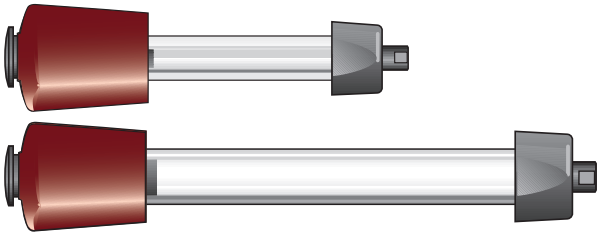
29275878 **Capto** HiRes Q 5/50
29275881 **Capto** HiRes Q 10/100
29275877 **Capto** HiRes S 5/50
29275879 **Capto** HiRes S 10/100

Instructions for Use



Read these instructions carefully before using the chromatography columns.

Intended use

Capto™ HiRes Q 5/50, Capto HiRes Q 10/100, Capto HiRes S 5/50, and Capto HiRes S 10/100 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 products in a safe way, refer to the *Safety Data Sheet*.

Product description

Capto HiRes Q and Capto HiRes S are available in Tricorn™ 5/50 and Tricorn 10/100 columns. The columns are prepacked glass columns for high resolution ion exchange chromatography of proteins, peptides, polynucleotides, and other biomolecules.

The columns are sealed with one 1/16" male stop plug and a Storage/Shipping Device at delivery. One extra 1/16" male stop plug, and two fingertight 1/16" male connectors for connecting to ÄKTA™ or other systems are also supplied.

Table 1. Resin data

	Capto HiRes Q	Capto HiRes S
Type of exchanger	Strong anion	Strong cation
Charged group	$-CH_2-N^+(CH_3)_3$	$-CH_2-SO_3^-$
Ionic capacity	~ 0.23 mmol Cl^-/mL resin	~ 0.12 mmol H^+/mL resin
Storage solution	20% ethanol	20% ethanol + 0.2 M sodium acetate

	Capto HiRes Q	Capto HiRes S
Matrix	Highly cross-linked agarose, spherical	
Particle size, d_{50V}[1]	~ 9 µm	
pH stability		
operational[2]	2 to 12	
CIP[3]	1 to 14	
pH ligand fully charged	Entire operational pH range	
Temperature		
operating	4°C to 40°C	
storage	4°C to 40°C	

[1] Median particle size of the cumulative volume distribution.

[2] pH range where resin can be operated without significant change in function.

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

Table 2. Column data

	5/50 column	10/100 column
Bed dimensions	5 mm × 50 mm	10 mm × 100 mm
Column volume	1 mL	8 mL
Average loading capacity[1]	50 mg	400 mg
Flow rate[2]		
recommended	0.5 to 2.0 mL/min	0.5 to 2.0 mL/min
maximum	2.0 mL/min	3.0 mL/min
Column hardware pressure limit	10 MPa	5 MPa
Maximum pressure over packed bed	4.0 MPa, (40 bar, 580 psi)	

[1] Sample dependent. For highest resolution, do not load more than 20% of the loading capacity.

[2] Recommended flow rates at room temperature for solutions with viscosity similar to water. Decrease the flow rate for solvents with higher viscosity like 20% ethanol or at lower temperature.

System recommendations

Capto HiRes Q and Capto HiRes S columns can be used on lab chromatography systems, for example ÄKTA and HPLC systems.

Setting column pressure limits

It is important to use correct settings for the pressure alarms to protect column hardware and packed bed of chromatography resin against too high pressures.

To protect the column hardware

The back pressure generated by the column together with the system flow path after the column affects the column hardware. The column hardware pressure limit is the maximum pressure the hardware can withstand without being damaged. This value is fixed for each column type.

To protect the packed bed

The maximum flow rate is the maximum flow rate that the packed bed of chromatography resin can withstand without risking gap formation or bed collapse to occur. The maximum flow rate is lower when working at low temperature or with viscous solutions.

The maximum pressure limit for Δp is the maximum pressure drop over the packed bed.

