Instructions 28-9372-10 AC

HiTrap™ Capto™ IEX Selection Kit

HiTrap Capto Q, 1 ml

HiTrap Capto S, 1 ml

HiTrap Capto DEAE, 1 ml

HiTrap Capto adhere, 1 ml

HiTrap Capto MMC, 1 ml

HiTrap Capto IEX Selection Kit includes five HiTrap 1 ml columns prepacked with different BioProcess™ chromatography media: Capto Q, Capto S, Capto DEAE, Capto adhere and Capto MMC. The kit is excellent for screening of method parameters such as selectivity, binding and elution conditions, as well as for small scale purifications. After choosing the optimal medium, prepacked columns and bulk media are available for larger scale preparative purifications.

Capto Q, Capto S and Capto DEAE are traditional ion exchange media for capture and intermediate purification of proteins. Capto adhere and Capto MMC are multimodal ion exchangers with a different type of selectivity due to the contribution of hydrogen bonding and hydrophobic interaction. In addition Capto MMC tolerates high salt concentrations during binding.

Table of contents

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap 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

HiTrap column characteristics

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

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

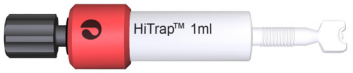


Fig 1. HiTrap, 1 ml column.

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

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

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)
Maximum flow rates[1]	4 ml/min
Recommended flow rates	1 ml/min

[1] Water at room temperature.

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

Supplied Connector kit with HiTrap column

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

Media characteristics

Capto Q is a strong anion exchanger (quaternary amine group).

Capto S is a strong cation exchanger (sulfoethyl group).

Capto DEAE is a weak anion exchanger (diethylaminoethyl group).

Capto adhere is a strong anion exchanger with multimodal functionality. The multimodal functionality gives a different selectivity compared to traditional anion exchangers. Capto adhere is designed for post protein A purification of monoclonal antibodies where the antibodies pass through the column while the contaminants are bound. Capto adhere can also be used for general purification where the different selectivity is utilized.

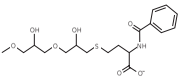
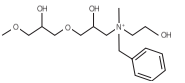
Capto MMC is a salt-tolerant weak cation exchanger with multimodal functionality. The multimodal functionality gives a different selectivity compared to other cation exchangers and also the possibility of binding proteins at high salt conditions.

High flow agarose matrix

The ligands, Q, S, DEAE, adhere and MMC, are coupled to a chemically modified, high flow agarose matrix. The high flow agarose matrix provides particle rigidity without compromising the pore size. In addition, dextran surface extenders coat the agarose matrix for Capto Q, Capto S and Capto DEAE. The combination allows for fast mass transfer, resulting in high dynamic binding capacities at high flow rates. This makes the media suitable for scale up to high volume process applications.

See Table 2 for characteristics of the different Capto IEX chromatography media.

Table 2. Characteristics of Capto IEX media

	Capto Q	Capto S	Capto DEAE	Capto MMC	Capto adhere
Matrix	High flow agarose with a dextran surface extender			Highly cross-linked agarose	
Particle size, d_{50v}[1]	90 µm	90 µm	90 µm	75 µm	75 µm
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	0.07 to 0.09 mmol H^+/ ml medium	0.09 to 0.12 mmol Cl^-/ ml medium
Dynamic binding capacity	> 100 mg BSA/ ml medium[2]	> 120 mg lysozyme/ ml medium[2]	> 90 mg ovalbumin/ ml medium[2]	> 45 mg BSA/ml medium at 30 mS/cm[3]	Not available
Recommended fluid velocity	700 cm/h in a 1 m column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)			At least 600 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)	
pH stability[4]					
short term	2 to 14	3 to 14	2 to 14	2 to 14	2 to 14
long term	2 to 12	4 to 12	2 to 12	2 to 12	3 to 12
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide[5], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol				
Storage	20% ethanol	20% ethanol, 0.2 M NaAc	20% ethanol	20% ethanol	20% ethanol

[1] d_{50v} is the medium 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 (Capto Q and Capto DEAE), or in a 30 mM sodium phosphate buffer, pH 6.8 (Capto S).

