

# Capto™ adhere ImpRes

## MULTIMODAL CHROMATOGRAPHY

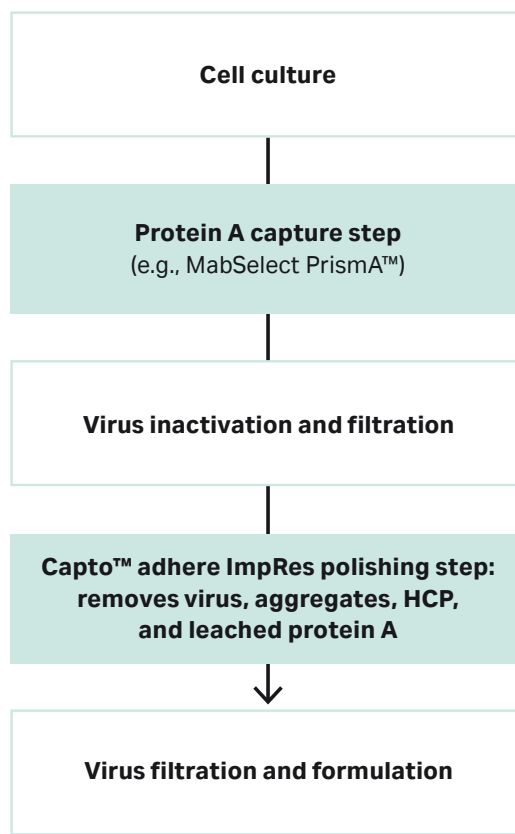
Capto™ adhere ImpRes is a BioProcess™ mixed mode chromatography resin for high-resolution polishing of monoclonal antibodies (mAbs) and other biomolecules. The strong anion exchange multimodal ligand displays high selectivity compared with traditional ion exchangers, which allows the possibility to solve challenging purification problems. Main contaminants in mAb processes such as DNA, host cell proteins (HCP), leached protein A, aggregates, and viruses are efficiently separated. Capto™ adhere ImpRes resin is an excellent choice for purification of mAb with high aggregate content. The efficient virus removal provided by the chromatography resin also allows two-step purification of mAbs with Capto™ adhere ImpRes resin as the final polishing step (Fig 1). Polishing can be performed in either bind and elute (binding) or flowthrough (nonbinding) modes.

### Key features and benefits of Capto™ adhere ImpRes resin are:

- Appropriate 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
- Allows separation of mAb charged variants

## 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 costs in process development. The mAb production toolbox from Cytiva's business employs protein A chromatography resins such as MabSelect PrismA™ for capture of the target.



**Fig 1.** Capto™ adhere ImpRes is a polishing resin for highly efficient removal of contaminants such as DNA, HCP, leached protein A, aggregates, and viruses.

Capto™ adhere ImpRes resin expands Cytiva's mAb purification toolbox. The resin is designed to be used after the capture step in a two- or three-step purification approach. Moreover, Capto™ adhere ImpRes resin enables work in bind/elute (B/E) or flowthrough (FT) modes to give good yield and purity of mAbs. In B/E mode, the high resolution of the small beads is utilized for separation of monomeric antibodies from contaminants during elution. In FT mode, mAbs pass directly through the column while contaminants are bound.

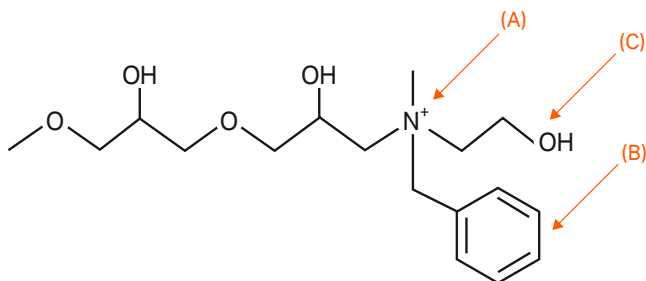
# Characteristics of Capto™ adhere ImpRes resin

## Bead size optimized for high-resolution polishing

Capto™ adhere ImpRes mixed-mode resin is based on the established high-flow agarose matrix, which gives excellent pressure/flow properties. The rigid matrix of Capto™ adhere ImpRes resin allows high flow velocities in mAb polishing 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 anion exchanger, Capto™ adhere.

