

Capto MMC

Capto™ MMC is a multimodal cation exchanger. It belongs to the Capto family of BioProcess™ media for fast, efficient and cost-effective protein purification.

Capto MMC gives increased productivity and reduced cost with

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

Salt-tolerant medium on high-flow agarose

Capto MMC combines recent base matrix developments with innovative ligand chemistry. The adsorption is salt tolerant, meaning that binding of proteins can be performed at the conductivity of the feed material. The medium is based on a highly rigid agarose base matrix that allows high flow rates and low back pressure at large scale. The characteristics of Capto MMC are summarized in Table 1.

Multimodal ligand

Capto MMC has a multimodal ligand (Fig 1) that may interact with target molecules in several different ways. It contains a carboxylic group and thus its features partly resemble those of a weak cation exchanger. However, in addition to the ionic interactions several other types of interactions are involved, including hydrogen bonding and hydrophobic interaction.

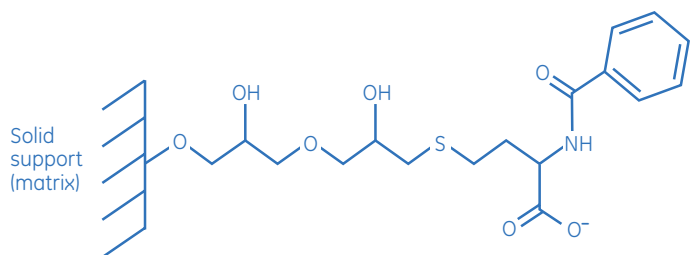


Fig 1. The multimodal ligand of Capto MMC.



Capto MMC allows high-flow processing and binding of proteins at high conductivity. It increases throughput and productivity in large-scale bioprocessing operations.

Table 1. Characteristics of Capto MMC.

Matrix	highly cross-linked agarose
Functional group	multimodal weak cation exchanger
Total ionic capacity	0.07-0.09 mmol H ⁺ /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 30 mS/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.



New selectivity and high salt tolerance

The multimodal functionality offers a selectivity different from traditional ion exchangers which includes binding of proteins at high salt concentration (Fig 2). The medium can

be used for direct load of clarified feed stocks, without prior dilution to reduce the conductivity of the starting material. The new selectivity (Fig 3) can also be used to solve specific purification problems, at high or at low conductivity.

High salt tolerance

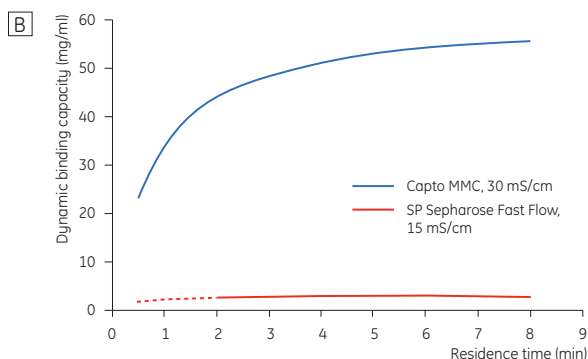
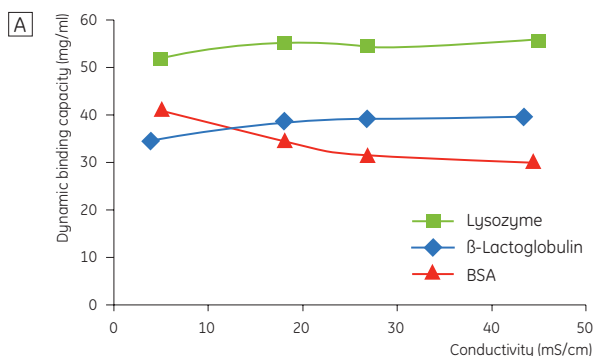


Fig 2. A) Dynamic binding capacity of Capto MMC at 1 min residence time for three different proteins at different conductivities. **B)** Dynamic binding capacity of bovine serum albumin (BSA) as a function of residence time. Capto MMC was run at 30 mS/cm, while SP Sepharose™ Fast Flow was run at 15 mS/cm. The SP Ligand is not salt tolerant and does not bind any BSA at this conductivity. Dotted lines refer to residence times that cannot be utilized in large scale columns, due to flow limits of the respective media.