[3] Dynamic binding capacity at 10% breakthrough as measured at a residence time of 2 minutes, 300 cm/h in a Tricorn 5/100 column with 10 cm bed height in 50 mM sodium acetate, 250 mM NaCl, pH 4.75.

[4] *Short term pH:* pH interval where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function. *Long term pH:* pH interval where the medium can be operated without significant change in function.

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

2 Selection of ion exchanger

Ion exchange chromatography is based on the binding of charged sample molecules to oppositely charged groups attached to an insoluble matrix. Substances are bound to ion exchangers when they carry a net charge opposite to that of the ion exchanger. This binding is electrostatic and reversible. The pH value at which a biomolecule carries no net charge is called the isoelectric point (pI). When exposed to a pH below its pI, the biomolecule will carry a positive net charge and will bind to a cation exchanger (Capto S). At pH's above its pI the biomolecule will carry a negative net charge and will bind to an anion exchanger (Capto Q and Capto DEAE). Due to the multimodal properties of Capto adhere and Capto MMC binding of the biomolecule can occur on both sides of its pI (Fig 2).

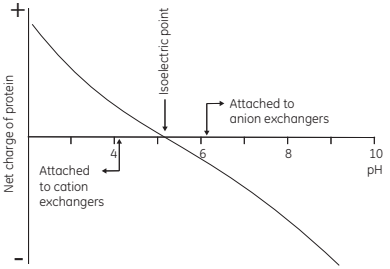


Fig 2. The net charge of a protein as a function of pH.

If the sample components are most stable below their pI's, a cation exchanger should be used and if they are most stable above their pI's, an anion exchanger is used. If stability is high over a wide pH range on both side of the pI, either type of ion exchanger can be used.

Weak ion exchangers have a limited pH working range. Information on the pI and how the net charge on the molecule varies with pH gives valuable information regarding the choice of starting conditions. Electrophoretic titration curves enable the determination of the charge/pH relationship for the molecules present across the pH range of interest.

3 Selection of buffer pH and ionic strength

Buffer pH and ionic strength are critical for the binding and elution of material (both target substances and contaminants) in ion exchange chromatography. Selection of appropriate pH and ionic strength for the start and elution buffers is essential for optimal performance.

Start buffer

The concentration of buffer required for efficient pH control varies with the buffer system. A list of suitable buffers and suggested starting concentrations is shown in Table 3 and Table 4. In the majority of cases a concentration of at least 20 mM is required to ensure adequate buffering capacity. The ionic strength of the buffer should be kept low (< 5 mS/cm) so as not to interfere with sample binding (this is not valid for Capto MMC which tolerates high salt concentrations during binding). Salts also play a role in stabilizing protein structures in solution and it is important that the ionic strength is high enough to prevent protein denaturation or precipitation.

The buffering ion should carry the same charge as the ion exchange group and should have a pKa within 0.5 pH units of the pH used in the separation. Buffering ions of opposite charge may take part in the ion exchange process and cause local disturbances in pH.

Starting pH

Cation exchangers (S)

At least 0.5 to 1 pH unit below the pI of substance to be bound.

Anion exchangers (Q, DEAE)

At least 0.5 to 1 pH unit above the pI of substance to be bound.

Multimodal ion exchangers (MMC, adhere)

At least 0.5 to 1 pH unit away from the pI of the substance to be bound.