## Multimodal ligand

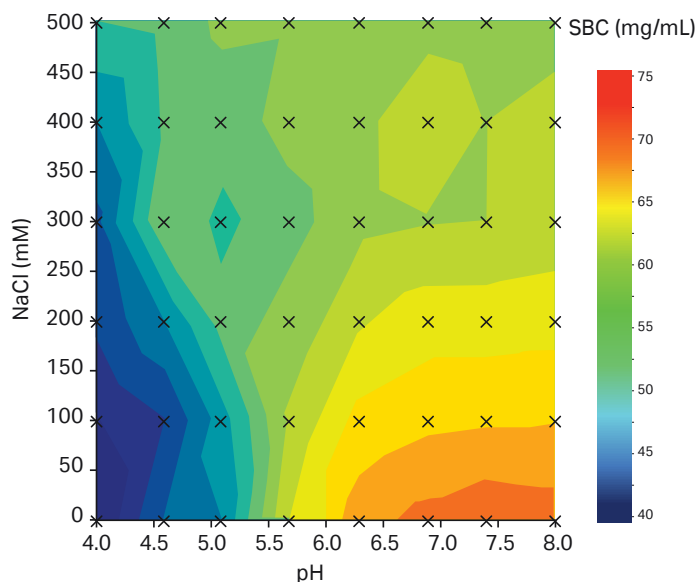
The multimodal anion exchanger ligand of Capto™ adhere ImpRes chromatography resin is immobilized to the base matrix (Fig 2) and interacts with the target molecule through multiple types of interactions. Ionic interactions are commonly involved, but others such as hydrogen bonding and hydrophobic interactions can be significant. The strength of these individual interactions often depends on the process conditions.



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

The combination of high resolution and high binding capacity provided by Capto™ adhere ImpRes resin results in higher load densities, small elution pool volumes, and improved impurity removal compared with the related multimodal anion exchanger, Capto™ adhere. Process economy is improved through higher yields, reduced buffer consumption, and the use of smaller columns and buffer tanks.

Figure 3 shows a result from a static binding capacity study of a mAb using Capto™ adhere ImpRes resin at different pH and sodium chloride concentrations. This example shows that with a sample applied at maximum binding capacity, achieving high yields by only changing NaCl concentration is difficult, because the electrostatic interaction would switch to a hydrophobic interaction as conductivity increases. Therefore, using same strategies as a regular ion exchange chromatography may lead to an unoptimized process. To get an understanding of how the ligand and the target interact, it is therefore recommended to screen a combination of pH and conductivity.



**Fig 3.** Static binding capacity at various pH and sodium chloride concentrations. Loading at the highest pH without salt gives the highest capacity. The surface plot also shows that increasing the salt for elution is not enough. Instead, the pH value should be lowered to increase the yield of the mAb. To maximize the purity, altering both pH and salt is needed.

Table 1 summarizes the characteristics of Capto™ adhere ImpRes mixed mode resin.

**Table 1.** Characteristics of Capto™ adhere ImpRes resin

Matrix	High-flow agarose
Ligand	Multimodal strong anion exchanger
Average particle size	36–44 µm ( $d_{50}$ )*
Binding capacity/mL chromatography resin	45–85 mg mAb/mL (residence time 4–5 min, pH ~8)
Ionic capacity	0.08–0.11 mmol Cl <sup>-</sup> /mL resin
pH stability	
working range	3–12 <sup>†</sup>
cleaning-in-place (CIP)	2–14 <sup>‡</sup>
Pressure/flow specification	300 kPa at min. 220 cm/h, 1 m diameter column, 20 cm bed height
Storage conditions	4°C to 30°C in 20% ethanol
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH, 2 M NaCl, 5% 1-propanol, 30% isopropanol, 70% ethanol
Avoid using	Oxidizing agents, anionic detergents