New selectivity

Column: Tricorn 5/100
 Medium: A) Capto MMC; B) SP Sepharose Fast Flow
 Sample: human blood plasma diluted 5 times, 10 CV
 Buffer A: 100 mM acetic acid, 50 mM Na-phosphate, 20 mM Na-succinate, pH 5.0.

Buffer B: 100 mM acetic acid, 50 mM Na-phosphate, 20 mM Na-succinate, pH 8.0 with 1 M NH₄Cl
 Flow: 150 cm/h
 Gradient: linear gradient 0 – 100% B over 10 column volumes (CV)
 System: ÄKTAexplorer™ 10

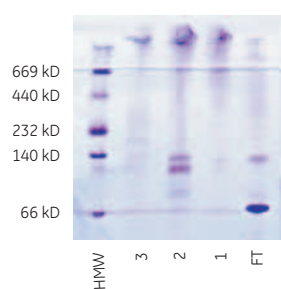
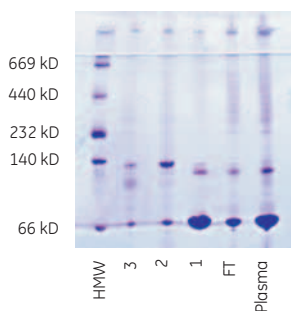
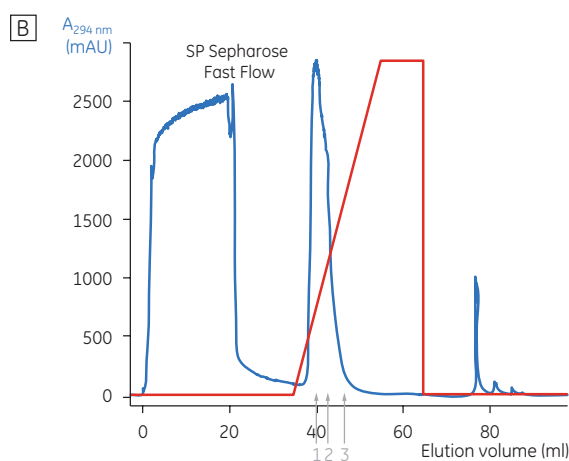
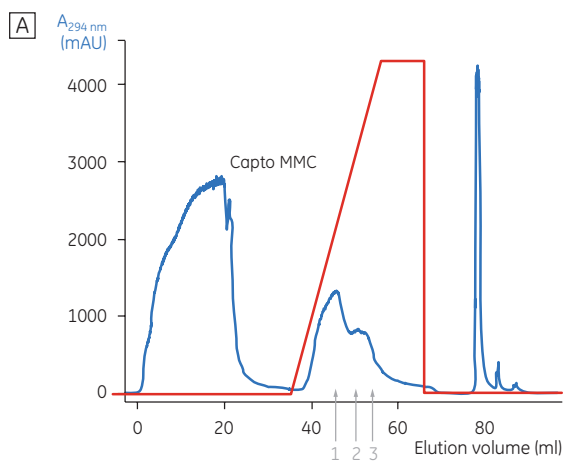


Fig 3. The selectivity of **A)** Capto MMC and **B)** SP Sepharose Fast Flow was investigated using human blood plasma. The sample was diluted 5 times to give a conductivity of 6 mS/cm and a pH of approximately 6. Fractions (indicated with arrows) and the flow-through pool (FT) were analyzed on native PhastGel™ gradient 8–25% and Coomassie™ stained. High molecular weight marker (HMW, GE Healthcare) and unfractionated plasma sample were also applied to the gels.

The elution profile on SP Sepharose Fast Flow revealed one peak whereas the elution profile on Capto MMC showed two, possibly three peaks. Native gel-electrophoresis also shows that the separation patterns differ between the media.

High flow rates and low backpressure in large scale

High flow velocities increase the productivity of large-scale bioprocessing operations and allow large volumes to be processed in one working shift. Capto MMC is characterized by high mechanical stability and low backpressure to allow columns to be operated at high flow velocities with a wide range of bed heights at large scale (Fig 4). Typical flow velocities for Capto MMC in a 1 m diameter column with 20 cm bed height are at least 600 cm/h, with a backpressure below 3 bar.