Table 3. Recommended buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C)[1]
4.3–5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8–5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5–6.5	L-Histidine	20	Cl^-	6.04
6.0–7.0	bis-Tris	20	Cl^-	6.48
6.2–7.2; 8.6–9.6	bis-Tris propane	20	Cl^-	6.65; 9.10
7.3–8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6–8.6	Tris	20	Cl^-	8.07
8.0–9.0	N-Methyldiethanol-amine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyldiethanol-amine	50	Cl^- or CH_3COO^-	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl^-	8.88
8.4–9.4	Propane 1,3-Diamino	20	Cl^-	8.88
9.0–10.0	Ethanolamine	20	Cl^-	9.50
9.2–10.2	Piperazine	20	Cl^-	9.73
10.0–11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6–11.6	Piperidine	20	Cl^-	11.12

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

Table 4. Recommended buffers for cation exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C)[1]
1.4–2.4	Maleic acid	20	Na^+	1.92
2.6–3.6	Methyl malonic acid	20	Na^+ or Li^+	3.07
2.6–3.6	Citric acid	20	Na^+	3.13
3.3–4.3	Lactic acid	50	Na^+	3.86
3.3–4.3	Formic acid	50	Na^+ or Li^+	3.75
3.7–4.7; 5.1–6.1	Succinic acid	50	Na^+	4.21; 5.64
4.3–5.3	Acetic acid	50	Na^+ or Li^+	4.75
5.2–6.2	Methyl malonic acid	50	Na^+ or Li^+	5.76
5.6–6.6	MES	50	Na^+ or Li^+	6.27
6.7–7.7	Phosphate	50	Na^+	7.20
7.0–8.0	HEPES	50	Na^+ or Li^+	7.56
7.8–8.8	BICINE	50	Na^+	8.33

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

4 Optimizing

Capto Q, Capto S and Capto DEAE are traditional ion exchangers where the elution of the target protein most often is performed by increasing the salt concentration in the binding buffer. The ion exchangers Capto adhere and Capto MMC have multimodal properties where elution is performed by changing the pH or by a combination of changes in pH, buffer concentration and eluting salt (see below). The multimodal ion exchangers offer a different type of selectivity which, depending on sample, may change the separation compared to traditional ion exchangers.

Linear gradient elution is recommended to be used for method development or when starting with an unknown sample. The results obtained can then serve as a basis from which to optimize the separation.

Note: *HiTrap columns are best suited for initial screening of binding and elution conditions. If scale up is considered, further optimization, as for example gradient slope, is preferably done on a larger column using the final bed height*.

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

Optimizing binding and elution conditions for Capto Q, Capto S and Capto DEAE

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 above the isoelectric point for Capto Q and Capto DEAE and 0.5 to 1 pH unit below pI for Capto S.

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 5 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.

Linear ionic strength gradients are recommended for optimizing the elution. The elution buffer is often the binding buffer + 1 M NaCl.

Linear ionic strength gradients are easy to prepare and very reproducible when generated by a suitable chromatography system.

Optimizing binding and elution conditions for Capto adhere and Capto MMC

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 for example, 0.5 to 1 pH unit away from the isoelectric point. Binding can occur on both sides of pI depending on the nature of the multimodal ion exchangers. Since Capto MMC allows binding at high conductivity, it may not be necessary to screen for optimal loading conductivity with respect to binding capacity. However, binding selectivity may still be affected by the loading conductivity.

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

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

Screen for optimal elution pH by using buffer at a pH of 0.5, 1.5 and 2.5 above the pI of the target protein for Capto MMC and below the pI of the target protein for Capto adhere. Use step elution or a pH gradient. Use the pH where the target molecule starts to elute as a basis if further optimization is needed in order to increase recovery.

- If required, continue with screening for eluting salt concentration using 0.5, 1.0, and 1.5 M salt at the earlier determined pH.

- If further optimization is needed, increase the concentration of buffering salt in elution buffer e.g., from 50 mM to 250 mM.
- Additives such as urea and organic modifiers can further increase the recovery of some proteins as hydrogen bonding is one of the binding mechanisms.

Note: *Design of Experiments (DoE) is a particularly useful optimization tool for multimodal ion exchangers. For more information on how to optimize the elution protocol see Application Note "Optimizing elution conditions on Capto MMC using Design of Experiments" (11-0035-48).*

Further optimization

The recommendations given above will give a sound basis for developing an efficient purification step. Details of how flow rate, sample loading and elution scheme may be optimized to meet the special needs can be found in the Handbook, *Ion Exchange Chromatography & Chromatofocusing, Principles and Methods*, see "Ordering information".

