Capto™ MMC ImpRes

Capto MMC ImpRes (Fig 1) is a BioProcess™ chromatography medium (resin) for high-resolution polishing of monoclonal antibodies (MAbs) and other biomolecules. The weak cation exchange multimodal ligand enables high binding selectivity in a broad pH/salt window compared with traditional ion exchangers, which allows the possibility to solve challenging purification problems. Capto MMC ImpRes is an excellent choice for removal of aggregates and host cell proteins (HCP) in MAb processes. The wide window of operation for conductivity and/or pH simplifies the process as conditioning after the protein A step can be omitted. When working with sensitive MAbs, the wide window of operation also simplifies optimization of conditions. Capto MMC ImpRes can be used in the initial polishing step in MAb purification processes, as well as for polishing of antibody fragments such as domain antibodies (DAbs).

Fig 1. Capto MMC ImpRes is a multimodal cation exchange polishing medium designed for initial polishing after the protein A capture step in MAb workflows.

Key features and benefits of Capto MMC ImpRes are:

- High yields achieved through the high-resolution beads and selectivity of the ligand
- Efficient removal of aggregates, viruses, and main contaminants in MAb processes
- Enables use of a platform approach to MAb process development
- Displays a broad pH/salt operational window
- Suitable for polishing of antibody fragments

Polishing medium for MAb platform processes

The relative homogeneity of MAbs makes them well-suited for use in platform technologies, which are sets of unit operations, conditions, and methods applied to molecules of a given class. A platform approach saves both time and money in process development. GE Healthcare Life Sciences´ platform approach to MAb production employs protein A chromatography media such as MabSelect SuRe™ or MabSelect SuRe LX for capture of the target. For the polishing purification steps, ion exchange or multimodal media are most commonly used. Multimodal media are powerful tools since they show improved selectivity and a wide window of operation in terms of conductivity and pH compared with traditional ion exchangers. As the elution from the protein A capture step is performed by lowering pH, a multimodal cation exchanger such as Capto MMC ImpRes can be an advantageous option for the first polishing step in a MAb purification process.

Characteristics of Capto MMC ImpRes

Bead size optimized for high-resolution polishing

Capto MMC ImpRes is based on the established high-flow agarose matrix, which gives excellent pressure/flow properties. The rigid matrix of Capto MMC ImpRes allows high flow velocities in MAb processes. The small bead size (~ 40 µm) results in higher resolution in polishing than is possible when using the larger beads (75 µm) of the related multimodal cation exchanger, Capto MMC.

Multimodal ligand

The multimodal cation exchanger ligand of Capto MMC ImpRes is immobilized to the base matrix (Fig 2) and interacts with the target molecule through multiple types of interactions. Ionic interactions are commonly involved, although hydrogen bonding and hydrophobic interactions can also be significant. The strength of these individual interactions often depends on the process conditions. Capto MMC ImpRes is designed to allow effective initial polishing of MAb after the protein A capture step. In order to fine-tune the protein/ligand interaction for optimal aggregate removal, the ligand density of Capto MMC ImpRes has been reduced significantly compared to the related multimodal cation exchanger, Capto MMC. The effect of this is improved selectivity between monomer and aggregates compared to Capto MMC. Another effect of the lower ligand density is reduced salt tolerance, which simplifies elution from Capto MMC ImpRes with salt leading to higher yield and smaller pool volumes. Capto MMC ImpRes still has a higher salt tolerance than traditional cation exchangers, which enables loading at moderate levels of salt, that is, direct loading after the protein A step without dilution.

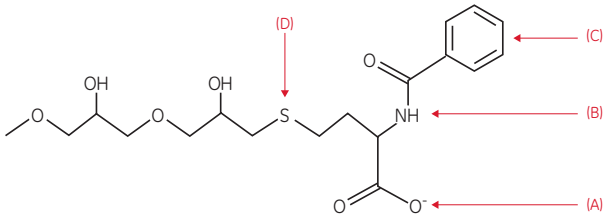

Fig 2. The Capto MMC ImpRes ligand exhibits multimodal functionality for interaction with a target molecule. The most pronounced of these interactions are (A) ionic, (B) hydrogen bonding, (C) hydrophobic interactions, and (D) thiophilic. The chromatography medium is designed for polishing, and is based on the high-flow agarose base matrix with small bead size, which gives good pressure-flow properties and high resolution.

