Capto™ MMC ImpRes resin

MULTIMODAL CHROMATOGRAPHY

Capto™ MMC ImpRes (Fig 1) is a BioProcess™ mixed-mode chromatography 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 resin 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 resin 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).

Key features and benefits of Capto MMC ImpRes resin are:

- Designed for mixed-mode chromatography (multimodal chromatography)
- 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 resin 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. Cytiva's platform approach to mAb production employs protein A chromatography resins such as

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

MabSelect PrismA™ for capture of the target. For the polishing purification steps, ion exchange or multimodal resins are most commonly used. Multimodal resins are powerful tools because 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 resin can be an advantageous option for the first polishing step in a mAb purification process.

Characteristics of Capto MMC ImpRes resin

Bead size optimized for high-resolution polishing

Capto MMC ImpRes mixed-mode resin is based on the established high-flow agarose matrix, which gives excellent pressure/flow properties. The rigid matrix of Capto MMC ImpRes resin 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 resin.

Multimodal ligand

The multimodal cation exchanger ligand of Capto MMC ImpRes resin 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, that needs to be optimized. Figure 3 shows a result from a static binding capacity (SBC) study for a mAb using Capto MMC ImpRes resin at various pH values and sodium chloride concentrations. Using the same strategies as regular ion exchange chromatography may lead to misleading conclusions during screening and lead to unoptimized processes. To understand how the ligand and the target interact, screening for pH and conductivity simultaneously is recommended in high-throughput process development (HTPD) formats. Capto MMC ImpRes resin 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 resin 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 resin. Another effect of the lower ligand density is reduced salt tolerance, which simplifies elution from Capto MMC ImpRes resin with salt leading to higher yield and smaller pool volumes. Capto MMC ImpRes resin 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.

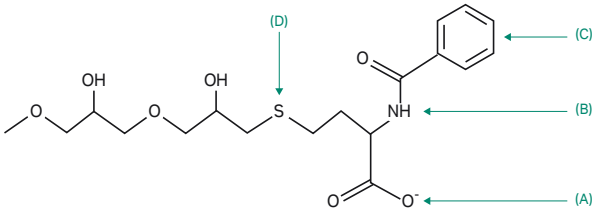

Fig 2. The Capto MMC ImpRes ligand exhibits multimodal functionality for interaction with a target molecule. The most pronounced of these interactions are (A) ionic, (B) hydrogen bonding, (C) hydrophobic interactions, and (D) thiophilic interactions. The chromatography resin is designed for polishing,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

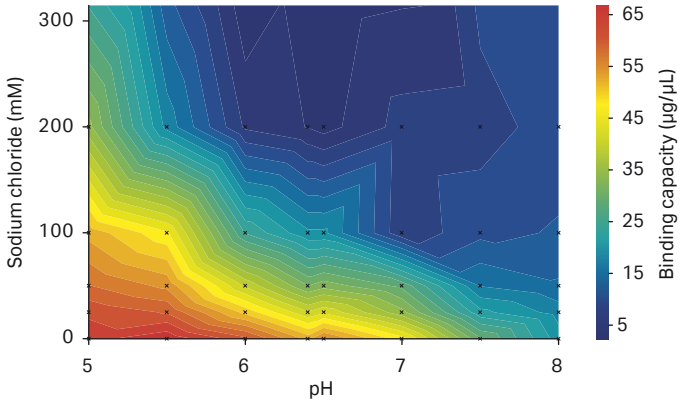

Fig 3. Binding capacity of Capto MMC ImpRes resin and for analyzed mAb when pH and conductivity are varied. Colors indicate a relative scale for the binding capacity of the resin, where red indicates a high binding capacity and blue a low binding capacity.

The combination of high resolution and high binding capacity provided by Capto MMC ImpRes resin 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 resin.

