

Capto MMC

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

Capto MMC increases productivity and reduces cost with:

- high dynamic binding capacity at high conductivity
- high volume throughput
- new selectivity
- smaller unit operations



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BioProcess Media

BioProcess media are developed and supported for production scale chromatography. All BioProcess media are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of 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.

Properties of Cpto MMC

Cpto MMC has a ligand with multimodal functionality (Fig 1). The multimodal functionality gives a different selectivity compared to traditional ion exchangers and also provides the possibility of binding proteins at high salt conditions.

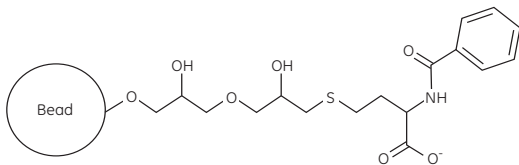


Fig 1. The Cpto MMC ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interaction, hydrogen bonding and hydrophobic interaction.

Cpto MMC is designed to increase speed and throughput in capture and intermediate purification. By offering high capacity at high salt concentrations, and high flow velocities with low backpressure, process cycle times may be reduced and productivity increased.

The medium is based on a high flow agarose matrix. Typical flow velocities at large-scale (1 m column diameter and 20 cm bed height) are 600 cm/h or over, with a backpressure below 3 bar (Fig 2). The highly cross-linked agarose base matrix gives the medium high chemical and physical stability. Characteristics such as capacity, elution behavior and pressure/flow properties are unaffected by the solutions commonly used in process chromatography (Table 1).

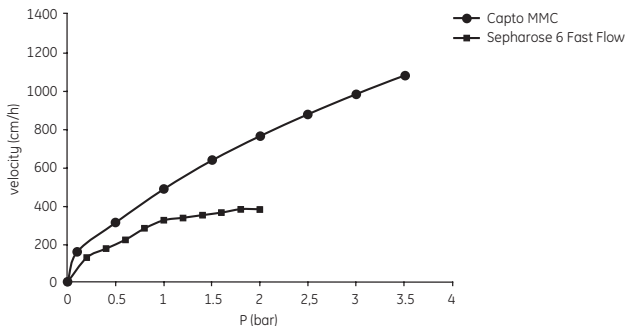


Fig. 2. Pressure-flow properties for Capto MMC compared to Sepharose™ 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.), open bed at settled bed height equal to 20 cm, with water at 20°C.

Table 1. Characteristics of Capto MMC.

Matrix	highly cross-linked agarose
Functional group	multimodal weak cation exchanger
Total ionic capacity	65 – 85 $\mu\text{mol H}^+/\text{ml}$ medium
Particle size*	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 [†]	> 45 mg BSA/ml medium at 30mS/cm
pH stability [‡]	
short term	2 – 14
long term	2 – 12
Working temperature	+4 to +30°C [§]
Chemical stability	all commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide [¶] , 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol
Avoid	oxidizing agents, cationic detergents

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

[†] 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.

[‡] 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.

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

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

Method design and optimization

The aim of designing and optimizing a method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery.

During binding Capto MMC behaves as a weak cation exchanger and pH of the start buffer should be the same as the sample and preferably at least 0.5 pH unit below the pI of the target protein. Since Capto MMC is a salt tolerant medium it is not necessary to scout for optimal salt conditions. The target molecule will adsorb at any ionic strength normally used.

Capto MMC allows efficient capture of proteins at high salt concentration. This disqualifies in many cases the use of increasing salt concentrations as an efficient way of eluting proteins. Therefore special attention has to be paid when optimizing elution conditions to avoid tailing and to increase recovery.

Suggested purification protocol

- Titrate the clarified feed to a pH that is 0.5-2.0 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 of 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. Scout for the optimal elution pH by using buffer at a pH of 0.5, 1.5 and 2.5 above the pI of the target protein 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. Scout for different NaCl concentrations using 0.5, 1.0, and 1.5 M NaCl 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 50mM to 250 mM. This will induce a faster change of the pH and also contribute to the increase in ionic strength.
4. If the target protein still elutes in an a symmetrical peak over a number of fractions, try to change the eluting salt. An alternative to NaCl could be NH_4Cl .
5. Use of additives e.g. urea and organic modifiers can increase the recovery of some proteins.

Optimization of throughput

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

Scaling up

After optimizing the method at laboratory scale, the process can be scaled-up.