The combination of high resolution and high binding capacity provided by Capto MMC ImpRes results in higher load densities, small elution pool volumes, and improved impurity removal in comparison to the multimodal cation exchanger, Capto MMC. Process economy is improved through higher yields, reduced buffer consumption, and the use of smaller columns and buffer tanks.

Table 1 summarizes the characteristics of Capto MMC ImpRes.

Table 1. Characteristics of Capto MMC ImpRes

Matrix	High-flow agarose
Ligand	Multimodal weak cation exchanger
Average particle size	36 to 44 µm (d_{50v})[1]
Binding capacity/mL chromatography medium	60 to 90 mg MAb/mL (residence time 4 to 5 min)
Ionic capacity	25 to 39 µmol/mL
pH stability working range cleaning-in-place (CIP)	 3 to 12[2] 2 to 14[3]
Pressure/flow specification	0.3 MPa (3 bar, 43.5 psi) at min. 220 cm/h, 1 m diameter column, 20 cm bed height
Storage conditions	4°C to 30°C in 20% ethanol, 0.2 M sodium acetate
Chemical stability	All commonly used aqueous buffers, 1 M NaOH, 2 M NaCl, 5% 1-propanol, 30% isopropanol, 70% ethanol
Avoid using	Oxidizing agents, cationic detergents

[1] d_{50v} is the median particle size of the cumulative volume distribution.
[2] pH interval where the medium can be operated without significant change in function.
[3] pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Efficient aggregate removal

High antibody titers often increase the amount of aggregates and other contaminants such as HCP in the cell culture feedstock. Chromatography media with ion exchange or multimodal properties designed for polishing need to offer high selectivity for effective removal of such contaminants from monomeric MAbs while retaining high yield.

To evaluate the binding selectivity of Capto MMC ImpRes for removal of MAb aggregates, a linear gradient elution experiment using two MAbs from post-protein A affinity chromatography was performed in Tricorn™ 5/50 columns. Fractions from the elution peaks were collected and analyzed by analytical size exclusion chromatography for aggregate content (Table 2).

Table 2. Results from elution of two MAbs using Capto MMC ImpRes

MAb	Sample load (mg/mL)	Elution pool vol. (column volumes, CV)	Aggregate content (%) at 90% yield	
			Start	Final
MAb 1	44	5.0	5.0	0.6
MAb 2	48	4.0	2.4	0.5

The results in Table 2 show effective removal of aggregates. MAb 1, which had the higher initial aggregate content (5% compared to 2.4% for MAb 2) was nonetheless effectively purified in terms of final aggregate content.

Aggregate removal using Capto MMC ImpRes compared with Capto SP ImpRes

To further evaluate the value of Capto MMC ImpRes, a study was performed to establish the efficiency of removal of high molecular-weight aggregates in comparison with a traditional cation exchanger, Capto SP ImpRes. Experiments were performed in Tricorn 5/20 columns with 4 min residence times and a high sample load corresponding to 70% of dynamic binding capacity (DBC) at 10% breakthrough.

Table 3. Summary of pool volumes, aggregate content at 90% yield, HCP-, and protein A concentrations in the purification of a MAb using Capto MMC ImpRes and Capto SP ImPres; sample load 30 mg/mL (Capto MMC ImpRes), 40 mg/mL (Capto SP ImpRes)

Chromatography medium	Elution pool vol. (CV)	Aggregate content (%) at 90% yield	HCP reduction factor	Protein A (ng/mL)	
				Start	Final
Capto MMC ImpRes	9	0.3	4.5	26	1
Capto SP ImpRes	8	0.5	4.8	26	4

The results exemplify the benefits of a multimodal cation exchange in terms of removal of aggregates compared with a traditional cation exchanger such as Capto SP ImpRes (Table 3). Capto MMC ImpRes delivered an aggregate concentration of 0.3% compared to 0.5% aggregates for Capto SP ImpRes. Both media reduced HCP equally effectively.

Chromatograms showing the relative resolution in removal of aggregates for Capto MMC ImpRes and Capto SP ImpRes are shown in Figure 3. The chromatograms show that aggregates elute at the tail for the peak for both media (green traces).

