Instructions 11-0035-06 AF

HiTrap™ Capto™ MMC, 1 ml and 5 ml

Capto MMC is a multimodal salt-tolerant BioProcess™ medium for capture and intermediate purification of proteins from large feed volumes by packed bed chromatography.

HiTrap Capto MMC are prepacked 1 ml and 5 ml columns for screening of selectivity, binding and elution conditions, and method scouting.

The column design, together with a modern chromatography medium, provides fast, reproducible, and easy separations in a convenient format. The columns are best used with liquid chromatography systems such as ÄKTA™, but can also be operated with a syringe or peristaltic pump.

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.

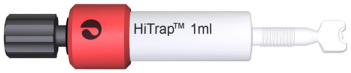

Fig 1. HiTrap, 1 ml column.

Fig 2. HiTrap, 5 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	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

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

Medium properties

Capto MMC has a ligand with multimodal functionality (Fig 3). The multimodal functionality gives a different selectivity compared to other ion exchangers and also the possibility of binding proteins at high salt conditions. The modified agarose matrix provides particle rigidity without compromising pore size.

The characteristics of Capto MMC are listed in Table 2.

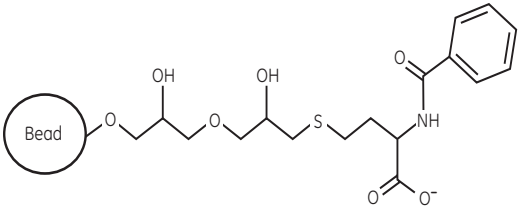

Fig 3. The Capto MMC ligand interacts in several ways with a target molecule to give an alternative selectivity compared to traditional media. The most pronounced interactions are ionic, hydrogen bonding and hydrophobic.

Table 2. Characteristics of Capto MMC

Matrix	highly cross-linked agarose
Functional group	multimodal weak cation exchanger
Total ionic capacity	0.07 to 0.09 mmol H^+/ml medium
Particle size[1]	75 µm (d_{50v})
Flow velocity	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)
Dynamic binding capacity[2]	> 45 mg BSA/ml medium at 30 mS/cm
pH stability[3]	
short term	2 to 14
long term	2 to 12
Working temperature[4]	4°C to 30°C
Chemical stability	all commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide[5], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol
Avoid	oxidizing agents, cationic detergents
Storage	4°C to 30°C 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 2 minutes, 300 cm/h in a Tricorn™ 5/100 column with 10 cm bed height in 50 mM Na-acetate, pH 4.75, 250 mM NaCl.

[3] **Short term pH:** pH interval where the medium can be subjected to, for cleaning- or sanitization-in-place (accumulated 90–300 hours at room temperature) without significant change in function.
Long term pH: pH interval where the medium can be operated without significant change in function.

[4] Capto MMC can be used under cold-room conditions, but for some proteins the capacity may decrease.

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

2 Sample preparation

Sample preparation with clarification of sample

Titrate the sample solution to a pH at least 0.5 units below the pI of the target molecule and clarify it before application by filtering through a 0.45 µm filter or by centrifugation.

Sample preparation without clarification of sample

The following sample preparation procedure is aimed to give a sample, sufficiently homogenized to be applied 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 5–10 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, approx. 10 min or homogenization with a French press or other homogenizer or freeze/thaw, repeated at least five times.
4. 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.
5. Adjust the pH of the lysate. The pH should be at least 0.5 units below the pI of the target molecule. Do not use strong bases or acids for pH-adjustment (precipitation risk). Apply the unclarified lysate on the column directly after preparation.

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.*

3 Start and elution buffer

During binding Capto MMC behaves as a weak cation exchanger and pH of the start buffer should be the same as the sample and preferable at least 0.5 pH unit below the pI of the target protein. The elution buffer should have a pH that is 0.5 units or more above the pI of the target protein and contain additional eluting salt. The buffer species and buffer concentration are important for reproducible and robust methods. Table 3 shows suitable buffers for cation exchangers and suggested starting concentrations. The buffer concentration should be at least 25 mM, but could sometimes be over 100 mM.

For samples with unknown charge properties, try the following:

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

Table 3. Buffers for cation exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C)
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

4 First time use or after long term storage

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml).

1. Remove the stopper and connect the column to the system with a drop-to-drop connection to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet and wash with 1 column volume of distilled water to remove the ethanol. This step avoids the risk of precipitation if buffer salts were to come into contact with the ethanol. The step can be omitted if precipitation is not likely to be a problem.
3. Wash with 5 column volumes of start buffer.
4. Wash with 5 column volumes of elution buffer.
5. Wash with 5 column volumes of start buffer.

5 Suggested purification protocol

Titrate the clarified or unclarified sample (*see* page 6) to a pH that is at least 0.5 pH units below the pI of the target molecule. The exact pH has to be determined for each target molecule.