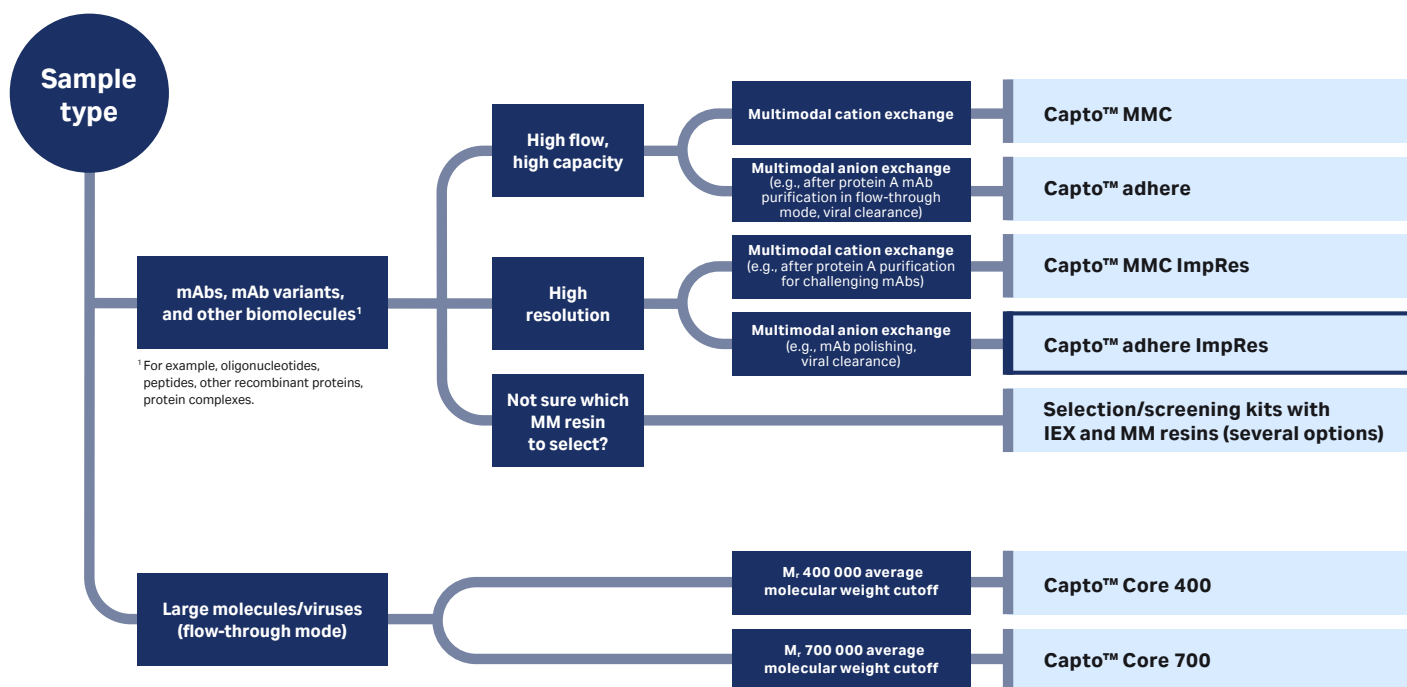
\*  $d_{50}$  is the median particle size of the cumulative volume distribution.

<sup>†</sup> pH interval where the resin can be operated without significant change in function.

<sup>‡</sup> pH interval where the resin can be subjected to cleaning-in-place without significant change in function.

## Capto™ adhere ImpRes resin is a member of the family of Cytiva Capto™ mixed mode resins

Figure 4 is an overview of Cytiva Capto™ multimodal resins. 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

Removal of contaminants such as aggregates, HCP, and leached protein A is performed in either B/E or FT mode.

#### Bind/elute mode

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 effective removal of such contaminants while retaining high yield.

Results from a step elution experiment using Capto™ adhere ImpRes resin in B/E mode for removal of contaminants from a mAb

partly purified by protein A affinity chromatography are shown in Table 2. Fractions from the polishing step were collected, pooled, and analyzed for yield, aggregate, protein A, and HCP content.

The results show the low elution volumes achieved using Capto™ adhere ImpRes resin in step-elution polishing while maintaining high yields. Removal of aggregates, leached protein A, and HCP in B/E mode with Capto™ adhere ImpRes resin was shown to be efficient. A longer residence time of 4 min improved removal of aggregates compared with a 2 min residence time. However, running with 2 min residence time and thus shorter bed heights allows shorter run times and higher productivity.