Table 1. Characteristics of Capto MMC ImpRes resin

Matrix	High-flow agarose
Ligand	Multimodal weak cation exchanger
Average particle size	36 to 44 µm (d_{50v})*
Binding capacity/mL chromatography resin	60 to 90 mg mAb/mL (residence time 4 to 5 min)
Ionic capacity	25 to 39 µmol/mL
pH stability	
working range	3 to 12†
cleaning-in-place (CIP)	2 to 14‡
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

* d_{50v} is the median particle size of the cumulative volume distribution.
† pH interval where the resin can be operated without significant change in function.
‡ pH interval where the resin can be subjected to cleaning-in-place without significant change in function.

Capto MMC ImpRes resin is a member of the Capto family of Cytiva mixed-mode resins

Figure 4 is an overview of Capto multimodal resins from Cytiva. Information about the other mixed-mode resins presented in this image can be found on their corresponding data files (see last page).

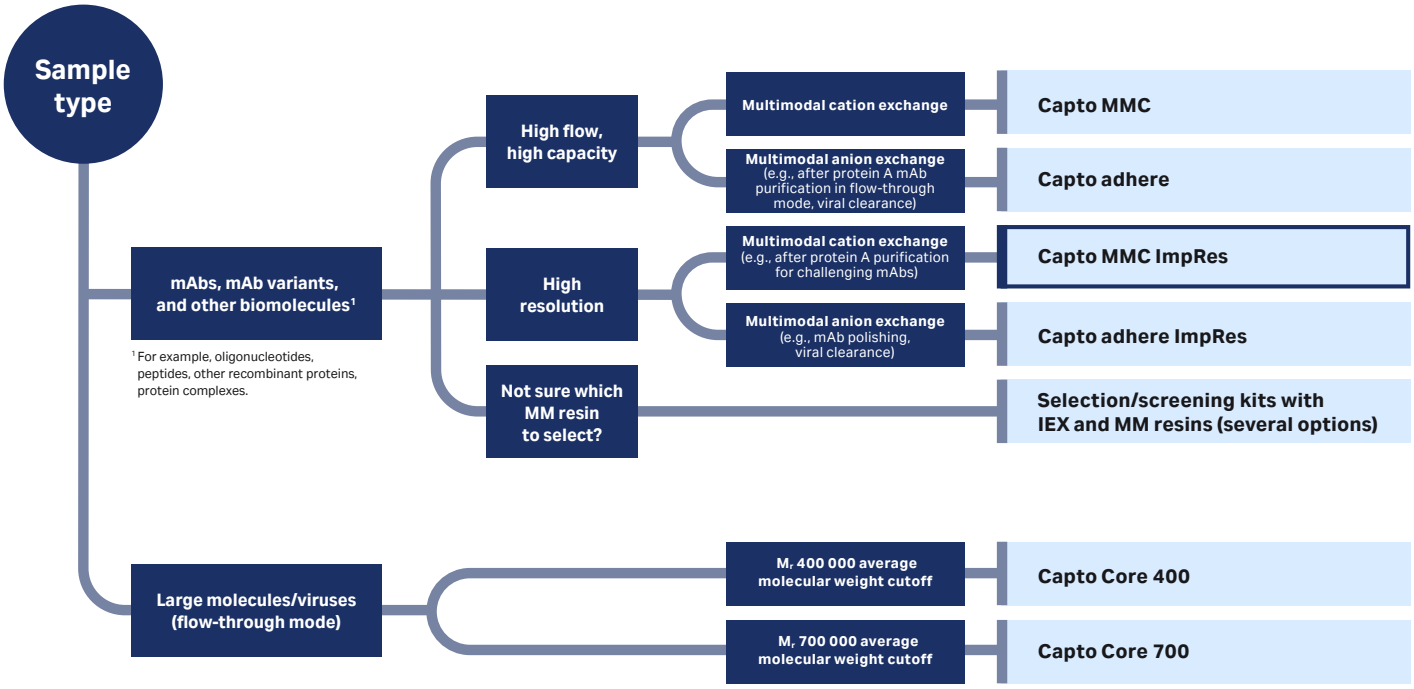

Fig 4. Overview of Capto multimodal resins.