Note: *To protect the packed bed, adjust the flow rate according to running conditions.*

For a system with multiple pressure sensors

The pressure alarm for precolumn pressure, **p1** (see Figure 1) should be set to the column hardware limit. This is affected by the pressure generated by the column plus the system flow path after the column. The pressure alarm for **Δp** should be set to the maximum pressure over the packed bed. The relationship between the pressures is:

Δp = p1 - p2

where **p1** is the pressure generated by the column and the flow path after the column), and **p2** is the pressure generated only by the flow path after the column.

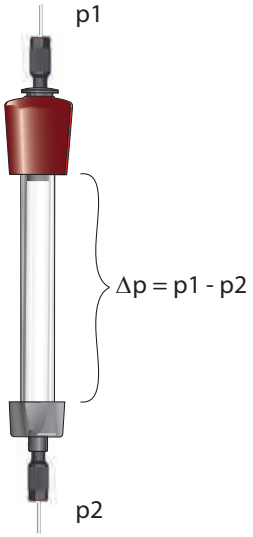


Fig 1. Δp is the pressure that affects the chromatography resin within the column. Maximum delta column pressure is defined as the pressure drop over the packed bed at maximum flow rate. Exceeding these limits can cause a gap formation or collapse of the packed bed.

For a system that measures the pressure only at the system pump

The total pressure is affected by the pressure generated by the column plus the pressure generated by the system flow path before and after the column:

Pressure limit = system pressure + column pressure limit

The system pressure is generated by all system flow paths. The column pressure limit is the maximum pressure over the packed bed.

For maximum pressure over the packed bed, see *Table 2, on page 1*. Determine the system pressure without column under running conditions at intended flow rate.

Note: *When using a high-pressure system like HPLC or a system with narrow tubing, the high system pressure will contribute significantly to the system pressure and must be considered when setting the pressure limit for the system pressure.*

Note: *The total pressure limit should never exceed the column hardware pressure limit.*

First-time use

Connect column to a system

Make sure that the top adapter is locked (locking ring pressed down, see Figure 2).

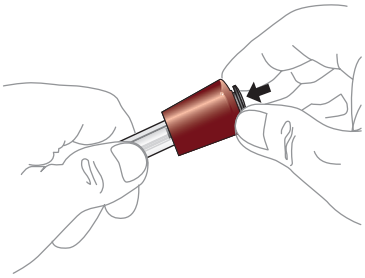


Fig 2. Illustration of how to lock the top adapter. The locking ring (black) must be in the down-position to prevent uncontrolled adjustment of the column bed height.

Make sure there is no air in the tubing and valves. Remove the stop plug and the Tricorn Storage/Shipping Device, see Section *Delivery/storage, on page 4*. Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system.

Note: *Buffer solution, column, and system should be kept at the same temperature during operation.*

To maintain the performance of Capto HiRes Q and S columns, there are two limits to consider: the maximum pressure limit and the maximum flow limit.

NOTICE
Do not exceed the column pressure limit (4.0 MPa). Do not exceed the maximum flow rate (2.0 mL/min for a 5/50 column and 3.0 mL/min for a 10/100 column). Decrease the flow rate at low temperatures, or when 20% ethanol or other viscous solutions are used.

Equilibrate the column

For first-time use or after long-term storage, equilibrate the column as described below. The flow rates are suitable at room temperature, decrease by half when working in a cold room.

Capto HiRes Q and Capto HiRes S 5/50 columns:

1. 5 column volumes (CV) of distilled water at 0.5 mL/min.
2. 5 CV binding buffer at 1 mL/min.
3. 5 CV elution buffer at 1 mL/min.
4. 5 CV binding buffer at 1 mL/min.

Capto HiRes Q and Capto HiRes S 10/100 columns:

1. 5 column volumes (CV) of distilled water at 1 mL/min.
2. 5 CV binding buffer at 2 mL/min.
3. 5 CV elution buffer at 2 mL/min.
4. 5 CV binding buffer at 2 mL/min.

For optimization of the separation, see Section *Optimization, on page 5*.