**Table 2.** Results from polishing of mAb using Capto™ adhere ImpRes in B/E mode; sample load 80 mg/mL

Residence time (min)	Elution pool volume (column volumes, CV)	Monomer yield (%)	Aggregate content (%)		HCP (log <sub>10</sub> reduction)	Protein A (ppm)
			Start	Final		
2	4.5	89.0	4.5	0.4	2.0	Below LOQ*
4	3.6	90.0	4.5	0.3	2.0	Below LOQ

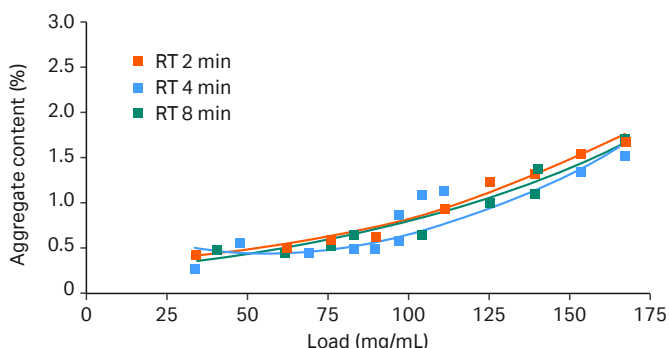
\* LOQ = limit of quantitation

## Flowthrough mode

Column experiments for Capto™ adhere ImpRes resin in FT mode were performed where fractions were analyzed by gel filtration during sample load. Figure 5 shows robust aggregate clearance over the range of residence times tested. Based on this experiment, it could be concluded that a sample load of 80 mg/mL would be suitable.

The efficiency of Capto™ adhere ImpRes resin for removal of aggregates and other contaminants in FT mode at different residence times was compared with that of Capto™ adhere resin. The flowthrough pools were collected and analyzed for yield, aggregate content, HCP content, and protein A concentration. Both resins displayed efficient mAb purification. However, Capto™ adhere ImpRes in FT mode resulted in higher yield and lower pool volumes with similar purity (Table 3). A shorter residence time (higher flow velocity) of 2 min could be used with Capto™ adhere ImpRes resin, although this resulted in slightly poorer clearance of aggregates.

**Column:** Tricorn™ 5/50, bed height 5 cm, column volume ~ 1 mL  
**Resin:** Capto™ adhere ImpRes  
**Sample:** mAb sample, partially purified using MabSelect SuRe™  
**Sample load:** 80 mg/mL chromatography resin  
**Start buffer:** 100 mM acetate, 335 mM NaCl, pH 5.5  
**Strip buffer:** 100 mM acetate, pH 3.0  
**Residence times:** 2, 4, 8 min  
**System:** ÄKTA™ system  
**Detection:** Protein-containing fractions detected at A<sub>280</sub> nm during sample load were analyzed by gel filtration



**Fig 5.** Concentration of aggregates as a function of sample load and residence times (RT) using Capto™ adhere ImpRes resin.

## Viral clearance

The capability of Capto™ adhere ImpRes resin for viral clearance from mAb was tested with two model viruses; the enveloped RNA retrovirus murine leukemia virus (MuLV) and the nonenveloped DNA parovirus minute virus of mice (MVM). mAb samples partially purified by protein A affinity chromatography were spiked with virus stock solution and were then applied to Capto™ adhere ImpRes resin in B/E or FT mode. Eluted fractions were analyzed for virus titer by endpoint titration and large-volume plating. Capto™ adhere ImpRes resin showed efficient viral clearance in both B/E and FT mode (Table 4). The log<sub>10</sub> virus reduction factor was approximately 5.0 in B/E mode for both MuLV and MVM. In FT mode, the log<sub>10</sub> virus reduction factor was > 4.0 for both MuLV and MVM.