Efficient aggregate removal

High antibody titers often increase the amount of aggregates and other contaminants such as HCP in the cell culture feedstock. Chromatography resins 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 resin 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 resin

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 resin compared with Capto SP ImpRes resin

To further evaluate the value of Capto MMC ImpRes resin, 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 resins; sample load 30 mg/mL (Capto MMC ImpRes resin), 40 mg/mL (Capto SP ImpRes resin)

Chromatography resin	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 resin delivered an aggregate concentration of 0.3% compared to 0.5% aggregates for Capto SP ImpRes resin. Both resins reduced HCP equally effectively.

Chromatograms showing the relative resolution in removal of aggregates for Capto MMC ImpRes and Capto SP ImpRes resins are shown in Figure 5. The chromatograms show that aggregates elute at the tail for the peak for both resins (green traces).

Column:	Tricorn 5/20, 0.5 mL
Resins:	Capto MMC ImpRes and Capto SP ImpRes
Sample:	mAb sample, partially purified by protein A chromatography step
Sample loads:	30 mg/mL chromatography resin (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:	ÄKTA™ system

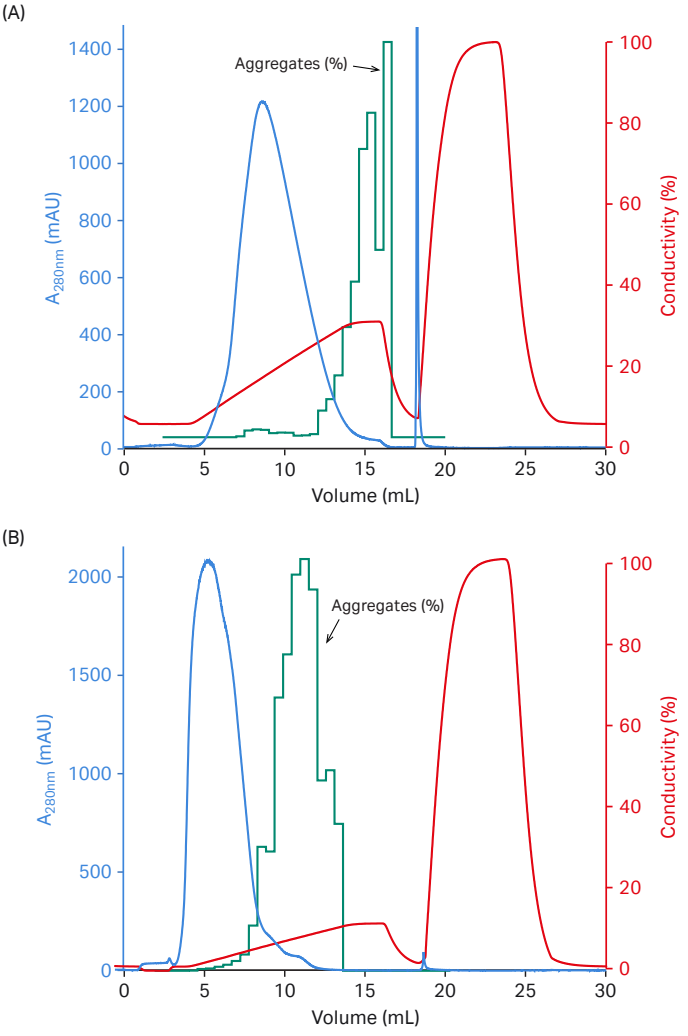

Fig 5. Chromatograms showing polishing of mAb using (A) Capto MMC ImpRes resin and (B) Capto SP ImpRes resin.

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 resins compared to ion exchangers (Fig 6). Depending on the required purity level, yield improvements by using Capto MMC ImpRes resin instead of conventional ion exchangers can in some cases be substantial, as shown in Figure 6. This translates directly to improved overall process yields and therefore also improved total process economy.