1. Select the bed volume according to required binding capacity.
2. Select a column diameter to obtain a bed height of 10 - 45 cm. To utilize the full potential of Copto MMC, we recommend bed heights of 20 cm or higher at large-scale.
3. Scale-up is typically done by keeping bed height and flow velocity constant, while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially done with small column volumes, in order to save sample and buffer, some parameters like 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 constant, the binding capacity for the target molecule remains the same. Other factors, like clearance of critical impurities, may change when column bed height is changed and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.

Packing process-scale columns

See Application Note, code number 11-0035-47 for process-scale column packing instructions.

Packing lab-scale columns

The following instructions are for packing lab-scale columns: Tricorn 5/100, Tricorn 10/100, XK 16/20 with 10 cm bed height and XK 16/70 with 40 cm bed height. For more details about packing Tricorn columns, please see the instructions "Tricorn Empty High Performance Columns" or for practical instruction in good packing techniques, the CD ROM "Column Packing - The Movie" code number: 18-1165-53.

Packing Tricorn 5/100 and 10/100 columns

Materials needed

Capto MMC
Glass filter funnel
Plastic spoon
Filtering flask
Measuring cylinder
20% ethanol

Amount of medium

The amount of Capto MMC medium needed can be calculated by: column cross sectional area (cm²) × bed height (cm) × compression factor (sedimented medium bed/packed medium bed). The compression factor is approximately 1.15 for Capto MMC. For example, a 10 cm bed height in a Tricorn 10/100 would require $0.78 \times 10 \times 1.15 = 9$ ml of sedimented medium.

Preparing the packing slurry

The amount of slurry needed is equal to the calculated sedimented medium divided by slurry concentration. The Capto MMC is delivered in approximately 75% slurry concentration in 20 % ethanol. When packing the Tricorn columns the slurry should have a concentration of approximately 60%.

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the medium into the funnel and wash with 6 ml 20% ethanol per ml medium. Move the sedimented medium from the funnel into a beaker and add the correct amount of 20% ethanol.

For a correct bed height, the slurry concentration should be checked in a measuring cylinder after either settling overnight or after centrifugation at 3000 rpm for 3 minutes, wait for 5 minutes before reading. Columns can also be packed with an excess of medium that can be removed after packing.

Equipment needed

For packing Tricorn 5/100 column

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

For packing Tricorn 10/100 column

Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector (with EPDM O-rings), 100 mm glass tube (to be used as a packing tube), and bottom unit with filter holder, cap and stop plug. ÄKTAdesign™ 100 system or a stand-alone pump such as P-901 that can deliver 20 ml/min.

When working with large volumes or when repeatedly loading, the Tricorn coarse filter kit is recommended to reduce the risk of clogging. Use Tricorn coarse filter kit 5 (11-0012-53) or Tricorn coarse filter kit 10 (11-0012-54).

Column packing procedure

To pack the column, use 20% ethanol and proceed as follows:

1. Insert a bottom filter into the filter holder.
2. Wet the O-ring on the filter holder by dipping the filter holder into 20% ethanol.
3. 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. Push the filter holder into place.
4. Screw the end cap onto the column tube. Insert a stop plug into the bottom unit.
5. 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).
6. Screw the Tricorn packing tube into the upper fitting of the Tricorn packing connector.
7. Mount the column and packing unit vertically.
8. Calculate how much chromatography medium is necessary.
9. Pour the chromatography medium into the top of the packing tube filling both column tube and packing tube. Remove all air bubbles.

10. Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column tube and connect a pump to the top of the packing unit, remove the stop plug from the bottom of the column tube.
11. Pack the column at 20 ml/min (Tricorn 10/100) or 13 ml/min (Tricorn 5/100) for 6 minutes.
12. When the medium is packed, switch off the pump, attach the stop plug into bottom of the column tube, disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess medium by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula.
13. Top off the column with the same fluid as used for packing the column.
14. Place a pre-wet filter on top of the fluid in the column.

Note: The top coarse filter is inserted by another procedure. See separate instruction included in the coarse filter kit.

15. Prepare the adapter unit by screwing the guiding ring inside the adapter unit down to its lower end position so that it is level with the bottom of the adapter unit.
16. Wet the O-ring on the adapter unit by dipping it into 20% ethanol.
17. Screw the guiding ring back 1.5 turns.
18. Screw the 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.
19. Screw the adapter halfway down. Press the adapter lock down into the locked position. It should now not be possible to turn the adapter if the adapter unit has been mounted correctly.
20. Unlock the adapter lock and screw the adapter unit down until the adapter meets the medium bed surface. Screw the adapter unit down a further 360 degrees so that the adapter is positioned slightly below the medium surface.
21. Connect the pump to the adapter unit. Remove the stop plug from the cap of the column tube.