Suggested conditions

If the running conditions are not developed, try these first:

Capto HiRes Q	Binding buffer:	20 mM Tris-HCl, pH 8.0
	Elution buffer:	20 mM Tris-HCl + 1.0 M NaCl, pH 8.0
Capto HiRes S	Binding buffer:	20 mM MES[1], pH 6.0
	Elution buffer:	20 mM MES + 1.0 M NaCl, pH 6.0

[1] 2-(N-morpholino)ethne-sulfonic acid

Separation by gradient elution

See *Table 2, on page 1* for recommended flow rates.

Step	Action
1	Equilibrate the column with 5 column volumes (CV) of binding buffer, or until UV baseline, pH, and conductivity are stable.
2	Adjust the sample to the chosen starting pH and ionic strength and apply to the column (see Section Sample recommendations).
3	Wash with 5 CV binding buffer, or until the UV baseline, pH, and conductivity are stable, that is, when all unbound material has been washed out of the column.
4	Elute using a gradient volume of 10 to 20 CV and an increasing ionic strength up to 0.5 M NaCl.
5	Wash with 2 to 5 CV 1 M NaCl to elute any remaining ionically bound material.
6	Equilibrate with at least 5 CV binding buffer, or until the UV baseline, pH, and conductivity are stable.

For optimization of the separation, see Section *Optimization, on page 5*.

Sample recommendations

Net charge of target molecule	Capto HiRes Q	Capto HiRes S
	Negative	Positive
Recommended sample load	Up to 20% of loading capacity, for optimal resolution.	

Preparation

Dilute the sample in binding buffer or perform buffer exchange using a desalting column. Filter through a 0.22 µm filter or centrifuge at 10 000 × g for 10 min.

Buffers and solvent resistance

Degas and filter all solutions through a 0.22 µm filter. Install an in-line filter before the injection valve.

Note: *Buffers and solvents with high viscosity will increase the back pressure. Reduce the flow rate accordingly.*

Operational stability

Operational stability refers to use where the resin is stable over a long period of time without significant change in chromatographic performance.

Capto HiRes Q and Capto HiRes S are stable in:

- All commonly used aqueous buffers, pH 2 to 12
- Urea, up to 8 M
- Guanidine hydrochloride, up to 6 M
- Acetonitrile, up to 30% in aqueous buffers
- Nonionic detergents
- Cationic detergents (Capto HiRes Q)
- Anionic detergents (Capto HiRes S)

Cleaning stability

Cleaning stability refers to the use during regeneration, cleaning-inplace, and sanitization procedures.

Capto HiRes Q and Capto HiRes S are stable in:

- Acetonitrile, up to 30%
- Sodium hydroxide, up to 1 M
- Ethanol, up to 70%
- Methanol, up to 70%
- Acetic acid, up to 1 M
- Isopropanol, up to 30%
- Hydrochloric acid, up to 0.1 M

Avoid

- Oxidizing agents
- Anionic detergents (Capto HiRes Q)
- Cationic detergents (Capto HiRes S)

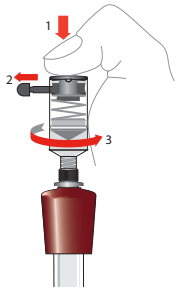
Delivery/storage

The column is delivered in storage solution, see *Table 1, on page 1*. For column storage, wash with 5 column volumes (CV) of distilled water, followed by 5 CV thoroughly degassed storage solution.

Note: *Apply the storage solution at a low flow rate due to the higher viscosity. Do not exceed the pressure limit or the maximum flow rate.*

Note: *Make sure that the column is sealed properly to prevent drying out. Do not freeze.*

The column is sealed with a Tricorn Storage/Shipping Device that prevents the column from drying out. For column storage, connect the device as described below.



How to remove the storage/shipping device

1. Push down the spring-loaded cap (1).
2. Remove the locking pin (2).
3. Release the cap and unscrew the device (3).

How to refill the storage/shipping device

1. Connect a syringe or pump to the storage/shipping device and fill with storage solution over the mark on the device. Remove the syringe or pump connection.
2. Tap out air bubbles and push the plunger to the mark on the device.

