11 all weak ion exchange ligands are uncharged and only permanently positively charged quaternary ammonium groups remain.

Note: *Capto DEAE may need a larger volume and/or an equilibration buffer with higher ionic strength after CIP and/or SIP compared to Capto Q and Capto S to achieve neutral pH as it is a weak ion exchanger.*

3 Method optimization

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 the highest possible product yield and purity. Design the method in laboratory scale.

For certain proteins, dynamic binding capacities increase at increased conductivity. Therefore, we recommend scouting for optimal dynamic binding conditions on Capto S, Capto Q and Capto DEAE, for the target protein at conductivities between 2 mS/cm and 15 mS/cm, as well as scouting 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.

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

Balancing product recovery against throughput is the major consideration when optimizing a method. The dynamic binding capacity for the target protein should be determined using process feedstock. Since the dynamic binding capacity is a function of the fluid velocity applied during sample application, the breakthrough capacity must be defined over a range of different residence times to show the optimal level of throughput.

When designing a process it is also important to take the cleaning of the media into consideration. Capto DEAE can be more challenging to clean than Capto S and Capto Q, for more details, see *Chapter 7 Maintenance, on page 27*.

For more information about method development and optimization, consult the handbook: *Ion exchange Chromatography & Chromatofocusing: Principles and Methods*, (11-0004-21).

4 Scale up

Principles for scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and linear flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the linear flow velocity (cm/h) applied during sample loading.

Suggested procedure for scale-up

- 1 Select the bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.
- 2 Select a column diameter to obtain a bed height of 10 cm to 40 cm. The high rigidity of the Capto S, Capto Q and Capto DEAE base matrix allows for bed heights well above 20 cm.
- 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 system and monitoring system for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems.

5 Packing columns

Recommended columns

Table 5. Recommended columns for Capto S, Capto Q and Capto DEAE.

Column	Inner diameter (mm)	Bed volume ¹	Bed height (cm)
Lab scale			
Tricorn 5/100	5	2 ml	10
Tricorn 10/100	10	8 ml	10
HiScale™ 16/20	16	20–40 ml	max 20
HiScale 16/40	16	20–70 ml	max 35
HiScale 26/20	26	53–106 ml	max 20
HiScale 26/40	26	53–186 ml	max 35
HiScale 50/20	50	196–393 ml	max 20
HiScale 50/40	50	196–687 ml	max 35
Production scale			
AxiChrom	50–200	0.2–12.5 l	max 40
AxiChrom	300–1000	7–314 l	max 50
BPG ^{2,3}	100–300	1–28 l	max 40
Chromaflo standard ^{3,4}	400–800	12–151 l	max 30

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

² The pressure rating of BPG 450 is too low to use with Capto media.

³ Packing instructions for Capto S, Capto Q and Capto DEAE in process scale columns are described in Application Note 28-9259-32.

⁴ Larger pack stations might be required at larger diameters.

All production scale columns can be supplied as variable bed height columns. Do not choose large diameter columns if the bed height is low.

For practical instructions in good packing techniques, see the CD-ROM Column Packing - The Movie (18-1165-33).

For more details about packing HiScale columns, see instructions HiScale™ columns (16, 26, 50) and accessories (28-9674-70).

Packing Capto S and Capto Q in Tricorn columns

Packing Capto S and Capto Q in Tricorn 5/100 and 10/100 columns requires a modified methodology compared to standard procedures.

Main differences in methodology

After packing the medium:

- Attach top adapter to column tube without flow.
- Remove the stop plug and start a flow.
- Turn the adapter head down to the surface of the chromatography media.

A more detailed instruction is provided below.

Materials needed

- Capto S or Capto Q
- Plastic spoon or spatula
- Glass filter funnel
- Filtering flask
- Measuring cylinder
- 10 mM NaCl in distilled water

Equilibrate all materials to room temperature.

Calculating amount of medium

The amount of chromatography medium needed can be calculated by: column cross-sectional area (cm^2) \times bed height (cm) \times compression factor (settled bed height/packed bed height).

Washing the medium

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the medium into the funnel and wash with approximately 5 to 10 ml 10 mM NaCl per ml medium.