22. Pack the column at 20 ml/min (Tricorn 10/100) or 13 ml/min (Tricorn 5/100) for 2 minutes.
23. Before switching off the pump mark the position of the medium surface with a pen on the column.
24. Switch off the pump, attach the stop plug into bottom of the column tube and disconnect the pump. Screw the adapter unit down to the marking and further 360 degrees so that the adapter is positioned slightly below the marking.
25. Press the adapter lock down into the locked position.
26. Screw a stop plug into 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 Evaluation of column packing, page 16.

Packing XK 16/20 columns and XK 16/70 columns

Materials needed

Capto MMC
Glass filter funnel
Plastic spoon
Filtering flask
Measuring cylinder
20% ethanol
Water
Lab stand

Amount of medium

The amount of Capto MMC medium needed can be calculated by: column cross sectional area (cm²) × bed height (cm) × compression factor (sedimented medium bed/packed medium bed). The compression factor is approximately 1.15 for Capto MMC. For example, a 10 cm bed height in a Tricorn 10/100 would require $0.78 \times 10 \times 1.15 = 9$ ml of sedimented medium.

Washing the medium

The washing step is only required for packing XK 16/20 columns. Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the medium into the funnel and wash. Wash with 6 ml purified water per ml medium.

Preparing the packing slurry

The slurry concentration should be 50-60% in water for XK 16/20 and 50-60% in 20% ethanol for the XK 16/70. The medium should be measured in a measuring cylinder after settling overnight or after centrifugation at 3000 rpm for 3 minutes, wait for 5 minutes before reading.

Equipment needed

An ÄKTAexplorer™ 100 system can be used for packing the column. The pump filter unit should be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure. Mount a pressure gauge at the inlet of the column to make sure that the operating pressure limit for the column is not exceeded.

The XK 16/20 columns should be packed without using a slurry reservoir, as it is important to lock the medium bed quickly at the end of the packing after flow stop. XK 16/20 is used for 10 cm bed heights.

XK 16/70 with two adaptors and an XK 16/20 column as packing reservoir are used for 40 cm bed heights.

XK 16/20 packing procedure

To pack the column, use water and proceed as follows:

1. Wet the bottom filter with 20% ethanol, with the aid of a syringe mounted on the outlet tubing. After the filter is wetted mount a stop screw on the outlet.
2. Mount the bottom piece in the column tube.
3. Wet the adapter filter with 20% ethanol, with the aid of a syringe mounted on the inlet tubing. After the filter is wetted mount a stop screw on the inlet.
4. Keep the adapter in a beaker with 20% ethanol.
5. Mount the column vertically on a stand.
6. Fill the glass tube with 60% medium slurry up to the upper edge of the glass tube.
7. Mount the adapter in the upper part of the glass tube with no air trapped below the filter.
8. Tighten the sealing-ring and connect the column inlet to the system outlet.
9. Pack the column at 40 ml/min for 10 min.

Note: Do not exceed the operating pressure limit for the columns (5 bar).

10. Stop the pump and mount a stop screw on the outlet.
11. Adjust the adapter quickly down to the medium surface and then a further 15 mm into the medium bed.
12. Lock the adapter at that level.

Testing the packed column

See Evaluation of column packing, page 16.

XK 16/70 packing procedure

To pack the column, use 20% ethanol and proceed as follows:

1. Wet the bottom adapter filter with 20% ethanol, with the aid of a syringe mounted on the outlet tubing. After the filter is wetted mount a stop screw on the outlet.

2. Mount the bottom adapter in the column tube.
3. Wet the top adapter filter with 20% ethanol, with the aid of a syringe mounted on the inlet tubing. After the filter is wetted mount a stop screw on the inlet.
4. Keep the adapter in a beaker with 20% ethanol.
5. Mount the column vertically on a stand and connect the packing reservoir with a packing connector.
6. Fill the glass tube with 50% medium slurry up to the upper edge of the glass tube.
7. Mount the adapter in the upper part of the glass tube with no air trapped below the filter.
8. Tighten the sealing-ring and connect the column inlet to the system outlet.
9. Pack the column at 33 ml/min for 10 min.

Note: Do not exceed the operating pressure limit for the columns (5 bar).