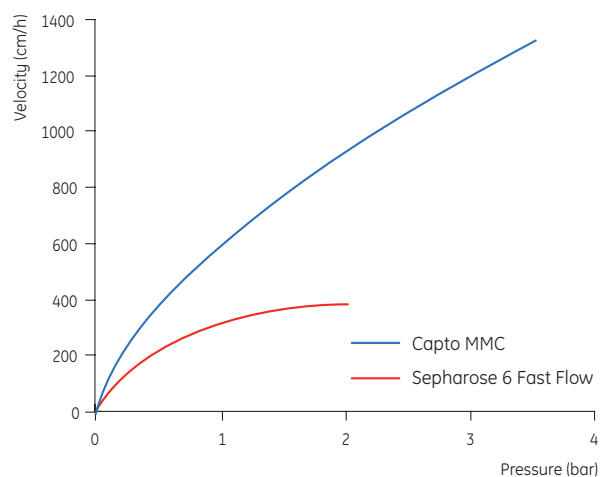


Fig 4. Pressure/flow properties of Capto MMC compared to Sepharose 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.), open bed at settled bed height equal to 20 cm, with water at 20°C.

Additional flexibility in process design

In addition to the flexibility in flow rates, bed heights and sample viscosities that all Capto media share, Capto MMC also gives flexibility in terms of conductivity of the start material. This increases productivity and enables a straight-forward process design, as the feed material can be loaded without the need for dilution (Fig 5).

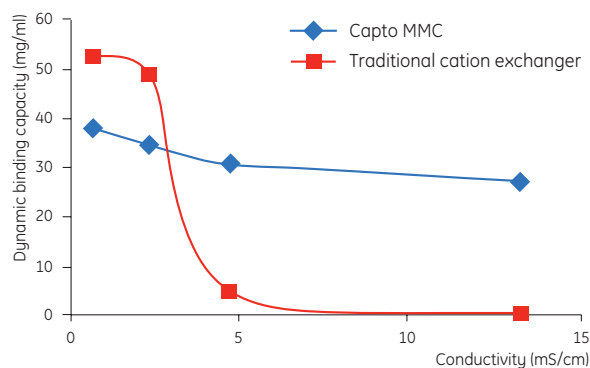


Fig 5. Capto MMC allows a much larger operating range (area below the curves) in terms of conductivity of the starting material than traditional cation exchangers.

HiTrap columns for fast screening

Time and sample can be saved in the early stages of development by using small, prepacked HiTrap™ columns to screen for optimal binding and elution conditions and to develop basic separation methods. To reduce tedious sample preparation, the HiTrap filter pore size has been optimized for loading sonicated lysate directly on the column without causing backpressure problems or leakage of Capto MMC beads. In combination with an automated system such as ÄKTAexplorer it is easy and fast to scout for optimum pH and/or buffer conditions. Capto MMC is available as HiTrap prepacked 1 ml and 5 ml columns. Basic characteristics of HiTrap Capto MMC columns are summarized in Table 2.

Table 2. Characteristics of HiTrap columns.

Column volumes	1 ml and 5 ml
Column dimensions	0.7 x 2.5 cm (1 ml); 1.6 x 2.5 cm (5 ml)
Maximum flow rates	HiTrap 1 ml: 4 ml/min, HiTrap 5 ml: 20 ml/min
Recommended flow rates	HiTrap 1 ml: 1ml/min, HiTrap 5 ml: 5 ml/min

Application

Optimization of binding and elution conditions

During binding Capto MMC behaves predominantly like a weak cation exchanger. Since it 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.

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. Optimal elution is often achieved by a combination of changes in pH, buffer concentration and eluting salt. Design of Experiments (DoE) is an effective tool for investigation of the effect of several parameters on protein recovery in order to establish the optimal elution protocol (Fig 6). An example of the DoE approach is described in application note 11-0035-48. Alternatively, a step-wise elution optimization protocol may be applied. An example is given in the instruction manual.