**Table 4.** Viral reduction factor (log<sub>10</sub>) of murine leukemia virus (MuLV) and minute virus of mice (MVM) purified using Capto™ adhere ImpRes resin in B/E and FT mode

Process purification mode	Minimal log <sub>10</sub> viral reduction factor	
	MuLV	MVM
B/E*	4.98	4.95
FT†	> 5.0	4.0

\* B/E binding conditions: phosphate/citrate, pH 7.9 (binding); phosphate/citrate + 45 mM NaCl, pH 5.4 (elution)

† FT binding conditions: phosphate/citrate, pH 5.5, 19 mS/cm conductivity

## Robust load and binding capacities

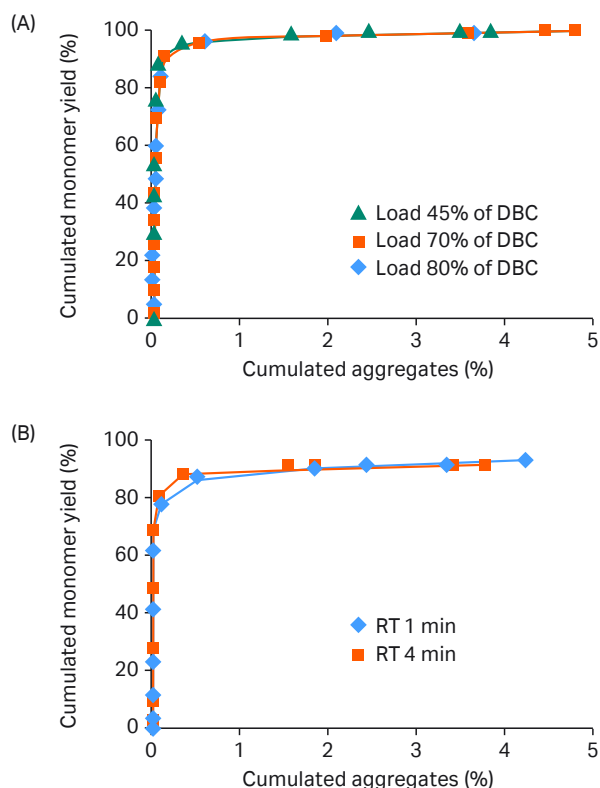
To evaluate the effect of load and residence time on the aggregate removal capacity of Capto™ adhere ImpRes resin, a study in B/E mode was performed. Sample load was varied between 45% and 80% of dynamic binding capacity (DBC) at residence times of 1 or 4 min. Fractions were collected and analyzed for aggregate concentration by analytical gel filtration. Cumulated yield of monomer was plotted against cumulated aggregates. The results show equivalent and robust separation of mAb monomers and aggregates, which were independent of load (Fig 6A) and residence time (Fig 6B).

The DBC at 10% breakthrough for Capto™ adhere ImpRes resin was also compared with that of Capto™ adhere resin at residence times of 2, 4, and 8 min. The results in Figure 7 show that Capto™ adhere ImpRes resin improved DBC compared with Capto™ adhere resin.

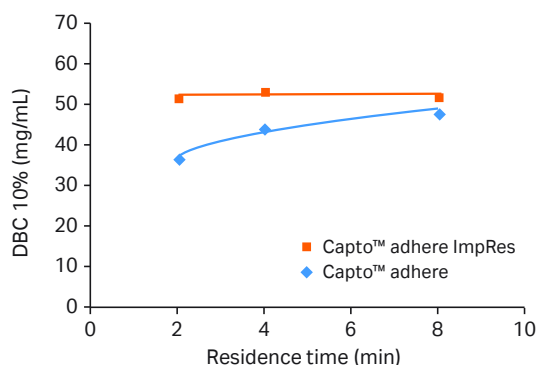
**Table 3.** Results from polishing of mAb using Capto™ adhere ImpRes and Capto™ adhere resins in FT mode; sample load 80 mg/mL

Chromatography resin	Residence time (min)	Elution pool volume (CV)	Monomer yield (%)	Aggregate content (%)	
				Start	Final
Capto™ adhere ImpRes	2	13.2	94	3.4	0.7
Capto™ adhere ImpRes	4	12.5	94	3.4	0.5
Capto™ adhere	4	14.8	91	3.4	0.5