Preparing the packing slurry

The slurry concentration should be 40% to 60% in 10 mM NaCl, measured in a measuring cylinder after settling overnight.

Equipment needed

An ÄKTA™ system or a stand-alone pump that can deliver 20 ml/min is used. The pump filter unit and the flow restrictor should be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure.

For packing Tricorn 5/100 column

Tricorn 5/100 column, Tricorn 5/100 glass tube (used as a packing reservoir), packing connector 5-5 and bottom unit.

For packing Tricorn 10/100 column

Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector, Tricorn 10/100 glass tube (used as a packing reservoir), and bottom unit with filter holder, cap and stop plug.

When working with large volumes, real feed or repeated loading, Tricorn coarse filter kits are recommended to reduce the risk of clogging. Use Tricorn 5 Coarse Filter Kit (11-0011-53) or Tricorn 10 Coarse Filter Kit (11-0012-54).

Prepare the column

To pack the column, use 10 mM NaCl in distilled water and proceed as follows:

- 1 Rinse the column and packing tube with 10 mM NaCl.
- 2 Insert a bottom filter into the filter holder and wet the filter.
- 3 Wet the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
- 4 Insert the filter holder into the column tube. Make sure that the “keyed” part of the filter holder fits into the slot on the threaded section on the column tube. Screw the end cap onto the column tube.
- 5 Pour the packing solution into the tube and make sure that the liquid drips from the column. Insert a stop plug into the bottom unit when approximately 1 cm of packing solution remains.

- 6 Screw a suitable Tricorn packing connector onto the top of the column tube. The Tricorn packing connector must be fitted with suitable O-rings (included with the Tricorn packing connector). Screw the Tricorn packing tube into the upper fitting of the Tricorn packing connector.
- 7 Mount the column and packing unit vertically on a lab stand.

Pack the column

- 1 Fill both column tube and packing tube with slurry. Avoid formation of air bubbles in the medium by pouring it along a thin capillary.
- 2 Attach an extra bottom unit or an adapter unit to the top of the packing tube. Fill the capillary from the pump with packing solution and connect the pump to the top of the packing unit, remove the stop plug from the bottom of the column tube.
- 3 Pack the medium at 540 cm/h (1.8 ml/min in Tricorn 5/100, 7.1 ml/min in Tricorn 10/100). When the liquid above the bed is clear, continue packing for 10 min.
- 4 Pack the medium for an additional 10 min at 3000 cm/h (Tricorn 5/100: 9.8 ml/min, Tricorn 10/100: 39.3 ml/min).
- 5 Switch off the pump and connect a stop plug into the bottom unit. Remove the packing tube and packing connector. If necessary, remove excess medium with a Pasteur pipette or spatula by re-suspending the top of the packed bed. Make sure that the medium surface is as even as possible.
- 6 Add packing solution to the upper edge of the column tube.
- 7 Place a pre-wetted filter on top of the fluid in the column.
Note: *The top coarse filter is inserted by another procedure. See separate instruction included in Tricorn coarse filter kits.*
- 8 Prepare the adapter unit by screwing the guiding ring inside the adapter unit down to the lower end position.
- 9 Wet the O-ring on the adapter unit by dipping it in water, packing solution, or 20% ethanol.
- 10 Screw the guiding ring back 1.5 turns.

- 11 Mount the top adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Ensure that there are no air bubbles.

Note: *The top adapter should be connected but not fully screwed down.*

- 12 Connect the pump, remove the stop plug and start a flow (Tricorn 5/100: 1 ml/min (300 cm/h), Tricorn 10/100: 5 ml/min (380 cm/h)).
- 13 Slowly screw the adapter unit down until the filter meets the bed surface. Make sure that the filter meets the bed horizontally.
- 14 Increase the flow to 3000 cm/h (Tricorn 5/100: 9.8 ml/min, Tricorn 10/100: 39.3 ml/min).
- 15 If the bed compresses, slowly screw the adapter unit down to the surface of the chromatography medium with maintained flow.
- 16 Pack the medium for 5 min. If the bed has compressed further, screw the adapter unit down to the bed surface.
- 17 Stop the flow and connect a stop plug to the bottom unit.
- 18 Disconnect the pump. Screw the adapter unit down for a further 2/3 turns. Lock the adapter and attach a stop plug. If the adapter lock is correctly attached, it should not be possible to turn the adapter unit.