How to connect the storage/shipping device

1. Seal the bottom unit of the column with a stop plug.
2. Fill the column inlet and luer connector with storage solution and connect the filled Storage/Shipping Device drop-todrop to the top of the column (1).
3. Attach the spring-loaded cap (2) and secure it with the locking pin (3).

Choice of buffers

Choose the binding buffer pH so that substances to be bound to the ion exchanger are charged, that is at least 1 pH unit above the isoelectric point (pI) for anion exchangers and at least 1 pH unit below the pI for cation exchangers. Figure 3 and Figure 4 shows a selection of standard aqueous buffers.

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select buffering ions of the same charge as the substituent groups on the ion exchanger.

Table 3 lists suggested volatile buffers that can be used for purification of material for mass spectrometry or freeze drying.

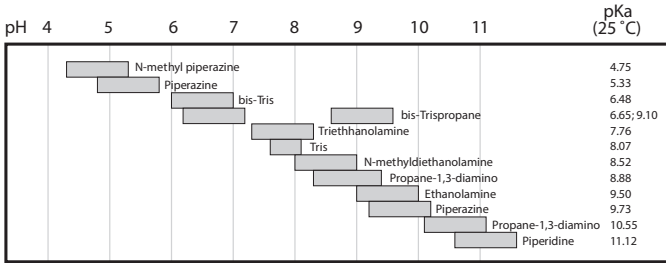


Fig 3. Recommended buffers for anion exchange chromatography.

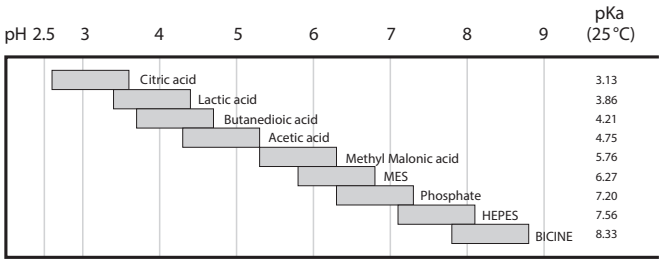


Fig 4. Recommended buffers for cation exchange chromatography.

Table 3. Volatile buffer systems

pH interval		Buffer system
3.3 to 4.3	4.8 to 5.8	Pyridine/formic acid
3.3 to 4.3	9.3 to 10.3	Trimethylamine/formic acid
4.3 to 5.8		Pyridine/acetic acid
3.3 to 4.3	8.8 to 9.8	Ammonia/formic acid
4.3 to 5.3	8.8 to 9.8	Ammonia/acetic acid
5.9 to 6.9	9.3 to 10.3	Trimethylamine/carbonate
5.9 to 6.9	8.8 to 9.8	Ammonium carbonate/ ammonia
4.3 to 5.3	7.2 to 8.2	N-ethylmorpholine/acetate

Optimization

Perform a first run as described in Section Separation by gradient elution. For optimization of the separation, see *Table 4*.

Table 4. Optimization

Action	Effect
Decrease sample load	Improved resolution.
Decrease flow rate	Improved resolution.
Change gradient slope	Shallower gradients improve selectivity, but broaden peaks (decrease efficiency). A steeper gradient will sharpen peaks but move them closer together.
Change pH/buffer	Changes selectivity, gives weaker/stronger binding (see Figure 3 and Figure 4 for buffers)
Change salt, counter ions and/or co-ions	Changes selectivity.
pH elution	Elution by pH-gradient gives different selectivity compared to salt elution.

For more information, refer to the *Handbook Ion Exchange Chromatography, Principles & Methods*.

Cleaning-in-place (CIP)

Perform the following regular cleaning cycle after 10 to 20 separation cycles, or when needed.