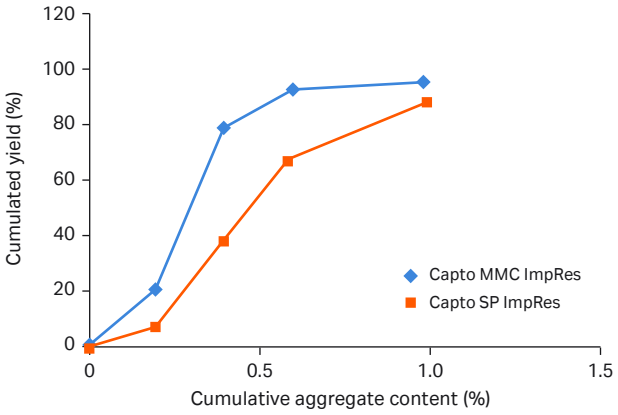

Fig 6. Cumulative yield vs cumulative aggregate content for mAb polishing using Capto MMC ImpRes and Capto SP ImpRes resins.

Different selectivity and higher salt tolerance

The multimodal functionality of Capto MMC ImpRes resin 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 resin 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 resin, 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 7. 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 resin
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

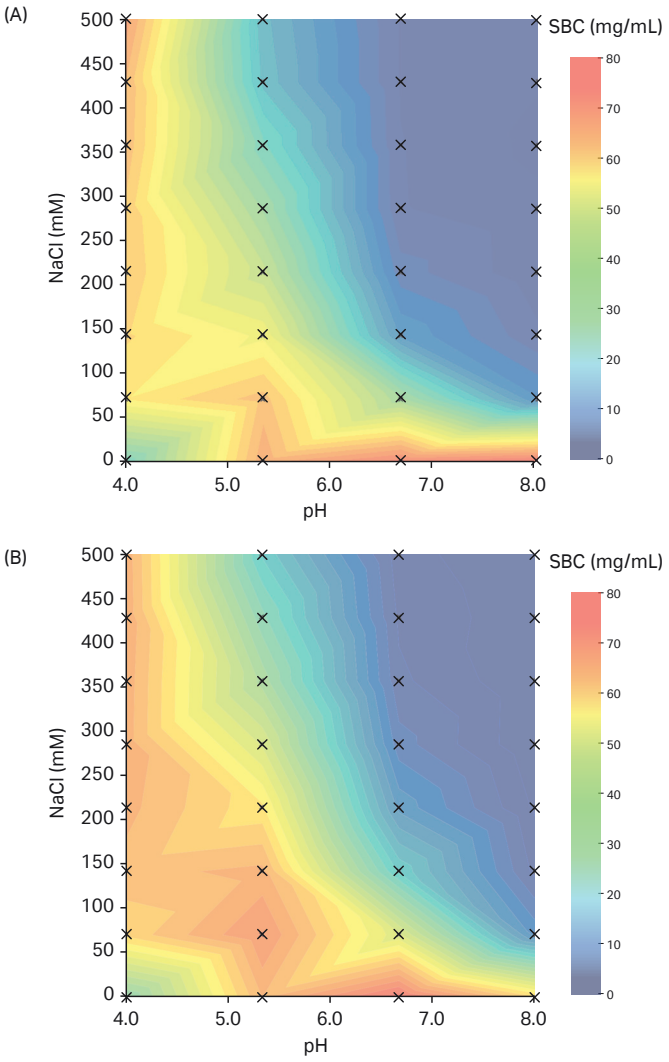

Fig 7. Contour maps showing SBC as affected by pH and NaCl concentration of (A) mAb 3 and (B) mAb 4 on Capto MMC ImpRes resin.

Robust dynamic binding capacity over a wide pH range

The high binding capacity of Capto MMC ImpRes resin 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 resin 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 8]) 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.

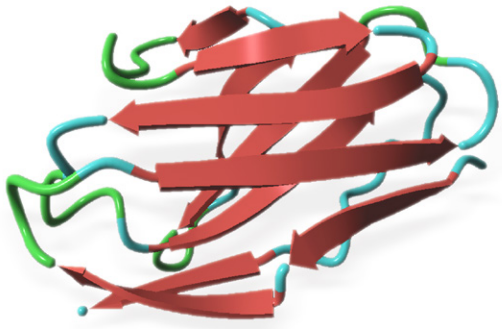

Fig 8. Structure of a recombinant DAb.