10. Stop the pump and mount a stop screw on the outlet.
11. Detach the packing reservoir and mount the top adaptor in the packing tube.
12. Pack the column at 33 ml/min for 5 additional minutes.
10. Stop the pump and mount a stop screw on the outlet.
11. Adjust the adapter quickly down to the medium surface and then a further 15 mm into the medium bed.
12. Lock the adapter at that level.

Testing the packed column

See Evaluation of column packing, page 16.

Evaluation of column packing

Test column efficiency to check the quality of the packing. Tests should be made directly after packing and at regular intervals during the working life of the column plus when separation performance is seen to deteriorate. 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 (A_s). 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 2.0 M NaCl in water with 0.5 M NaCl in water as eluent.

Note: The calculated plate number will vary according to the test conditions and it 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.

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow velocity between 15 and 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:

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

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

where:

L = Bed height (cm)

N = number of theoretical plates

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

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 is calculated as follows:

$$\frac{\text{HETP}}{d}$$

where:

d is the diameter of the bead

As a guideline, a value of <3 is normally acceptable.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (values between 0.8–1.5 are usually acceptable).

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

Peak asymmetry factor calculation:

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

where:

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height.

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

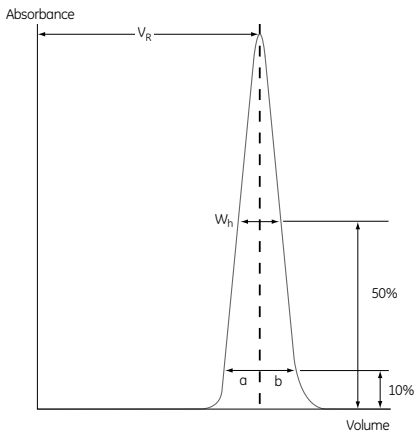


Fig. 3. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

Maintenance

For the best performance from Capto MMC over a long working life, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g. 2 M NaCl in buffer) and at the same time increase pH to about 10-11. Regenerate the medium by washing with at least 5 bed volumes of buffer, 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. These types of contamination occur frequently when working with crude feedstock. Regular CIP prevents the build-up of contaminants in the medium bed and helps to maintain the capacity, flow properties and general performance of Capto MMC.

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.

CIP protocols

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH at 40 cm/h with reversed flow direction. Contact time 1–2 hours, dependent on feed.
Ionically bound proteins	Wash with 0.5-2 column volumes of 2 M NaCl with reversed flow direction. Contact time 10–15 min.
Lipids and very hydrophobic proteins	Wash with 2–4 column volumes of up to 70% ethanol* or 30% iso-propanol with reversed flow direction. Contact time 1–2 hours, dependent on feed. Alternatively, wash with 2–4 column volumes of 0.1% non-ionic detergent with reversed flow direction. Contact time 1–2 hours, dependent on feed.

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

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5–1.0 M NaOH with a contact time of 1 hour is recommended. The CIP protocol for precipitated, hydrophobic bound proteins or lipoproteins removes bound contaminants and sanitizes the medium effectively.

Storage

Store unused medium in the container at a temperature of +4 to +30 °C. Ensure that the screw top is fully tightened. Packed columns should be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

Ordering information

Product	Pack size	Code No
Capto MMC	25 ml	17-5317-10
	500 ml	17-5317-01
	5 L	17-5317-04
	10 L	17-5317-05
	60 L	17-5317-60

All bulk media products are supplied in suspension in 20% ethanol. For additional information, including data file, please contact your local GE Healthcare representative.

Related products

Product	Quantity	Code No
HiTrap™ Capto MMC	5x1 ml	11-0032-73
HiTrap Capto MMC	5x5 ml	11-0032-75
Tricorn 5/100 column	1	18-1163-10
Tricorn 10/100 column	1	18-1163-15
XK 16/20 column	1	18-8773-01
XK 16/70 column	1	18-8775-01

Accessories

Product	Quantity	Code No
Tricorn 5/100 glass tube	1	18-1153-06
Tricorn 5 coarse filter kit	1	11-0012-53
Tricorn 10 coarse filter kit	1	11-0012-54
Tricorn packing connector 5-5	1	18-1153-21
Tricorn packing equipment 10/100	1	18-1153-25

Literature

Product	Code No
Column Packing - The Movie	18-1165-53
Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Data file: Capto MMC	11-0035-45
Application note: Methods for packing Capto MMC in production-scale columns	11-0035-47
Application note: Elution optimization for Capto MMC using Design of Experiment	11-0035-48

Further information

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