Note: *Reverse the flow during CIP to prevent the contaminants from passing through the entire length of the column.*

Note: *For storage after CIP, equilibrate the column to neutral pH with a neutral buffer before transferring to 20% ethanol.*

Note: *Never exceed the column pressure limit.*

Regular cleaning

Flow rate at room temperature:

0.5 mL/min for 5/50 columns

2.0 mL/min for 10/100 columns

1. Wash with 2 column volumes (CV) of 2 M NaCl.
2. Wash with 4 CV 1 M NaOH.
3. Wash with 2 CV 2 M NaCl.
4. Rinse with 2 CV distilled water.
5. Wash with at least 4 CV binding buffer or storage solution until pH and conductivity values are stable.

Rigorous cleaning

Flow rate at room temperature:

0.25 mL/min for 5/50 columns

1.0 mL/min for 10/100 columns

Remove strongly hydrophobically bound proteins, lipoproteins, and lipids by washing with 4 CV 30% isopropanol or 70% ethanol.

CAUTION
Specific regulations may apply when using 70% ethanol or 30% isopropanol since it can require the use of explosion-proof areas and equipment.

Remove precipitated proteins by washing the column with 2 CV 6 M guanidine hydrochloride.

Choose an appropriate cleaning solution depending on the nature of the contaminant, see Section Buffers and solvent resistance. After cleaning the column: wash with at least 2 CV distilled water, and 4 CV binding buffer or storage solution. For more information on how to clean your column, refer to *Handbook Ion Exchange Chromatography, Principles & Methods*.

Replacing the top filter

If column performance is not restored after rigorous cleaning, consider replacing the top filter. Since contaminants are introduced with the liquid flow, many of them are caught by the filter which might be clogged. Instructions for replacing the filter are supplied with Tricorn Filter Kit (see Section *Ordering information, on page 7*). Clean the column after filter change according to the procedure described in Section *Regular cleaning*.

Column performance control

It is recommended to run an initial function test on a new column. Check column performance at regular intervals and whenever you suspect a problem, and compare the resulting chromatogram with the initial run under the same conditions.

Figures 5 to 7 show typical separations of proteins on Capto HiRes Q and Capto HiRes S columns.

Functional test of Capto HiRes Q 5/50

Sample:	1. Apotransferrin, human (1.1 mg/mL) 2. α-Lactalbumin, bovine milk (0.6 mg/mL) 3. β-Lactoblobulin, bovine milk (2.2 mg/mL)[1] 4. Amyloglucosidase, Aspergillus niger (1.1 mg/mL)
Sample volume:	500 µL
Sample load:	2.5 mg
Gradient:	0 to 50% elution buffer in 20 CV
Binding buffer:	20 mM Tris-HCl, pH 8.0
Elution buffer:	20 mM Tris-HCl + 1 M NaCl, pH 8.0
Flow rate:	1.0 mL/min (room temperature)

[1] Contains β-Lactoglobulins A and B

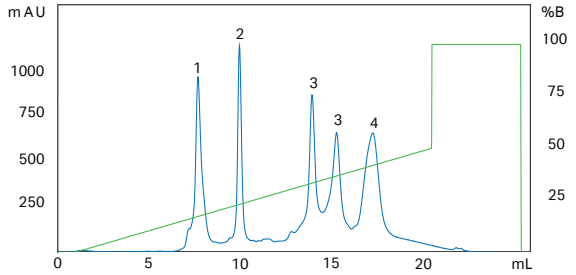


Fig 5. Typical chromatogram from a functional test of Capto HiRes Q 5/50.

Functional test of Capto HiRes S 5/50 and HiRes S 10/100

Sample:	1. Ribonuclease A, bovine pancreas (2.1 mg/mL) 2. α-Chymotrypsinogen A, bovine pancreas (0.7 mg/mL) 3. Cytochrome C, horse heart (1.1 mg/mL) 4. Lysozyme, chicken egg white (1.1 mg/mL)	
Sample volume:	5/50 column: 100 µL	10/100 column: 800 µL
Sample load:	5/50 column: 0.5 mg	10/100 column: 4.0 mg
Gradient:	0 to 50% elution buffer in 20 CV	
Binding buffer:	20 mM phosphate, pH 6.8	
Elution buffer:	20 mM phosphate + 1 M NaCl, pH 6.8	
Flow rate:	1.0 mL/min (5/50 column) 2.0 mL/min (10/100 column) (room temperature)	