Wide window of operation for DAbs

The performance of Capto MMC ImpRes resin was evaluated in a study where the resin was used after an initial DAb capture step using Capto L resin. 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 resins 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 9), with the red areas on the contour maps showing optimal binding conditions while blue areas show optimal elution conditions.

Figure 9 shows the DAb binding capacities for Capto MMC ImpRes and Capto SP ImpRes resins. Both resins showed a large pH range for binding. However, Capto MMC ImpRes resin had a larger window of operation for 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 resin
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

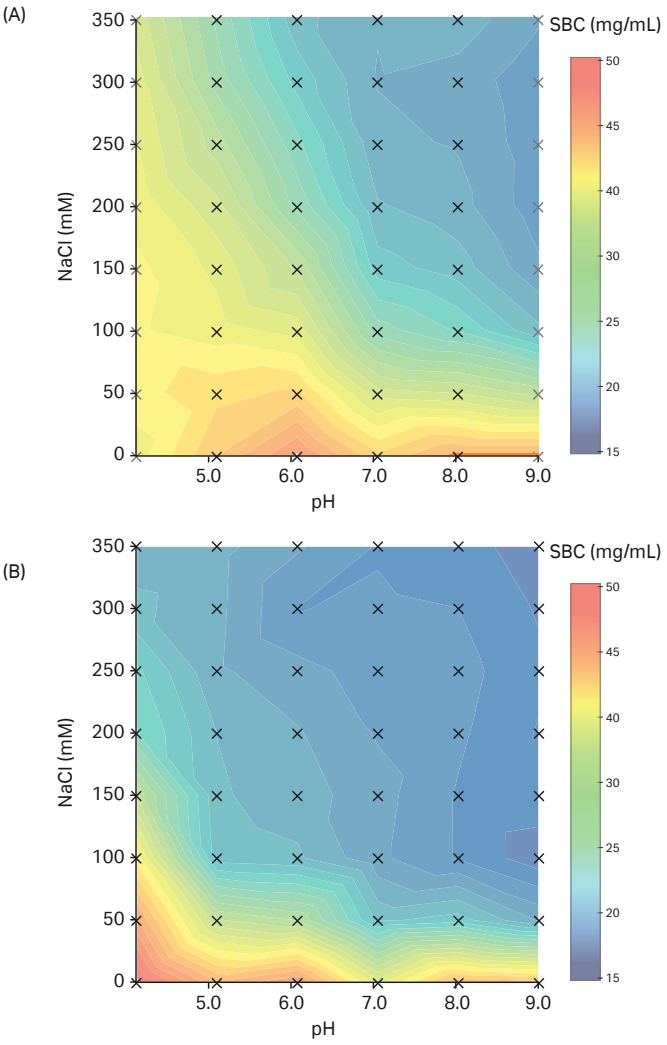

Fig 9. Contour maps showing binding capacity of DAb on (A) Capto MMC ImpRes resin and (B) Capto SP ImpRes resin at different pH levels and NaCl concentrations.

Removal of *E. coli* protein contaminants

Capto MMC ImpRes resin 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 resin, DAb sample was applied to Capto MMC ImpRes resin at a load of 20 mg/mL, pH 5.0. As shown in Figure 9, 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 10. The results showed improved ECP clearance at 125 mM NaCl without major impact on yield.

Column:	Tricorn 5/50, 1 mL
Resin:	Capto MMC ImpRes
Sample:	Capto L purified DAb
Sample loads:	20 mg/mL chromatography resin
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:	ÄKTA™ system