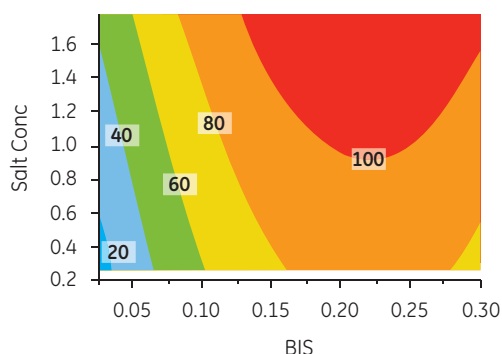


Fig 6. Response surface plots for the recovery of BSA. Recovery is plotted as a function of salt concentration and buffer ionic strength (BIS). Labels correspond to recovery in percent. For more information see application note 11-0035-48.

Improved productivity with Capto MMC

Capto MMC, SP Sepharose Fast Flow and SP Sepharose XL were used to capture recombinant human serum albumin (rHSA, pI 5.5) from cell culture supernatant (CCS) of *Pichia pastoris*. The CCS was clarified by centrifugation and used directly as a feed material (conductivity 15 mS/cm) to measure dynamic binding capacities of the three media. In parallel experiments the feed was diluted to a conductivity of 3 mS/cm in order to test dynamic binding capacities of SP media. Figure 7 shows straightforward scale-up to pilot scale on Capto MMC.

Dynamic binding capacities at 10% breakthrough for all media and different dilutions of the feed were determined at different loading step residence times. The results obtained at residence times attainable in large diameter columns at 20 cm bed height were used for productivity calculations. The results calculated show (Table 3) that Capto MMC gives a productivity of 19 kg/m³,h which is approximately 3 times higher than that obtained with SP Sepharose Fast Flow and SP Sepharose XL when the diluted feed is used and more than 13 times higher than when the undiluted feed is used. The latter result is expected since the SP ligand is not salt tolerant.

This example shows how high dynamic binding capacity at high conductivity combined with high flow velocities can improve the overall productivity of a capture step.

Column: A) Tricorn 5/100, 10 cm bed height (CV 2 ml)
 B) AxiChrom 50, 10 cm bed height (CV 208 ml)
 Medium: Capto MMC
 Sample: rHSA in *P. pastoris* CCS
 Buffer A: 25 mM sodium acetate, pH 4.5
 Buffer B: 50 mM sodium phosphate, pH 7.2 + 1 M NH₄Cl
 Flow rate: 600 cm/h
 Gradient: 100% B, 10 CV
 System: A) ÄKTAexplorer 100; B) ÄKTApilot™

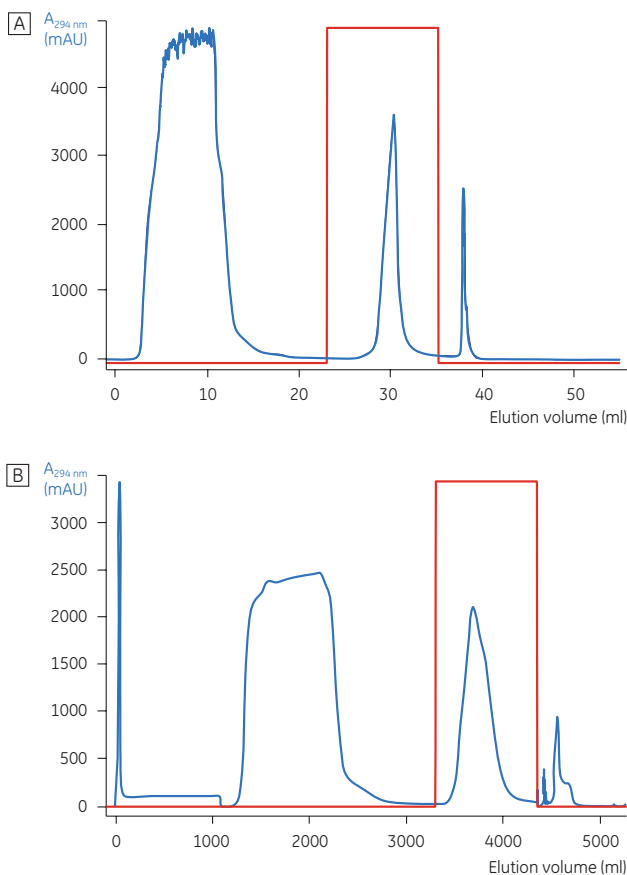


Fig 7. Straightforward scale-up from A) Tricorn 5/100 to B) AxiChrom 50 (100 times). In both cases the purification factor was 4 and the recovery was 93%.