Note: *Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.*

Testing the packed column

See Chapter 6 Evaluation of column packing, on page 24.

Packing Capto DEAE in Tricorn columns

The following instructions are for packing Tricorn 5/100 and Tricorn 10/100 with 10 cm bed height. For more details about packing Tricorn columns, please see the instructions Tricorn Empty High Performance Columns or for practical instructions in good packing techniques, the CD-ROM Column Packing – The Movie, code number: 18-1165-33.

Note: *The packing procedure for Tricorn 10/100 columns is different for Capto S and Capto Q compared to Capto DEAE.*

Materials needed

- Capto Q
- Plastic spoon or spatula
- Glass filter funnel
- Filtering flask
- Measuring cylinder
- 0.2 M NaCl in 20% ethanol

Equilibrate all materials to room temperature.

Calculating amount of medium

The amount of medium needed can be calculated by: column cross-sectional area (cm^2) \times bed height (cm) \times compression factor (settled bed height/ packed bed height).

Washing the medium

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the medium into the funnel and wash with approximately 5 to 10 ml 0.2 M NaCl in 20% ethanol per ml medium.

Preparing the packing slurry

The slurry concentration should be 40% to 60% in 0.2 M NaCl in 20% ethanol, measured in a measuring cylinder after settling overnight.

Equipment needed

An ÄKTA system or a stand-alone pump that can deliver 20 ml/min is used. The pump filter unit and the flow restrictor should be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure.

For packing Tricorn 10/100 column

Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector, Tricorn 10/100 glass tube (used as a packing reservoir), and bottom unit with filter holder, cap and stop plug.

When working with large volumes, real feed or repeated loading, Tricorn coarse filter kits are recommended to reduce the risk of clogging. Use Tricorn 10 Coarse Filter Kit.

Packing procedure

To pack the column, use 0.2 M NaCl with 20% ethanol and proceed as follows:

- 1 Rinse the column and packing tube in 0.2 M NaCl in 20% ethanol.
- 2 Insert a bottom filter into the filter holder and wet the filter.
- 3 Wet the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
- 4 Insert the filter holder into the column tube. Ensure that the "keyed" part of the filter holder fits into the slot on the threaded section on the column tube. Screw the end cap onto the column tube.
- 5 Pour the packing solution into the tube and ensure that the liquid drips from the column. Insert a stop plug into the bottom unit when approximately 1 cm of packing solution remains.
- 6 Screw a suitable Tricorn packing connector onto the top of the column tube. The Tricorn packing connector must be fitted with suitable O-rings (included with the Tricorn packing connector). Screw the Tricorn packing tube into the upper fitting of the Tricorn packing connector.
- 7 Mount the column and packing unit vertically on a lab stand.

- 8 Fill both column tube and packing tube with slurry. Avoid formation of air bubbles in the medium by pouring it along a thin capillary.
- 9 Attach an extra bottom unit or an adapter unit to the top of the packing tube. Fill the capillary from the pump with packing solution and connect the pump to the top of the packing unit, remove the stop plug from the bottom of the column tube.
- 10 Pack the column at 20 ml/min for 6 minutes.
- 11 Switch off the pump and connect a stop plug into the bottom unit. Remove the packing tube and packing connector. If necessary, remove excess medium with a Pasteur pipette or spatula by re-suspending the top of the packed bed. Ensure that the surface of the chromatography medium is as even as possible.
- 12 Add packing solution to the upper edge of the column tube.
- 13 Place a pre-wetted filter on top of the fluid in the column.
Note: *The top coarse filter is inserted by another procedure. See separate instruction included in Tricorn coarse filter kits.*
- 14 Prepare the adapter unit by screwing the guiding ring inside the adapter unit down to the lower end position.
- 15 Wet the O-ring on the adapter unit by dipping it in water, packing solution, or 20% ethanol.
- 16 Screw the guiding ring back 1.5 turns.
- 17 Mount the top adapter unit onto the column tube, making sure that the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there are no air bubbles.
- 18 Screw the adapter unit down until the adapter meets the bed surface. Screw the adapter unit down a further 360 degrees so that the adapter is positioned slightly below the surface of the medium.
- 19 Connect the pump to the adapter unit. Remove the stop plug from the cap of the column tube.
- 20 Pack the column at 20 ml/min for 2 minutes.