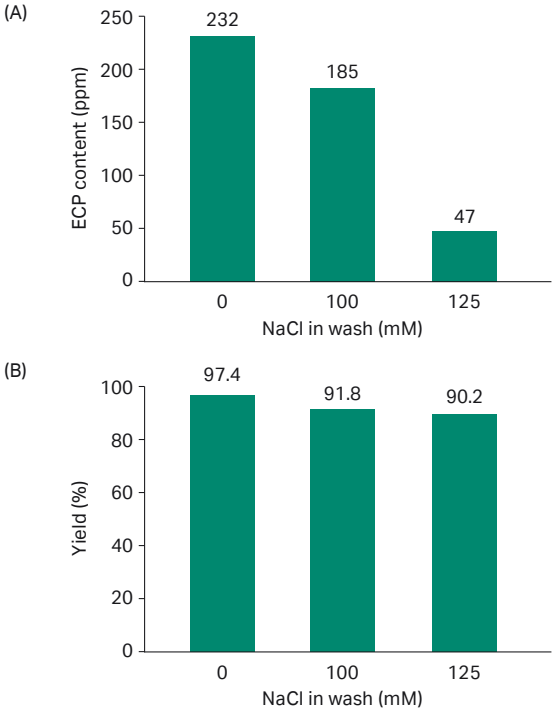

Fig 10. Purification of a recombinant DAb using Capto MMC ImpRes resin. (A) *E. coli* protein contaminants in the elution pool and (B) DAb yield using different NaCl concentrations in the binding and wash buffers.

Operation

Bed heights and flow velocities

The freedom available in process design for a given chromatography resin can be illustrated as its "window of operation." Figure 11 shows the relationship between column bed height and operating flow velocity for Capto ImpRes and Sepharose™ High Performance matrices. Both resins 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 resins are composed of larger beads and do not possess the high resolution provided by Capto MMC ImpRes resin. The size of the area below the pressure-limit curves represents the window of operation, that is, the available operating range for the respective resin. As Figure 11 shows, the window of operation of Capto MMC ImpRes resin suits most needs both in terms of bed height and flow velocities.

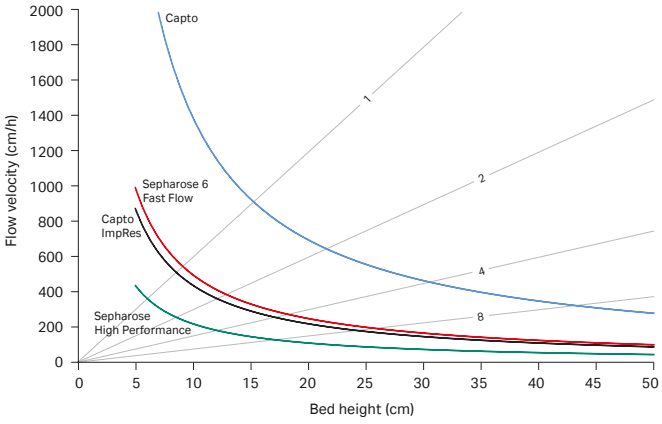

Fig 11. The window of operation (area below the curves) of different resins from Cytiva. Data correspond to a process diameter column at 20°C and viscosity equivalent to water. Gray contours give the residence time in the column in minutes.

Columns

Capto MMC ImpRes resin can be used with most modern chromatography equipment from laboratory to production scale. Due to the higher rigidity of Capto MMC ImpRes resin, packing procedures in pilot- and process-scale columns differ slightly compared to Sepharose High Performance resins (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 Cytiva.

Table 5. Cytiva column families for packing with Capto MMC ImpRes resin

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.

Small-scale formats for fast screening and method development

Using small-scale format to screen for the most suitable chromatography process conditions in the early stages of process development saves both time and sample. Capto MMC ImpRes resin is available in multiple formats that are suitable for process development.

Prepacked formats for high-throughput process development (HTPD):

- PreDictor 96-well filter plates (96 purifications in parallel under static conditions)
- PreDictor RoboColumn® units (8 purifications in parallel under dynamic conditions)

Prepacked formats for method optimization and parameter screening:

- HiTrap™ columns (1 or 5 mL)
- HiScreen™ columns (4.7 mL)

Kits for resin variability studies:

- Process Characterization Kits (3 bottles of 25 mL, with 3 different ligand densities).