Column:	Tricorn 5/20, 0.5 mL
Media:	Capto MMC ImpRes and Capto SP ImpRes
Sample:	MAb sample, partially purified by protein A chromatography step
Sample loads:	30 mg/mL chromatography medium (Capto MMC ImpRes); 40 mg/mL (Capto SP ImpRes)
Start buffers:	40 mM sodium citrate, pH 6.0 (Capto MMC ImpRes); 50 mM sodium citrate, pH 5.0 (Capto SP ImpRes)
Elution buffers:	Respective start buffer + 1 M NaCl
Residence time:	4 min
Gradients:	0% to 50% elution buffer in 20 CV (Capto MMC ImpRes); 0% to 25% elution buffer in 15 CV (Capto SP ImpRes)
System:	ÄKTAexplorer 10

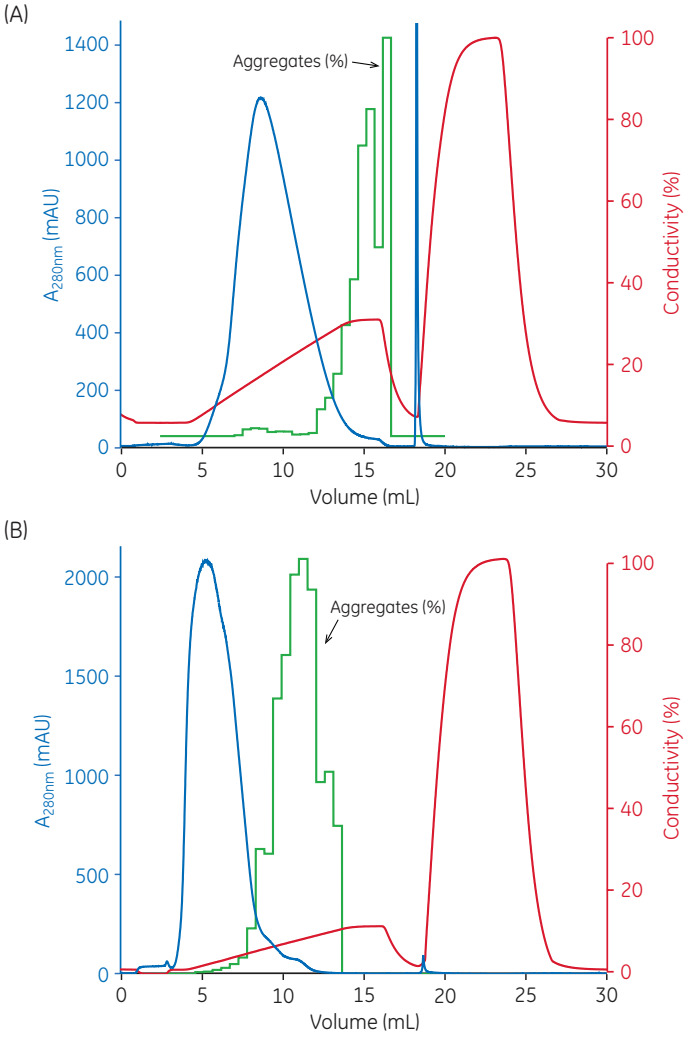

Fig 3. Chromatograms showing polishing of MAb using (A) Capto MMC ImpRes and (B) Capto SP ImpRes.

Improved process economy through high yields

The selectivity of the MMC ligand can result in improved aggregate removal at similar yields when comparing with ion exchangers. For a given purity level, higher yields can be observed when using multimodal chromatography media compared to ion exchangers (Fig 4). Depending on the required purity level, yield improvements by using Capto MMC ImpRes instead of conventional ion exchangers can in some cases be substantial, as shown in Figure 4. This translates directly to improved overall process yields and therefore also improved total process economy.

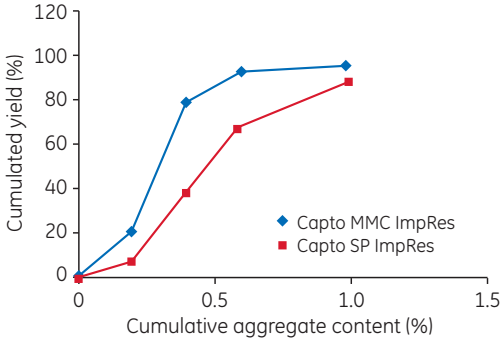

Fig 4. Cumulative yield vs cumulative aggregate content for MAb polishing using Capto MMC ImpRes and Capto SP ImpRes.