- 21 Before switching off the pump, mark the position of the medium surface on the column with a pen.
- 22 Switch off the pump, attach the stop plug into the bottom of the column tube and disconnect the pump. Screw the adapter unit down to the marking and then turn a further 360 degrees, so that the adapter is positioned slightly below the marking.
- 23 Lock the adapter and attach a stop plug. If the adapter lock is correctly attached, it should not be possible to turn the adapter unit. The column is now ready for use or storage.

Note: *Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.*

Testing the packed column

See Chapter 6 Evaluation of column packing, on page 24.

Packing HiScale columns

Materials needed

- Capto S, Capto Q or Capto DEAE
- HiScale column
- HiScale packing tube (depending on bed height)
- Plastic spoon or spatula
- Glass filter G3
- Vacuum suction equipment
- Filter flask
- Measuring cylinder
- 20% ethanol with 0.4 M NaCl

Equipment

ÄKTA system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at a given flow (consolidated). Therefore, the compression factor (CF) has to be separated from the packing factor (PF).

L_{settled}	Bed height measured after settling by gravity.
L_{cons}	Consolidated bed height Bed height measured after settling the medium at a given flow velocity.
L_{packed}	Packed bed height
CF	Compression factor $CF = L_{\text{settled}}/L_{\text{packed}}$
PF	Packing factor $PF = L_{\text{cons}}/L_{\text{packed}}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{\text{packed}} \times A_C$
C_{slurry}	Concentration of the slurry

Preparation of the slurry

To measure the slurry concentration, let the medium settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28-9259-32. This method can also be used for HiScale columns.

Washing the medium

Mount a glass filter funnel onto a filtering flask. Suspend the medium by shaking and pour into the funnel and wash according to the following instructions:

- 5 times with 5 ml 20% ethanol with 0.4 M NaCl/ml medium.
- Gently stir with a spatula between additions.
- Move the washed medium from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.

Packing the column

Table 6. Main features of the packing method for HiScale 16/20 and HiScale 16/40.

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.10	1.10	1.06
Packing velocity (cm/h)	750	750	750
Packing flow rate (ml/min)	25	25	25
Flow condition (cm/h)	750	750	420
Flow condition (ml/min)	25	25	14

Table 7. Main features of the packing method for HiScale 26/20 and HiScale 26/40.

Column	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.10	1.06

Column	HiScale 26/20	HiScale 26/40	
Packing velocity (cm/h)	750	750	750
Packing flow rate (ml/min)	66	66	66
Flow condition (cm/h)	750	750	420
Flow condition (ml/min)	66	66	37

Table 8. Main features of the packing method for HiScale 50/20 and HiScale 50/40.

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.15	1.10
Packing velocity (cm/h)	750	750	750
Packing flow rate (ml/min)	250	250	250
Flow condition (cm/h)	750	750	420
Flow condition (ml/min)	250	250	140

Packing procedure

- 1 Assemble the column according to the column instructions (HiScale columns (16, 26, 50) and accessories, code no 28-9674-70).
- 2 Mount the column tube in a stand.
- 3 Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest if the nets are dry, but if air is trapped under the net it can be removed by a light suction with a syringe.
- 4 Mount the bottom adapter unit in the bottom of the column tube and tighten the O-ring.
- 5 Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet.
- 6 Mount the packing tube on top of the column tube.