**Column:** Tricorn™ 5/50, bed height 5 cm, column volume ~ 1 mL  
**Resin:** Capto™ adhere ImpRes  
**Sample:** mAb sample (9.4 mg/mL), partially purified by protein A affinity chromatography  
**Sample load:** Fig 6A, 20 to 35 mg/mL chromatography resin (corresponding to 45 to 80% of DBC at 10% breakthrough); Fig 6B, 45% of DBC  
**Start buffer:** 35 mM Tris, pH 8.0  
**Elution buffer:** 20 mM Tris, 20 mM citrate, 20 mM phosphate, 100 mM NaCl, pH 4.0  
**Gradient elution:** 0% to 100% in 20 to 30 column volumes (CV)  
**Residence times:** 4 min in Fig 4A; 1 min or 4 min in Fig 4B  
**System:** ÄKTA™ system



**Fig 6.** Cumulated yield of mAb monomer (%) vs cumulated mAb aggregates at (A) different sample loads and (B) residence times (RT) of 1 or 4 min during polishing on Capto™ adhere ImpRes resin.



**Fig 7.** Dynamic binding capacity at 10% breakthrough as a function of residence time. Comparison of Capto™ adhere ImpRes with Capto™ adhere resins.

## Detection of more mAb charged variants

The high resolution of the Capto™ ImpRes base matrix combined with the selectivity of the ligand allows detection of charged variants of mAb and allows their removal in B/E mode. In a gradient elution of mAb on Capto™ adhere ImpRes resin in B/E mode, the main peak, containing monomeric mAb, was divided into two peaks. The last peak (or shoulder) contained mAb aggregates (Fig 8A). Fractions from the elution peak were analyzed by analytical cation exchange chromatography for acidic, main, and alkaline charge isoforms. The relative recovery of each is shown in Figure 8B. During optimization for removal of aggregates, several more peaks of charged variants were observed (data not shown). With additional fine-tuning, it should therefore be possible to resolve more charged variants than shown in this study.

## Process economy

The smaller bead size of Capto™ adhere ImpRes resin offers a higher resolution than Capto™ adhere resin, and this impacts the yield. Improvements in yield for different mAbs with Capto™ adhere ImpRes resin have been greater than 3% (up to 12%) compared with Capto™ adhere resin when operated in B/E mode using a step elution protocol (Table 5). To assess the process economic impact of the higher yield, the process economic simulation tool BioSolve from Biopharm Services Limited was used with a typical mAb process as template. The impact of the yield improvement was studied, and the 3% improvement using Capto™ adhere ImpRes resin increased throughput from 17.5 kg per year to 18.5 kg and reduced production cost from USD \$372/g mAb to \$360/g mAb compared with Capto™ adhere resin (Table 6).

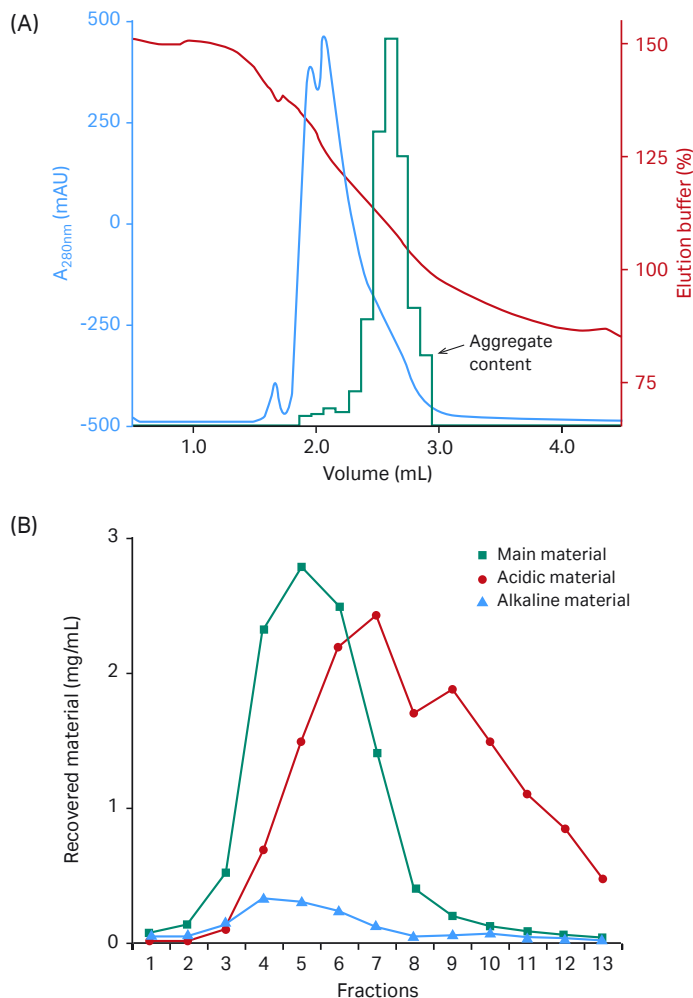
**Table 5.** Monomer yield from purification of three different mAbs on Capto™ adhere and Capto™ adhere ImpRes resins using a step-elution protocol