Different selectivity and higher salt tolerance

The multimodal functionality of Capto MMC ImpRes offers a different binding selectivity compared with traditional ion exchangers, which includes binding of proteins at higher salt concentrations and over a wider pH range. The improved salt tolerance and ability to work over a large range of pH conditions gives Capto MMC ImpRes a wide window of operation, which allows direct loading of clarified feed without dilution to reduce conductivity of the start material.

To determine optimal conditions for binding to Capto MMC ImpRes, the static binding capacity (SBC) was determined in prefilled PreDictor™ Capto MMC ImpRes 96-well plates over a wide range of conductivity and pH values. For comparison, two MAbs were used—MAb 3 and MAb 4.

Contour maps to describe the effect of pH and NaCl concentration on SBC are shown in Figure 5. The red areas on the contour maps indicate high SBC, and the blue areas low SBC. Both MAbs showed similar binding patterns with high binding capacity over a wide pH range between 5.0 and 8.0 and a high salt tolerance at low pH values between 4.0 and 5.0. This indicates that the two MAbs evaluated could be purified using a similar protocol, thereby enabling a platform approach for the purification.

96-well plate:	PreDictor Capto MMC ImpRes, 6 µL
Sample:	100 µL MAb 3 or MAb 4, partially purified by protein A chromatography step
Sample load:	100 mg/mL chromatography medium
Binding buffers:	25 mM sodium acetate (pH 4.0 to 5.3), sodium phosphate, or Tris (pH 6.7 to 8.0); 0 to 500 mM NaCl

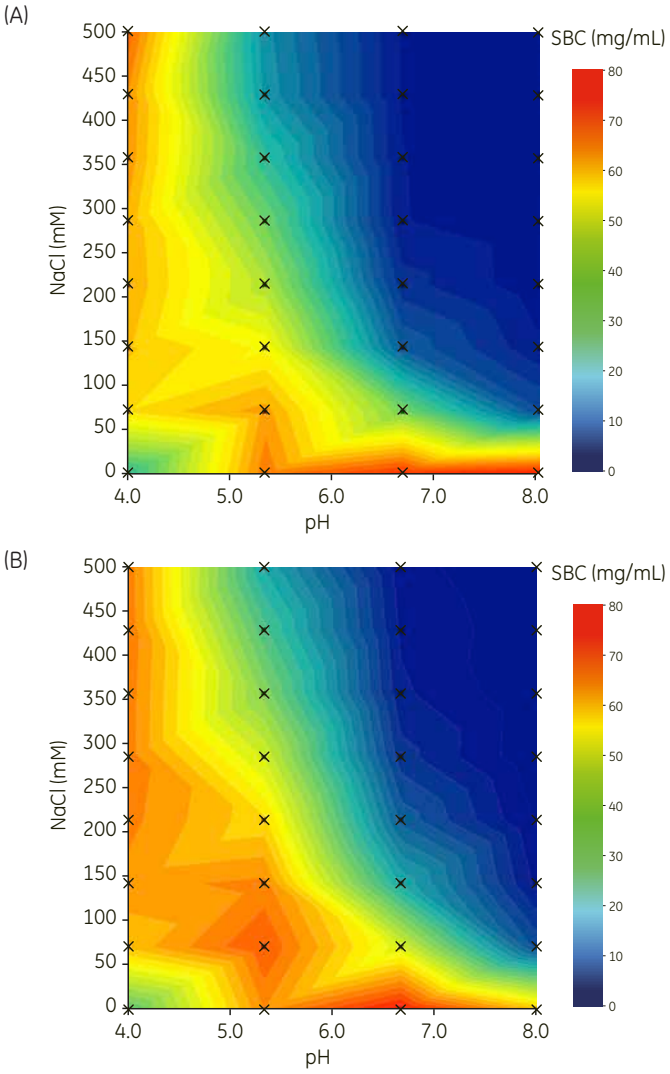

Fig 5. Contour maps showing SBC as affected by pH and NaCl concentration of (A) MAb 3 and (B) MAb 4 on Capto MMC ImpRes.