- 7 Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing upwards as this is done. If air is trapped under the net it can be removed by a light suction with a syringe.
- 8 Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
- 9 Mount the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.
- 10 Start a downward flow with packing velocity according to *Table 6*, *Table 7* and *Table 8*.
- 11 Let the flow run until the bed has consolidated.
- 12 Use the scale on the column to measure the bed height. There might be a build up of medium at the column wall after the bed is consolidated and to make it easier see where the top of the bed is, a light source can be used.
- 13 Calculate the final bed height by dividing the consolidated bed height with the desired packing factor. $L_{\text{packed}} = L_{\text{cons}}/\text{PF}$. See *Table 6*, *Table 7* and *Table 8*.
- 14 Turn off the flow and put a stop plug in the bottom.
- 15 Remount the top adapter from the packing tube.
- 16 Over a beaker or a sink, detach the packing tube from the column.
- 17 Remount the top adapter into the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.
- 18 Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While liquid spills out through the bottom, keep turning until the calculated final bed height is reached.
- 19 Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the media.
- 20 Start a downward flow to flow condition the bed. The flow rates are shown in *Table 6*, *Table 7* and *Table 8*.

- 21 Let the flow run for about 10 column volumes. The column is ready to be tested.

Testing the packed column

See Chapter 6 Evaluation of column packing, on page 24.

6 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing should be done after packing and at regular intervals during the working life of the column, and also when separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note Column efficiency testing (28-9372-07).

Note: *The calculated plate number will vary according to the test conditions and should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)^2$$

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h , is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

d_{50v} = mean diameter of the beads (cm)

As a guideline, a value < 3 is very good.

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

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

Peak asymmetry factor calculation:

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

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 3 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

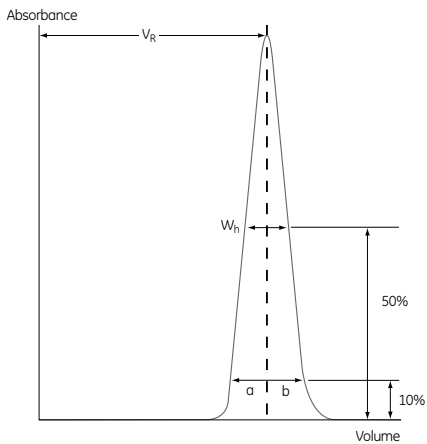


Fig 3. A typical test chromatogram showing the parameters used for HETP.

7 Maintenance

For best performance from Capto S, Capto Q and Capto DEAE, and to maximize the working life time of the media, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with equilibration buffer by washing with at least five bed volumes for Capto S and Capto Q and at least 10 bed volumes for Capto DEAE, or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with equilibration buffer until the conductivity and pH values are stable.

Note: *Capto DEAE may need a larger volume and/or an equilibration buffer with higher ionic strength after CIP and/or SIP compared to Capto Q and Capto S to achieve a neutral pH as to it is a weak ion exchanger.*

Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1–2 M NaCl in buffer). Regenerate the medium by washing with at least five bed volumes of equilibration buffer for Capto S, Capto Q and Capto DEAE, or until the column effluent shows stable conductivity and pH values.

Cleaning-In-Place

Cleaning-In-Place (CIP) is a procedure that removes contaminants such as lipids, endotoxins, and precipitated or denatured proteins that remain in the packed column after regeneration. This type of contamination occurs frequently when working with feedstock. Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain the capacity, flow properties, and general performance of Capto S, Capto Q and Capto DEAE.

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends on the nature and the condition of the feedstock, but for capture steps CIP is recommended after each cycle.

Note: *For some contaminants a more rigorous CIP procedure can be required for Capto DEAE than for Capto S and Capto Q, see CIP protocols, below.*

CIP protocols

When...	Then...
Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH at 40 cm/h with reversed flow direction. Contact time 1 to 4 h, dependent on feed. If the removal of contaminants is not satisfactory for Capto DEAE, use 1 M NaOH containing 1 M NaCl as CIP solution.
Ionically bound proteins	Wash with 0.5 to 2 column volumes (CV) of 2 M NaCl with reversed flow direction. Contact time 10 to 15 min.
Lipids and very hydrophobic proteins	Wash with 2 to 4 CV of up to 70% ethanol ¹ or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h, dependent on feed. Alternatively, wash with 2 to 4 CV of 0.1% nonionic detergent with reversed flow direction. Contact time 1 to 2 h, dependent on feed.

¹ Specific regulations may apply when using 70% ethanol, since the use of explosion-proof areas and equipment may be required.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 h is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins sanitizes the medium effectively.