	Monomer yield (%)	
	Capto™ adhere	Capto™ adhere ImpRes
mAb 1	86	90
mAb 2	79	91
mAb 3	90	93

**Table 6.** Process economy for purification of mAb using Capto™ adhere compared with Capto™ adhere ImpRes resin

	Capto™ adhere	Capto™ adhere ImpRes
Product titer (g/L)	3	3
Target capacity utilization (%)	80	80
Production bioreactor working volume (L)	1000	1000
Estimated number of batches per year	12	12
Throughput per year (kg)	17.5	18.5
Production cost (\$/g mAb)	372	360

**Column:** Tricorn™ 5/100, bed height ~ 10 cm, volume ~ 2 mL  
**Resin:** Capto™ adhere ImpRes  
**Sample:** mAb sample, partially purified using MabSelect SuRe™ LX  
**Sample load:** 30 mg mAb/mL  
**Start buffer:** 35 mM Tris, pH 8.0  
**Elution buffer:** 20 mM Tris, 20 mM phosphate, 20 mM citrate, pH 4.0  
**Residence time:** 4 min  
**System:** ÄKTA™ system

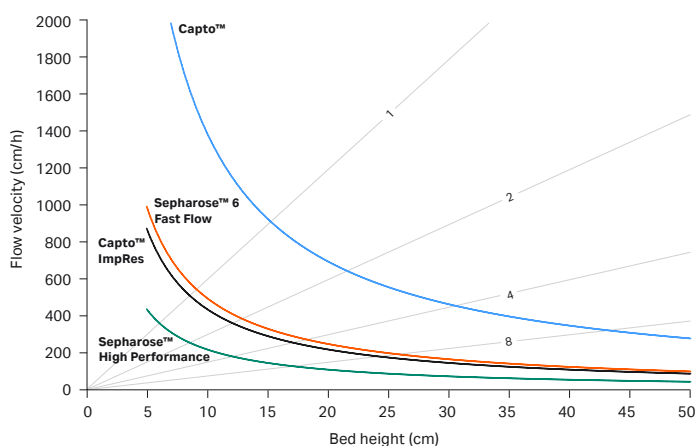


**Fig 8.** (A) Chromatogram showing gradient elution of mAb aggregates on Capto™ adhere ImpRes resin. (B) Relative recovery of main-, acidic-, and alkaline-charged variants in the pH gradient.

## 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 9 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  $\mu$ m vs 34  $\mu$ m) and therefore display high resolution, which is used 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™ ImpRes resin. The size of the area below the pressure-limit curve represents the window of operation, that is, the available operating range for the respective resin. As Figure 9 shows, the window of operation of Capto™ adhere ImpRes resin suits most needs both in terms of bed height and flow velocities.



**Fig 9.** The window of operation (area below the curve) of different chromatography 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™ adhere ImpRes resin can be used with most modern chromatography equipment from laboratory to production scale. Due to the higher rigidity of Capto™ adhere ImpRes resin, packing procedures in pilot- and process-scale columns differ slightly compared with 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 8 lists suitable empty columns from Cytiva.



**Table 8.** Cytiva column families for packing with Capto™ adhere ImpRes resin

Lab scale columns	Inner diameter (mm)
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 columns	Inner diameter (mm)
AxiChrom™	50 to 200
AxiChrom™*	300 to 1000
BPG	100 to 300
Chromaflo™	400 to 600

\* Maximum bed height for AxiChrom™ 1000 is 20 cm.