Robust dynamic binding capacity over a wide pH range

The high binding capacity of Capto MMC ImpRes over a wide pH range was exemplified in a study to determine DBC at different pH values using a HiScreen™ Capto MMC ImpRes, 4.7 mL prepacked column (Table 4). Residence time on the column was constant. As can be seen in Table 4, DBC at 5% breakthrough was between 81 and 97 mg/mL over a range of pH values from pH 5.0 to pH 8.0.

Table 4. Dynamic binding capacity of Capto MMC ImpRes at 5% breakthrough over a range of pH

	Dynamic binding capacity at 5% breakthrough (mg/mL)			
	pH 5.0	pH 6.0	pH 7.0	pH 8.0
Capto MMC ImpRes	81	93	97	95

Effective polishing of domain antibodies (DAbs)

Antibody fragments (e.g., Fab, scFv, and DAbs, Fig 6) are becoming an important class of protein-based products. The structure and smaller size give antibody fragments properties to suit a range of applications (e.g., easier tissue penetration) and their effective purification is therefore of great interest for manufacturers of biopharmaceuticals.

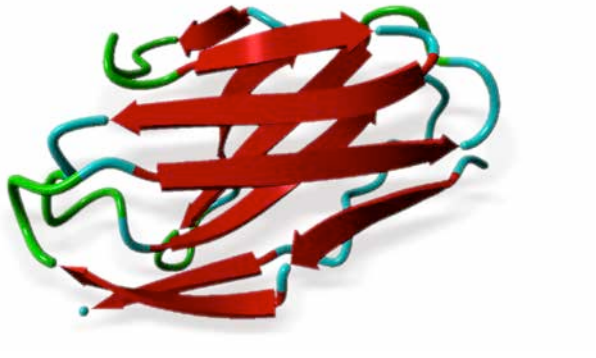

Fig 6. Structure of a recombinant DAb.

Wide window of operation for DAbs

The performance of Capto MMC ImpRes was evaluated in a study where the medium was used after an initial DAb capture step using Capto L. The recombinant DAb, expressed in *E. coli*, included the kappa light chain (V_L). Binding and elution conditions for Capto MMC ImpRes and Capto SP ImpRes were screened using PreDictor 96-well plates using a high-throughput process development (HTPD) approach. The binding capacity calculated using Assist software revealed information on binding and elution conditions (Fig 7), with the red areas on the contour maps showing optimal binding conditions while blue areas show optimal elution conditions.

Figure 7 shows the DAb binding capacities for Capto MMC ImpRes and Capto SP ImpRes. Both media showed a large pH range for binding. However, Capto MMC ImpRes had a larger window of operation regarding NaCl concentration.

96-well plates: PreDictor Capto MMC ImpRes and PreDictor Capto SP ImpRes

Sample: Domain antibody, DAb (M_r 12 900; pI 9.2)

Sample load: 100 mg/mL chromatography medium

Binding buffers: 25 mM sodium citrate (pH 4.1 to 5.1), sodium phosphate (pH 6.1 to 7.1), and Tris-HCl (pH 8.1 to 9.1); 0 to 350 mM NaCl