Table 3. Productivity calculations for purification of rHSA from *P. pastoris* CCS. Flow rates and residence times are based on the restrictions of the respective media in a large scale column at 20 cm bed height. In all cases, 93% recovery and 70% loading safety factor were used.

	Dilution factor	Capacity (g/L)	Residence time (min)	max flow rate (cm/h)	Productivity (kg/m ³ ,h)	Productivity (kg/24h)*
Capto MMC 15 mS/cm	no dil	44	2	600	19	102
SP Sepharose XL 3 mS/cm	6.4	195	6	200	6.4	35
SP Sepharose XL 15 mS/cm	no dil	0	6	200	0	0
SP Sepharose Fast Flow 3 mS/cm	6.4	135	6	200	6.0	33
SP Sepharose Fast Flow 15 mS/cm	no dil	6	6	200	1.4	7.4

* Assuming column dimensions of 120 cm diameter, 20 cm bed height (CV ≈ 225 L)

Operation

Fast method development

Fast method development can easily be achieved on ÄKTAexplorer with quick initial screening for selectivity and process conditions using prepacked HiTrap columns. Further optimization and method development using Tricorn or XK columns allows straight-forward scale-up. The UNICORN™ software on ÄKTAdesign™ systems makes it simple to transfer the optimized method to a production scale process system.

Fully scalable

Capto MMC belongs to the BioProcess range of media that are developed and supported for production-scale chromatography. This includes validated manufacturing methods, secure supply and Regulatory Support Files to assist process validation and submission to regulatory authorities.

Scale-up is typically done by keeping bed height and flow velocity constant, while increasing column bed diameter and flow rate. However, since optimization is preferentially done with small column volumes, to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time** is 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 calculated by dividing the bed height (cm) by the flow velocity (cm/h) applied during sample loading.

Cleaning and sanitization

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media 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 of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 1–5 separation cycles. Capto MMC withstands all standard CIP procedures, e.g. 1 M NaOH, 2 M NaCl or 70% ethanol.

Equipment

Capto MMC can be used together with most equipment available for chromatography from lab scale to production scale. Appropriate columns from GE Healthcare are shown in Table 4.

Table 4. Appropriate columns

Column family	Range (inner diameters)
Tricorn	5 mm, 10 mm
XK	16 mm, 26 mm
FineLINE™	35 mm – 350 mm
BPG	100 mm – 300 mm*
BioProcess LPLC	100 mm – 1200 mm
Chromaflo™	400 mm – 2000 mm
AxiChrom	50 mm – 100 mm

* The pressure rating of BPG 450 is too low to use it with Capto MMC.

Storage

Store unused media and prepacked columns at +4 to +30 °C in 20% ethanol.

Ordering information

Product	Pack size	Code No
Capto MMC	25 ml	17-5317-10
Capto MMC	500 ml	17-5317-01
Capto MMC	5 l	17-5317-04
Capto MMC	10 l	17-5317-05
Capto MMC	60 l	17-5317-60
HiTrap Capto MMC	5 x 1ml	11-0032-73
HiTrap Capto MMC	5 x 5ml	11-0032-75

All bulk media and prepacked column products are supplied in 20% ethanol. For more information, contact your local GE Healthcare representative.

Application Notes	Code No
Optimizing elution conditions on Capto MMC using Design of Experiments	11-0035-48

Related Product Literature

Data File	Code No
Capto Q	11-0025-76

Application Notes	Code No
Methods for packing Capto Q in production-scale columns	11-0026-21
High productivity capture of green fluorescent protein on Capto Q	11-0026-20

Handbook	Code No
Ion exchange chromatography and chromatofocusing: principles and methods	11-0004-21

Further information

For the latest news, instructions or handbooks visit:

www.chromatography.amershambiosciences.com

www.bioprocess.amershambiosciences.com

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