Multimodal chromatography

Capto™ adhere ImpRes

Capto adhere ImpRes is a BioProcess™ chromatography medium (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 is an excellent choice for purification of MAb with high aggregate content. The efficient virus removal provided by the chromatography medium also allows two-step purification of MAbs with Capto adhere ImpRes 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 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
- Allows separation of MAb charged variants

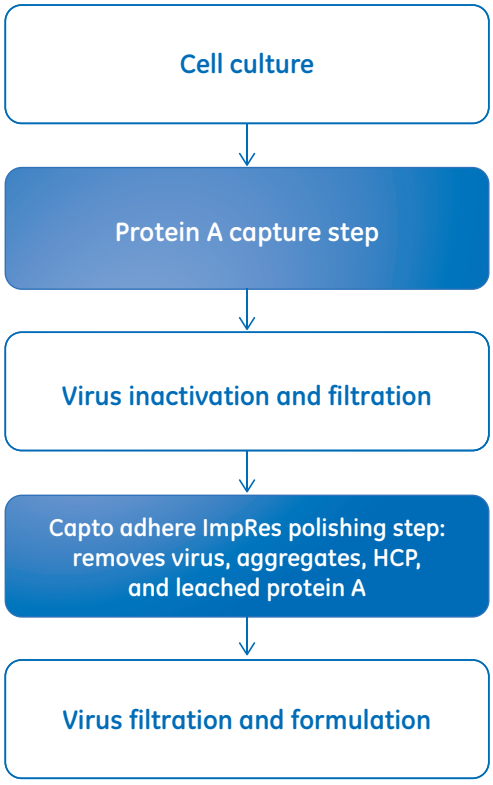

Fig 1. Capto adhere ImpRes is a polishing medium for highly efficient removal of contaminants such as DNA, HCP, leached protein A, aggregates, and viruses.

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. The MAb production toolbox from GE Healthcare's Life Sciences business employs protein A chromatography media such as MabSelect SuRe™ or MabSelect SuRe LX for capture of the target.

Capto adhere ImpRes expands GE's MAb purification toolbox. The medium is designed to be used after the capture step in a two- or three-step purification approach. Moreover, Capto adhere ImpRes 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

Bead size optimized for high-resolution polishing

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

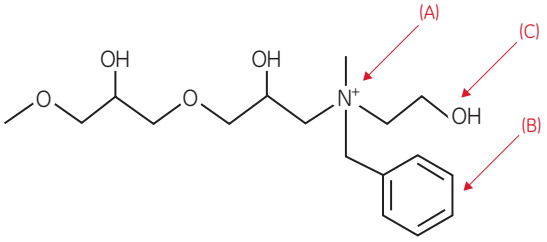

Fig 2. The Capto adhere ImpRes ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interactions (A), hydrophobic interactions (B), and hydrogen bonding (C). The chromatography medium is designed for polishing and is based on the high-flow agarose 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 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.

Table 1 summarizes the characteristics of Capto adhere ImpRes.

Table 1. Characteristics of Capto adhere ImpRes

Matrix	High-flow agarose
Ligand	Multimodal strong anion exchanger
Average particle size	36–44 µm (d_{50v})[1]
Binding capacity/mL chromatography medium	45–85 mg MAb/mL (residence time 4–5 min, pH ~8)
Ionic capacity	0.08–0.11 mmol Cl^-/mL medium
pH stability	
working range	3–12[2]
cleaning-in-place (CIP)	2–14[3]
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

[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

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 media 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 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.

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_{10} reduction)	Protein A (ppm)
			Start	Final		
2	4.5	89.0	4.5	0.4	2.0	Below LOQ[1]
4	3.6	90.0	4.5	0.3	2.0	Below LOQ

[1] LOQ = limit of quantitation

The results show the low elution volumes achieved using Capto adhere ImpRes in step-elution polishing while maintaining high yields. Removal of aggregates, leached protein A, and HCP in B/E mode with Capto adhere ImpRes 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.

Flowthrough mode

Column experiments for Capto adhere ImpRes in FT mode were performed where fractions were analyzed by gel filtration during sample load. Figure 3 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 for removal of aggregates and other contaminants in FT mode at different residence times was compared with that of Capto adhere. The flowthrough pools were collected and analyzed for yield, aggregate content, HCP content, and protein A concentration. Both media 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, although this resulted in slightly poorer clearance of aggregates.