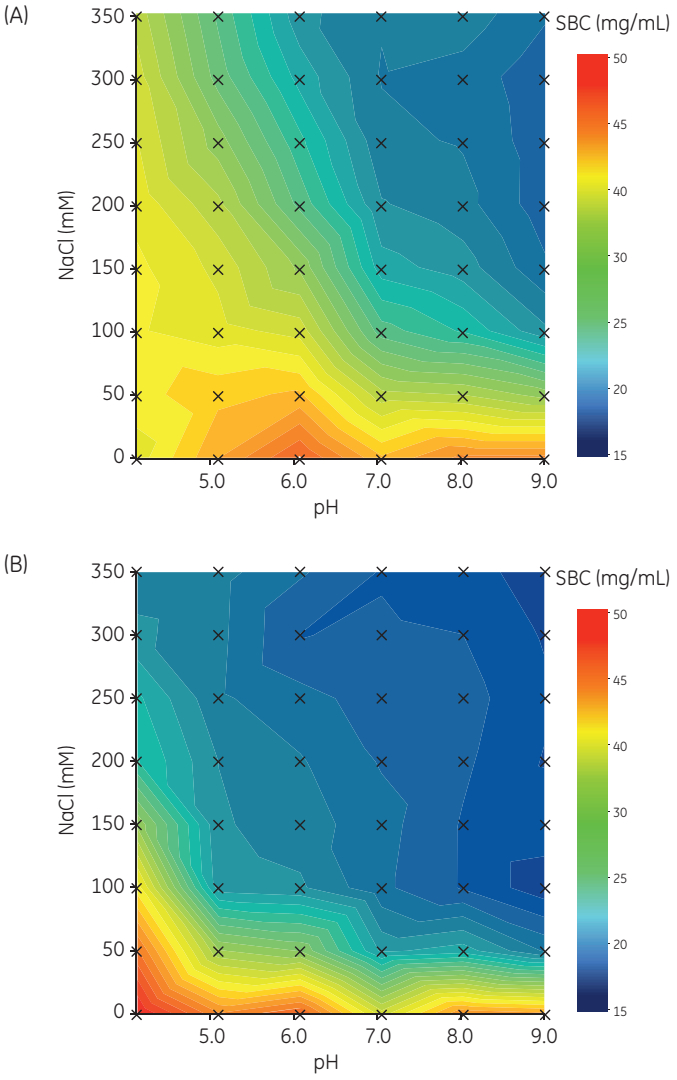

Fig 7. Contour maps showing binding capacity of DAb on (A) Capto MMC ImpRes and (B) Capto SP ImpRes at different pH and NaCl concentrations.

Removal of *E. coli* protein contaminants

Capto MMC ImpRes is effective in removing *E. coli* protein (ECP) contaminants in the polishing step of DAb purification processes. To study ECP removal using Capto MMC ImpRes, DAb sample was applied to Capto MMC ImpRes at a load of 20 mg/mL, pH 5.0. As shown in Figure 7, the salt tolerance at pH 5.0 is high. Three different wash conditions were investigated—0, 100, and 125 mM NaCl. DAb was eluted with 500 mM NaCl and the ECP content in the elution pool and DAb yield are shown in Figure 8. The results showed improved ECP clearance at 125 mM NaCl without major impact on yield.

Column:	Tricorn 5/50, 1 mL
Medium:	Capto MMC ImpRes
Sample:	Capto L purified DAb
Sample loads:	20 mg/mL chromatography medium
Start buffers:	20 mM sodium citrate, pH 5.0
Wash buffers:	Start buffer including 0, 100, and 125 mM NaCl
Elution buffer:	Start buffer + 500 mM NaCl
Residence time:	4 min
System:	ÄKTAexplorer 10

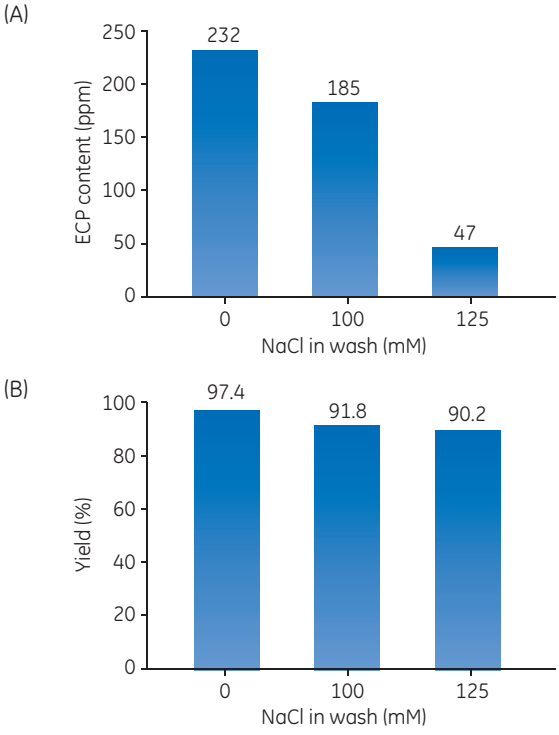

Fig 8. Purification of a recombinant DAb using Capto MMC ImpRes. (A) *E. coli* protein contaminants in the elution pool and (B) DAb yield using different NaCl concentrations in the binding and wash buffers.