### Small-scale format provides 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™ adhere ImpRes resin is available in multiple formats that are suitable for process development.

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

- PreDicator™ 96-well filter plates (96 purifications in parallel under static conditions)
- PreDicator™ 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 format for scale-up and GMP manufacturing

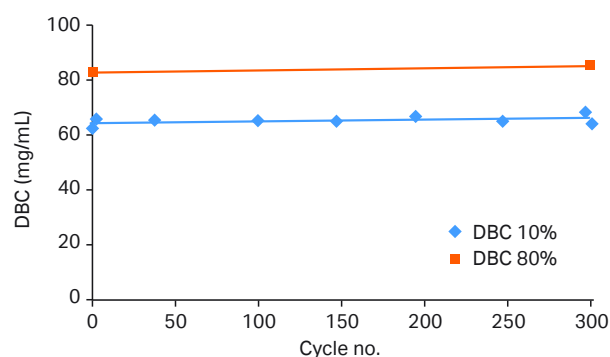
Capto™ adhere ImpRes resin is also available in ReadyToProcess™ column formats, which are validated high performance prepacked columns for scale-up and GMP biomanufacturing.

## 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™ adhere 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™ adhere 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™ adhere ImpRes resin lifetime study, the DBC remained stable over 300 cycles of CIP using 1 M NaOH. The results 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.

**Fig 10.** Resin lifetime study of dynamic binding capacity over multiple CIP cycles.

## BioProcess™ chromatography resins

Capto™ adhere ImpRes 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. 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](#)
- [ReadyToProcess™ columns](#)

# Ordering information

## Capto™ adhere ImpRes resin and prepacked formats

Format	Quantity	Product code
Bulk	25 mL	17371501
	100 mL	17371502
	1 L	17371503
	5 L	17371504
	10 L	17371505
PreDicator™ plate	6 µL 4 × 96-well filter plates	17371530
	20 µL 4 × 96-well filter plates	17371531
PreDicator™	200 µL 8 columns	17371540
RoboColumn unit	600 µL 8 columns	17371541
HiTrap™ column	1 × 1 mL	29400464
	5 × 1 mL	17371510
HiScreen™ column	1 × 4.7 mL	17371520
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17371570
<i>f</i> (x) column for mechanistic modeling	10/100 mm	29723329
	10/200 mm	29723450
ReadyToProcess™ columns	1 L (80/200)	29101703
	2.5 L (126/200)	29101704
	3.7 L (178/150)	29287573
	5 L (178/200)	29146151
	6.2 L (178/250)	29287574
	7.4 L (251/150)	29287572
	10 L (251/200)	29101705
	12.4 L (251/250)	29287575
	15 L (359/150)	29304093
	20 L (359/200)	29101706
	25 L (359/250)	29304094
	57 L (600/200)	29376125

## Related literature

### Application notes

Polishing of monoclonal antibodies using Capto™ adhere ImpRes in bind and elute mode	CY13429
Polishing of monoclonal antibodies using Capto™ MMC ImpRes in bind and elute mode	CY13430
A platform approach for the purification of domain antibodies (Dabs)	CY13501
Purification of monoclonal antibodies using modern chromatography media and membranes	CY13545
Three-step monoclonal antibody purification processes using modern chromatography media	CY13645
mAb polishing step development using Capto™ adhere ImpRes in bind-elute mode	CY20571

### Data files

Capto™ MMC ImpRes	CY13699
Capto™ adhere	CY11848
Capto™ MMC	CY13468
Capto™ SP ImpRes, Capto™ Q ImpRes	CY12674
PreDicator™ 96-well filter plates and Assist software	CY13663
PreDicator™ RoboColumn	CY13689
HiScreen™ prepacked columns	CY13473
HiScale™ columns	CY13664
Process Characterization Kits	CY8047
AxiChrom™ columns	CY10003
BPG columns	CY978
ReadyToProcess™ columns	CY11724

### Handbook

Multimodal Chromatography Handbook	CY14738
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### Selection guide

Prepacked chromatography columns for ÄKTA™ systems Selection guide	CY14019
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### White paper

Navigate the road mAb	CY13545
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CY11775-13Apr23-DF