- Equilibrate the column with start buffer having the same pH as the titrated feed.
- Apply the sample to the column.
- Wash out unbound sample using start buffer.
- Elute the target protein as described below:

1. 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 using step elution, or by using a pH gradient. Use the pH where the target molecule starts to elute as a base if further optimization is needed in order to increase recovery.
2. Screen for eluting salt concentration using 0.5, 1.0, and 1.5 M salt at the pH determined in step 1.

3 If further optimization is needed, increase the concentration of buffering salt in elution buffer e.g. from 50 mM to 250 mM.

4 If the target protein still elutes in an asymmetrical peak over a number of fractions, the eluting salt can be changed, e.g. from NaCl to NH_4Cl.

5 Additives such as urea and organic modifiers can further increase the recovery of some proteins.

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).

Separation by step elution

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml). Collect fractions throughout the separation.

1 Equilibrate column with 5–10 column volumes of start buffer or until the baseline, eluent pH and conductivity are stable.

2 Adjust the sample to the chosen start pH and apply to the column.

3 Wash with 5–10 column volumes of start buffer or until all unbound material has washed through the column.

4 Elute with 5 column volumes of elution buffer.

5 Wash with 5 column volumes of 1 M NaOH to elute any remaining bound material.

6 Re-equilibrate with 5–10 column volumes of start buffer or until eluent pH and conductivity reach the required values.

Separation by pH gradient elution

For proteins with pI below 7 scouting of the most favorable pH for elution can be done with a stable and reproducible linear pH-gradient in the pH range 3–7, *see* buffer preparation below.

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml). Collect fractions throughout the separation.

1 Equilibrate column with 5–10 column volumes of start buffer or until the baseline, eluent pH and conductivity are stable.

2 Adjust the sample to the chosen starting pH and ionic strength and apply to the column.

3 Wash with 5–10 column volumes of start buffer or until all unbound material has washed through the column.

4 Begin elution using a gradient volume of 10–20 column volumes of elution buffer.

5 Wash with 5 column volumes of 1 M NaOH to elute any remaining ionically-bound material.

6 Re-equilibrate with 5–10 column volumes of start buffer or until eluent pH and conductivity reach the required values.

Buffer preparation for pH gradient elution:

- Prepare 1 liter of start buffer: 100 mM Na-phosphate/citrate, pH 3 by dissolving 29.4 g tri-Na-citrate × 2 H_20 (M_r = 294) and 13.8 g NaH_2PO_4 × H_20 (M_r = 138) in 800 ml distilled water, then adjust the pH to 3 with 1 M HCl and the volume to 1 liter.
- Prepare 1 liter of elution buffer: 100 mM Na-phosphate/citrate, pH 7.5 by dissolving 29.4 g tri-Na-citrate × 2 H_20 (M_r = 294) and 17.8 g Na_2HPO_4 × 2 H_20 (M_r = 178) in 800 ml distilled water, then adjust the pH to 7.5 with 1 M HCl and the volume to 1 liter.

6 Further optimization

HiTrap columns are best suited for initial screening of binding and elution conditions, further optimization is preferably done on a longer column such as Tricorn and XK columns.

7 Cleaning

When reduced performance or increasing backpressure is seen the column needs cleaning.

The following procedure removes common contaminants:

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml).

1. Wash with at least 2 column volumes of 2 M NaCl in a buffer with a pH higher than the pI of the target protein.
2. Wash with at least 4 column volumes of 1 M NaOH.
3. Rinse with at least 2 column volumes of distilled water.
4. Wash with at least 4 column volumes of start buffer until eluent pH and conductivity have reached the required values or storage solution.

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 4. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

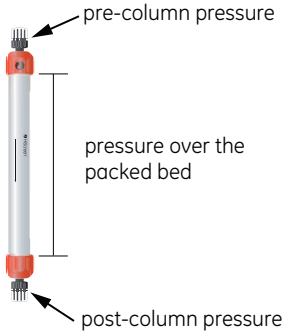

Fig 4. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

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

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

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

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

Wash with 2 column volumes of distilled water followed by 2 column volumes of 20% ethanol. Store at 4°C to 30°C.

Do not freeze. Seal the column with the supplied stoppers.

10 Further information

Further information about optimization, scale-up, troubleshooting, cleaning, etc. can be found in Ion Exchange Chromatography & Chromatofocusing, Principles and Methods 11-0004-21, also available at: *www.gelifesciences.com/protein-purification or www.gelifesciences.com/hitrap*

11 Ordering information

Product	Quantity	Code No.
HiTrap Capto MMC	5 × 1 ml	11-0032-73
	5 × 5 ml	11-0032-75

Related products	Quantity	Code No.
Capto MMC	25 ml	17-5317-10
	500 ml[1]	17-5317-01

[1] Capto MMC is available in process scale quantities. Please contact your local representative.

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

Accessories	Quantity	Code No.
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

Literature	Code No.
Data file: Capto MMC	11-0035-45
Application Note: Optimizing elution conditions on Capto MMC using Design of Experiment	11-0035-48
Ion Exchange Chromatography & Chromatofocusing, Principles and Methods	11-0004-21

For local office contact information, 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

11-0035-06 AF 03/2014