Automated buffer preparation

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

5 Operation

Prepare buffers

Table 3 and Table 4 show suitable buffers for anion and cation exchangers and suggested starting concentrations, see Section 3 for details. Try the following buffers for samples with unknown charge properties.

Anion exchange (Capto Q and Capto DEAE)

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

Cation exchange (Capto 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

Multimodal cation exchange (Capto MMC)

Start buffer: 25 mM sodium acetate, pH 4.5
Elution buffer: 25 mM phosphate buffer, 1 M NaCl, pH 7.5

Multimodal anion exchange (Capto adhere)

Start buffer: 0.5-1 pH unit away from pI.
Elution buffer: 0.5-2.5 pH units below pI (NaCl may be included).

Note: *Water and chemicals used for buffer preparation should be of high purity.*

Prepare the sample

Adjust the sample to the composition of the start buffer (pH and conductivity), using one of following methods:

- Dilute the sample with start buffer.
- Exchange buffer using for example HiPrep™ 26/10 Desalting or HiTrap Desalting columns (see Table 5).

Clarification of the sample by filtering or centrifugation is not necessary. HiTrap Capto columns are designed with a top filter in the column allowing unclarified samples to be loaded directly on the column.

Table 5. Prepacked columns for desalting and buffer exchange.

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

Example of lysis procedure

The following lysis procedure is aimed to get the pH adjusted sample, sufficiently homogenized to be loaded directly to the column without prior clarification. The protocol below has been used successfully in our own laboratories, but other established procedures may also work.

1. Dilution of cell paste: Add 5 to 10 ml of start buffer for each gram of cell paste.
2. Enzymatic lysis: 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 min at room temperature or 4°C depending on the sensitivity of the target protein.
3. Mechanical lysis: Sonication on ice, approximately 10 min or homogenization with a French press or other homogenizer or freeze/thaw, repeated at least 5 times.

   Mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the column and back pressure problems). Different proteins have different sensitivity to cell lysis and care must be taken to avoid frothing and overheating of the sample.
4. Adjust the pH of the lysate. Do not use strong bases or acids for pH-adjustment (precipitation risk). Apply the unclarified lysate on the equilibrated column directly after preparation (see Purification protocol below).

   Note: *If the sonicated or homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New sonication of the lysate can then prevent increased backpressure problems when loading on the column.*

First time starting up or after long term storage

Flow rate: 1 ml/min

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).*

2. Wash with 1 column volume (CV) of 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. Wash with 5 CV start buffer.
4. Wash with 5 CV elution buffer.
5. Wash with 5 CV start buffer.

Purification

Note: *If it is the first time the column is used, see section "First time starting up or after long term storage", page 16.*

Flow rate: 1 ml/min.

Collect fractions throughout the separation.

1. Equilibrate the column with at least 5 to 10 CV start buffer until the UV baseline, eluent pH and conductivity are stable.
2. Adjust the sample to the chosen starting pH and conductivity and load on the column.
3. Wash with 5 to 10 CV start buffer or until the UV trace of the effluent returns to near baseline.

4 Elute, either by linear gradient elution or a step elution. Elution is performed by a change in ionic strength for Capto Q, Capto S and Capto DEAE, while elution by a change in pH is recommended for Capto adhere and Capto MMC (see Section 4).

- *Linear gradient elution:* Elute with 0 to 100% elution buffer in 10 to 20 CV.
- *Step elution:* Elute with 5 CV elution buffer at chosen concentration and pH. If more than one elution buffer, elute with 5 CV of the next elution buffer and continue until the target protein has been eluted.

If required, the collected eluted fractions can be buffer exchanged or desalted using columns listed in Table 5.

5 Wash with 5 CV 100% elution buffer to elute any remaining bound material.

6 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 the high salt wash and re-equilibration steps can be used.

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

6 Cleaning-in-place

Correct preparation of samples and buffers, including a high salt wash (1 to 2 M NaCl) after each separation, should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the chromatography medium needs cleaning. Cleaning-in-place (CIP) may be performed regularly after every run or between the runs when different proteins are purified on the same column (to prevent possible cross-contamination).