Operation

Bed heights and flow velocities

The freedom available in process design for a given chromatography medium can be illustrated as its "window of operation". Figure 9 shows the relationship between column bed height and operating flow velocity for Capto ImpRes and Sepharose™ High Performance matrices. Both media are composed of small beads (40 µm vs 34 µm) and therefore display high resolution, making them suitable for the intermediate purification/polishing step in large-scale purification schemes. Sepharose 6 Fast Flow and Capto media are composed of larger beads and do not possess the high resolution provided by Capto MMC ImpRes. The size of the area below the pressure-limit curves represents the window of operation, that is, the available operating range for the respective medium. As Figure 9 shows, the window of operation of Capto MMC ImpRes suits most needs both in terms of bed height and flow velocities.

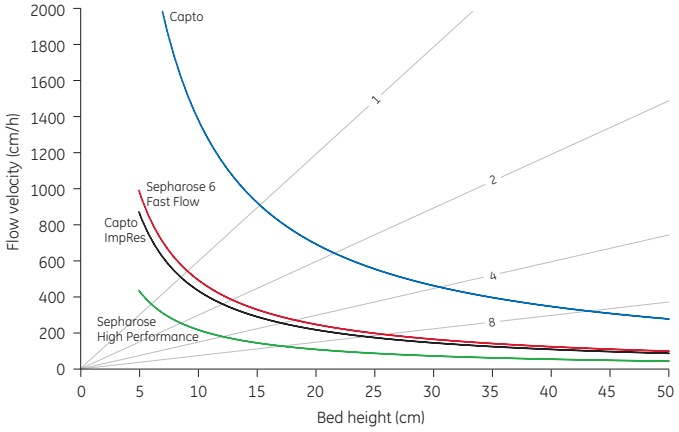

Fig 9. The window of operation (area below the curves) of different media from GE Healthcare. Data correspond to a process diameter column at 20°C and viscosity equivalent to water. Gray contours give the residence time in the column in minutes.

Columns

Capto MMC ImpRes can be used with most modern chromatography equipment from laboratory to production scale. Due to the higher rigidity of Capto MMC ImpRes, packing procedures in pilot- and process-scale columns differ slightly compared to Sepharose High Performance media (for details of packing laboratory-scale columns, see the appropriate instructions). There are also differences in packing procedures between pilot- and production-scale columns. Table 5 lists suitable empty columns from GE Healthcare.

Table 5. GE Healthcare column families for packing with Capto MMC ImpRes

Column	Inner diameter (mm)
Lab scale	
Tricorn 5/100	5
Tricorn 10/100	10
HiScale™ 16/20	16
HiScale 16/40	16
HiScale 26/20	26
HiScale 26/40	26
HiScale 50/20	50
HiScale 50/40	50
Production scale	
AxiChrom™	50 to 200
AxiChrom[1]	300 to 1000
BPG	100 to 300
Chromaflow™	400 to 600

[1] Maximum bed height for AxiChrom 1000 is 20 cm.

High-throughput process development

Using small-scale formats to screen for the most suitable chromatography media and/or process conditions in the early stages of process development saves both time and sample. Capto MMC ImpRes is available in prefilled 96-well PreDictor plates, which support HTPD by allowing convenient parallel screening of chromatographic conditions such as pH and conductivity. The medium is also available in PreDictor RoboColumn™ format. These miniaturized columns are prepacked with different BioProcess media for HTPD using robotic liquid handling workstations. Capto MMC ImpRes is also available in the small prepacked column formats, HiTrap™ (1 mL) and HiScreen (4.7 mL). Together with a chromatography system such as ÄKTA™ avant or ÄKTA pure, prepacked HiTrap and HiScreen columns are convenient to use when developing an efficient and robust purification method. Further development and optimization using Tricorn or HiScale columns then permits straightforward scale-up.

Cleaning and sanitization

Cleaning-in-place (CIP) is a procedure that removes contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Capto MMC ImpRes withstands the following CIP agents at the concentrations given: 1 M NaOH, 2 M NaCl, 5% 1-propanol, 30% isopropanol, 70% ethanol.