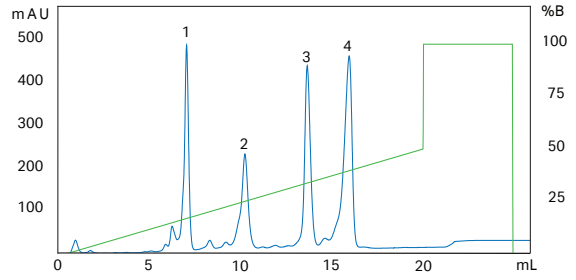


Fig 6. Typical chromatogram from a functional test of Capto HiRes S 5/50.

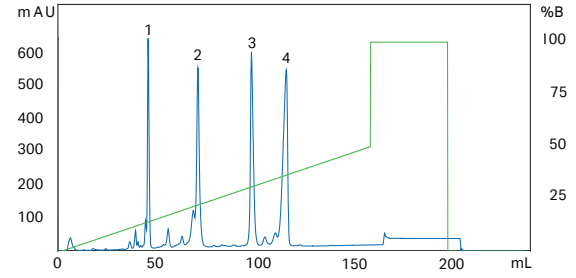


Fig 7. Typical chromatogram from a functional test of Capto HiRes S 10/100.

Troubleshooting

Situation	Possible cause / Corrective action
Proteins of interest not sufficiently resolved.	Check required conditions. Check pH and ionic strength. Decrease sample load, use a shallower gradient, or reduce flow rate (see Section *Optimization, on page 5*).
Target molecule elutes during application/ wash.	**Concentration of salt in sample is too high:** Dilute the sample in binding buffer or perform buffer exchange. **Target molecule not properly charged:** Increase pH for Capto HiRes Q. Decrease pH for Capto HiRes S. **Equilibration not sufficient:** Extend column equilibration.
Target molecule elutes late or not at all.	Increase salt concentration in elution buffer. Check pH conditions. Use a buffer pH closer to pI of the target molecule.
Target molecule precipitates on the column.	Check pH and salt conditions, adjust to improve sample solubility. Use gradient elution.
Pressure alarm above maximum limit even at low flow rate.	Consider the system pressure when using high pressure systems like HPLC, see Section Pressure limit in In-depth information.
Increased back pressure over the column.	Confirm that the column is the cause. If so, reverse the flow direction and wash with elution buffer at a low flow rate. Return to normal flow direction and run for 5 minutes at recommended flow rate. If the back pressure is still high, clean the column according to the procedure described in Section *Regular cleaning, on page 5*.
Loss of resolution and/or decreased sample recovery.	Reverse the flow direction and clean the column according to the procedure described in Section *Rigorous cleaning, on page 5*.
High back pressure, loss of resolution even after rigorous cleaning.	**The top filter might be clogged.** Follow the instruction for Tricorn Filter Kit to replace the top filter, see *Ordering information, on page 7*.

Situation	Possible cause / Corrective action
Air bubbles in the packed bed.	Small amounts of air will normally not affect the performance of the column. Degas buffers thoroughly. Remove small air bubbles with 3 to 4 column volumes of well degassed buffer in reversed direction at a low flow rate.

Ordering information

Product	Quantity	Product code
Capto HiRes Q 5/50	1	29275878
Capto HiRes Q 10/100	1	29275881
Capto HiRes S 5/50	1	29275877
Capto HiRes S 10/100	1	29275879

Accessories

Product	Quantity	Product code
Tricorn 10 Filter Kit	1	29053612
Tricorn 5 Filter Kit	1	29053586
Filter tool	1	18115320
Fingertight connector, 1/16" male	10	18111255
Tricorn storage/shipping device	1	18117643

Literature

Document	Product code
Handbook: Ion Exchange Chromatography, Principles and Methods	11000421

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