CIP is strongly recommended:

- When an increase in back pressure is seen.
- If reduced column performance is observed.

CIP protocol

Flow rate: 0.5 ml/min.

For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during the purification.

1 Wash with at least 2 column volumes (CV) of 2 M NaCl.

2 Wash with at least 4 CV 1 M NaOH.

3 Wash with at least 2 CV 2 M NaCl.

4 Wash with at least 2 CV distilled water.

5 Wash with 5 to 10 CV start buffer or until eluent pH and conductivity have reached the required values.

7 Scaling up

The high capacity combined with high flow rate and low back pressure makes Capto media particularly useful for larger scale purifications. Purifications can be scaled up using prepacked HiTrap Capto 5 ml columns or prepacked HiScreen™ columns (10 cm bed height, 4.7 ml) containing the same media. Two HiScreen columns can easily be connected in series to increase the bed length to 20 cm. 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 linear fluid velocity (cm/h) constant while increasing bed diameter and volumetric flow rate (ml/min or l/h). Larger quantities of media for further scale-up are also available, see ordering information.

All media included in HiTrap Capto IEX Selection Kit are BioProcess media with full technical and regulatory support.

8 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. 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 1) may damage the column.*

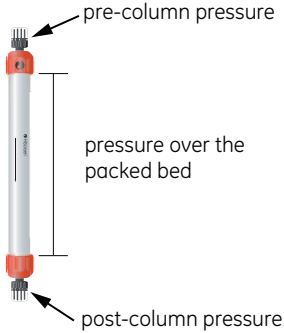


Fig 3. Pre-column and post-column measurements.

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:

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.*

9 Storage

HiTrap Capto Q, HiTrap Capto DEAE, HiTrap Capto adhere and HiTrap Capto MMC

- Wash with 2 column volumes (CV) of distilled water followed by 2 CV 20% ethanol.
- Store at 4°C to 30°C. Do not freeze.
- Make sure that the column is tightly sealed to avoid drying out.

HiTrap Capto S

- Wash with 2 CV distilled water followed by 2 CV 20% ethanol containing 0.2 M sodium acetate.
- Store at 4°C to 30°C. Do not freeze.
- Make sure that the column is tightly sealed to avoid drying out.

10 Ordering information

Product	Quantity	Code No
HiTrap Capto IEX Selection Kit	5 × 1 ml	28-9343-88

Related product	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
HiTrap Capto MMC	5 × 1 ml	11-0032-73
	5 × 5 ml	11-0032-75
HiTrap Capto adhere	5 × 1 ml	28-4058-44
	5 × 5 ml	28-4058-46
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 product	Quantity	Code No.
HiScreen Capto MMC	1 × 4.7 ml	28-9269-80
HiScreen Capto adhere	1 × 4.7 ml	28-9269-81
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
Capto adhere	25 ml	17-5444-10
	100 ml[1]	17-5444-01
Capto MMC	25 ml	17-5317-10
	100 ml[1]	17-5317-01
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[1] Other quantities are also available. Please contact your local representative or visit www.gelifesciences.com/protein-purification

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Data File: HiTrap Capto IEX Selection Kit	28-9359-95
Data File: Capto Q, Capto ViralQ, Capto S and Capto DEAE	11-0025-76
Data File: Capto MMC	11-0035-45
Data File: Capto adhere	28-9078-88
Handbook: Ion Exchange Chromatography & Chromatofocusing, Principles and methods	11-0004-21
Ion Exchange Chromatography Columns and Media, Selection guide	18-1127-31
Prepacked chromatography columns for ÄKTAdesign systems, Selection guide	28-9317-78
HiTrap - Column guide	18-1129-81

For contact information for your local office, please visit: www.gelifesciences.com/contact

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

www.gelifesciences.com/hitrap
www.gelifesciences.com/protein-purification

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

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

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

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

imagination at work

28-9372-10 AC 01/2014