Prepacked and precharacterized formats for mechanistic modeling:

- *f*(x) columns (10 × 100 mm or 10 × 200 mm)

Prepacked formats for scale-up and GMP manufacturing

Capto MMC ImpRes resin is also available in ReadyToProcess™ column formats, which are validated high performance prepacked columns for scale-up and GMP biomanufacturing.

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 resin 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 resin bed and helps to maintain the capacity, flow properties, and general performance of Capto MMC ImpRes resin. 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 resin lifetime study, the DBC at 10% breakthrough remained stable over 300 cycles of CIP using 1 M NaOH (Fig 12). 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 resin 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
Resin:	Capto MMC ImpRes
Sample:	Human IgG (hIgG), Gammanorm (Octapharma)
Sample load:	30 mg hIgG/mL chromatography resin
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:	ÄKTA™ system

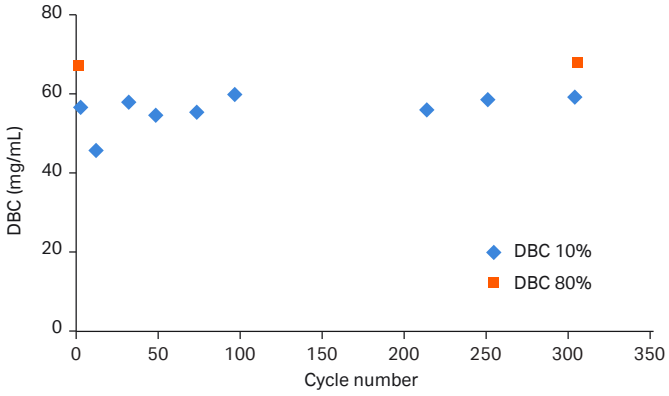

Fig 12. Capto MMC ImpRes resin lifetime study of dynamic binding capacity over multiple CIP cycles.

BioProcess chromatography resins

Capto MMC ImpRes resin is a BioProcess chromatography resin, a family of purification resins widely used by biopharmaceutical manufacturers. Support for these products includes validated manufacturing methods, secure long-term resins 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.

Additional reading

Visit our website to explore our application notes showcasing our multimodal resins.

- Mixed-mode chromatography resource center
- Capto high productivity resins
- Antibody production workflow

Ordering information

Format	Quantity	Product code
Bulk	25 mL	17371601
	100 mL	17371602
	1 L	17371603
	5 L	17371604
	10 L	17371605
PreDictor™ plate	6 µL 4 × 96-well filter plates	17371630
	20 µL 4 × 96-well filter plates	17371631
PreDictor RoboColumn™ unit	200 µL 8 columns	17371640
	600 µL 8 columns	17371641
HiTrap™ column	1 × 1 mL	29401108
	5 × 1 mL	17371610
HiScreen™ column	1 × 4.7 mL	17371620
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17371670
f(x) column for mechanistic modeling	10/100 mm	29723328
	10/200 mm	29722931
ReadyToProcess™ columns	1 L (80/200)	29101707
	2.5 L (126/200)	29101708
	5 L (178/200)	29146153
	10 L (251/200)	29101709
	20 L (359/200)	29101710
	57L (600/200)	29505248

Related literature

Application notes	
Polishing of monoclonal antibodies in bind and elute mode using Capto MMC ImpRes resin	CY13430
Polishing of monoclonal antibodies in bind and elute mode using Capto adhere ImpRes resin	CY13429
Tools and solutions for separation of charged mAb variants	CY13974
Data files	
Capto adhere ImpRes	CY11775
Capto adhere	CY11848
Capto MMC	CY13468
Capto SP ImpRes, Capto Q ImpRes	CY12674
PreDictor 96-well filter plates and Assist software	CY13663
PreDictor RoboColumn	CY13689
HiScreen prepacked columns	CY13473
HiScale columns	CY13664
AxiChrom columns	CY10003
BPG columns	CY978
Process Characterization Kits	CY8047
Selection guide	
Prepacked chromatography columns for ÄKTA systems	CY14019
Handbook	
Multimodal Chromatography Handbook	CY14738

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