Regular CIP prevents the accumulation of contaminants in the medium bed and helps to maintain the capacity, flow properties, and general performance of Capto MMC ImpRes. An acidic strip using, for example, 0.1 M sodium acetate, pH 3.0, is recommended before CIP. Cleaning-in-place is normally recommended after each cycle. 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.

In a Capto MMC ImpRes lifetime study, the DBC at 10% breakthrough remained stable over 300 cycles of CIP using 1 M NaOH (Fig 10). The chromatographic method consisted of sample application of 30 mg human IgG (hIgG), elution of adsorbed material, and CIP with 1 M NaOH corresponding to 30 min contact time. This was repeated 300 times and DBC, yield, and carry-over were measured every 50 cycles. The results clearly demonstrate the stability of the medium over many purification cycles, which contributes to an overall improved process economy. No carry-over between different purification cycles was noted.

Column:	Tricorn 5/20
Medium:	Capto MMC ImpRes
Sample:	Human IgG (hIgG), Gammanorm™ (Octapharma)
Sample load:	30 mg hIgG/mL chromatography medium
Start buffer:	50 mM sodium acetate, pH 5.0
Elution buffer:	25 mM sodium citrate, 25 mM sodium phosphate, 1 M NaCl, pH 8.0
CIP:	1 M NaOH
System:	ÄKTAexplorer 10

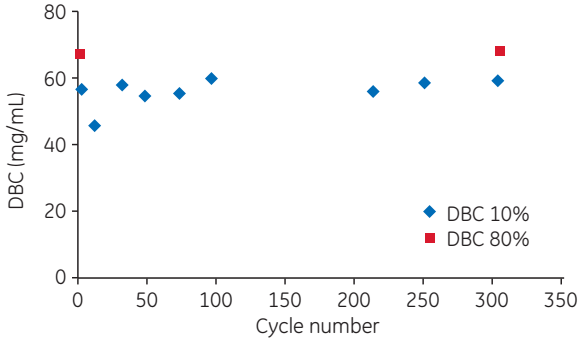

Fig 10. Capto MMC ImpRes lifetime study of dynamic binding capacity over multiple CIP cycles.

BioProcess chromatography media

Capto MMC ImpRes is a BioProcess chromatography medium, a family of purification media widely used by biopharmaceutical manufacturers. Support for these products includes validated manufacturing methods, secure long-term media supply, safe and easy handling, and Regulatory Support Files (RSF) to assist process validation and submissions to regulatory authorities. Further, the Fast Trak Training & Education team provide high level, hands-on training for all key aspects of BioProcess development and manufacturing.

Ordering information

Product	Quantity	Code number
Capto MMC ImpRes	25 mL	17-3716-01
	100 mL	17-3716-02
	1 L	17-3716-03
	5 L	17-3716-04
	10 L	17-3716-05
PreDictor Capto MMC ImpRes, 6 µL	4 × 96-well filter plates	17-3716-30
PreDictor Capto MMC ImpRes, 20 µL	4 × 96-well filter plates	17-3716-31
PreDictor RoboColumn Capto MMC ImpRes, 200 µL	One row of 8 columns	17-3716-40
PreDictor RoboColumn Capto MMC ImpRes, 600 µL	One row of 8 columns	17-3716-41
HiTrap Capto MMC ImpRes	5 × 1 mL	17-3716-10
HiScreen Capto MMC ImpRes	1 × 4.7 mL	17-3716-20

Related literature

Application notes

Polishing of monoclonal antibodies in bind and elute mode using Capto MMC ImpRes	29-0273-49
Polishing of monoclonal antibodies in bind and elute mode using Capto adhere ImpRes	29-0273-38

Data files

Capto adhere ImpRes	29-0344-97
Capto adhere	28-9078-88
Capto MMC	11-0035-45
Capto SP ImpRes, Capto Q ImpRes	28-9837-63
PreDictor 96-well filter plates and Assist software	28-9258-39
PreDictor RoboColumn	28-9886-34
HiScreen prepacked columns	28-9305-81
HiScale columns	28-9755-23
AxiChrom columns	28-9290-41
BPG columns	18-1115-23

Selection guide

Prepacked chromatography columns for ÄKTA systems	28-9317-78

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