Storage

Store unused medium in the container at a temperature of 4°C to 30°C. Ensure that the cap is fully tightened. Packed media and bulk media should be stored in 20% ethanol containing 0.2 M sodium acetate (Capto S) or 20% ethanol (Capto DEAE and Capto Q). After storage, equilibrate with at least five column volumes of starting buffer for Capto S and Capto Q and at least 10 bed volumes of starting buffer for Capto DEAE before use.

8 Ordering information

Quantity	Product code number		
	Capto S	Capto Q	Capto DEAE
25 ml	17-5441-10	17-5316-10	17-5443-10
100 ml	17-5441-01	17-5316-02	17-5443-01
1 l	17-5441-03	17-5316-03	17-5443-03
5 l	17-5441-04	17-5316-04	17-5443-04
10 l	17-5441-05	17-5316-05	17-5443-05
60 l	17-5441-60	17-5316-60	17-5443-60

Capto S media products are supplied in 20% ethanol containing 0.2 M sodium acetate. Capto Q and Capto DEAE media products are supplied in 20% ethanol. For additional information, including a Data File, please contact your local GE Healthcare representative.

Related products	Quantity	Product code no
PreDictor™ Capto Q, 2 µl	4 × 96-well filter plates	28-9257-73
PreDictor Capto Q, 20 µl	4 × 96-well filter plates	28-9258-06
PreDictor Capto Q, 50 µl	4 × 96-well filter plates	28-9258-07
PreDictor Capto S, 2 µl	4 × 96-well filter plates	28-9258-08
PreDictor Capto S, 20 µl	4 × 96-well filter plates	28-9258-09
PreDictor Capto S, 50 µl	4 × 96-well filter plates	28-9258-10
PreDictor Capto DEAE, 2 µl	4 × 96-well filter plates	28-9258-11
PreDictor Capto DEAE, 20 µl	4 × 96-well filter plates	28-9258-12
PreDictor Capto DEAE, 50 µl	4 × 96-well filter plates	28-9258-13
PreDictor RoboColumn™ Capto Q, 200 µl	One row of eight columns	28-9860-72
PreDictor RoboColumn Capto Q, 600 µl	One row of eight columns	28-9861-75
PreDictor RoboColumn Capto S, 200 µl	One row of eight columns	28-9860-81
PreDictor RoboColumn Capto S, 600 µl	One row of eight columns	28-9861-76
PreDictor RoboColumn Capto DEAE, 200 µl	One row of eight columns	28-9860-82
PreDictor RoboColumn Capto DEAE, 600 µl	One row of eight columns	28-9861-77
HiTrap™ Capto S	5 × 1 ml	17-5441-22
HiTrap Capto S	5 × 5 ml	17-5441-23
HiTrap Capto Q	5 × 1 ml	11-0013-02
HiTrap Capto Q	5 × 5 ml	11-0013-03
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
HiTrap Capto DEAE	5 × 5 ml	28-9165-40
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
Tricorn 5/100 column	1	18-1163-10
Tricorn 10/100 column	1	18-1163-15
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

Related literature		Product code no
Movie	Column Packing – The Movie	18-1165-33
Handbook	Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Data file	Capto S, Capto Q and Capto DEAE	11-0025-76
Application notes	High productivity capture of α -chymotrypsin on Capto S cation exchange	28-4078-15
	Screening and optimization of the loading conditions on Capto S	28-4078-16
	Capto S cation exchanger for post-Protein A purification of monoclonal antibodies	28-4078-17
	High-productivity capture of Green Fluorescent Protein on Capto Q	11-0026-20
	Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates	28-9258-40
	Process-scale purification of monoclonal antibodies - polishing using Capto Q	28-9037-16
	Purification of a monoclonal antibody using ReadyToProcess columns	28-9198-56
	Use of Capto ViralQ for the removal of genomic DNA from influenza virus produced in MDCK cells	28-9769-69
	Two-step purification of monoclonal IgG1 from CHO cell culture supernatant	28-9078-92
	Methods for packing Capto S, Capto Q and Capto DEAE in production-scale columns	28-9259-32
Instructions	Tricorn Empty High Performance Columns	28-4094-88
	HiScale columns (16, 26, 50) and accessories	28-9674-70

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