Column: Tricorn™ 5/50, bed height 5 cm, column volume ~ 1 mL
Medium: Capto adhere ImpRes
Sample: MAb sample, partially purified using MabSelect SuRe
Sample load: 80 mg/mL chromatography medium
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: ÄKTAexplorer system
Detection: Protein-containing fractions detected at A_{280} nm during sample load were analyzed by gel filtration

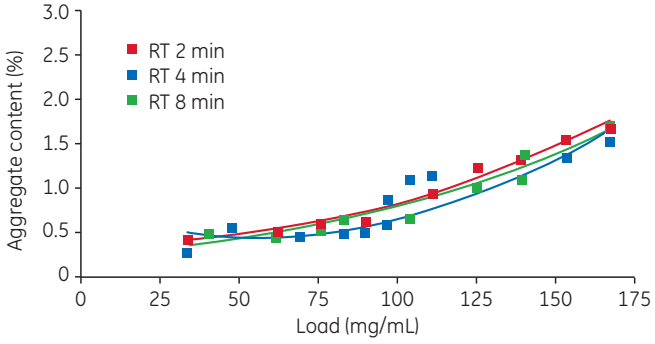

Fig 3. Concentration of aggregates as a function of sample load and residence times (RT) using Capto adhere ImpRes.

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

Chromatography medium	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

Viral clearance

The capability of Capto adhere ImpRes 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 in B/E or FT mode. Eluted fractions were analyzed for virus titer by endpoint titration and large-volume plating. Capto adhere ImpRes showed efficient viral clearance in both B/E and FT mode (Table 4). The $\log_{10}$ virus reduction factor was approximately 5.0 in B/E mode for both MuLV and MVM. In FT mode, the $\log_{10}$ virus reduction factor was > 4.0 for both MuLV and MVM.

Table 4. Viral reduction factor ($\log_{10}$) of murine leukemia virus (MuLV) and minute virus of mice (MVM) purified using Capto adhere ImpRes in B/E and FT mode

	Minimal $\log_{10}$ viral reduction factor	
Process purification mode	**MuLV**	**MVM**
B/E[1]	4.98	4.95
FT[2]	> 5.0	4.0

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

[2] 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, 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 4A) and residence time (Fig 4B).

Column:	Tricorn 5/50, bed height 5 cm, column volume ~ 1 mL
Medium:	Capto adhere ImpRes
Sample:	MAb sample (9.4 mg/mL), partially purified by protein A affinity chromatography
Sample load:	Fig 4A, 20 to 35 mg/mL chromatography medium (corresponding to 45 to 80% of DBC at 10% breakthrough); Fig 4B, 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:	ÄKTAexplorer 10 system

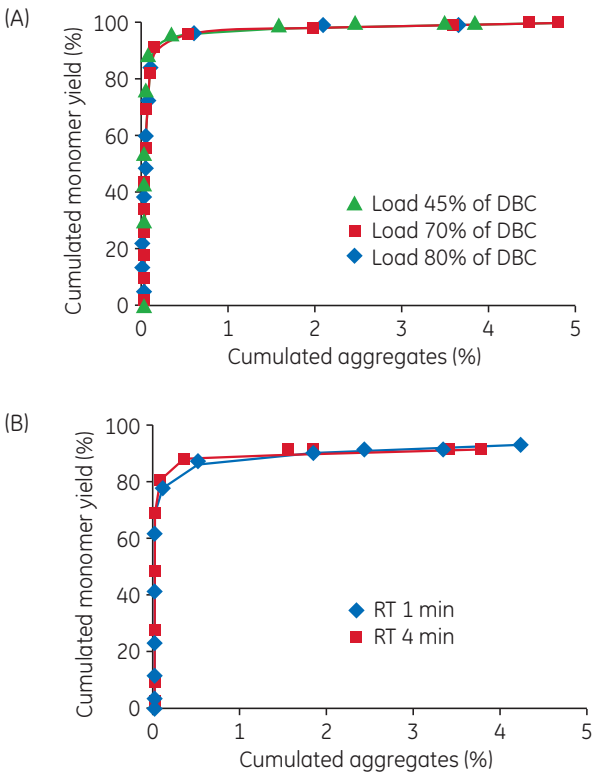

Fig 4. Cumulated yield of MAb monomer (%) vs cumulated MAb aggregates at (A) different sample loads and (B) residence times (RT) of 1 or 4 min during polishing on Capto adhere ImpRes.

The DBC at 10% breakthrough for Capto adhere ImpRes was also compared with that of Capto adhere at residence times of 2, 4, and 8 min. The results in Figure 5 show that Capto adhere ImpRes improved DBC compared with Capto adhere.

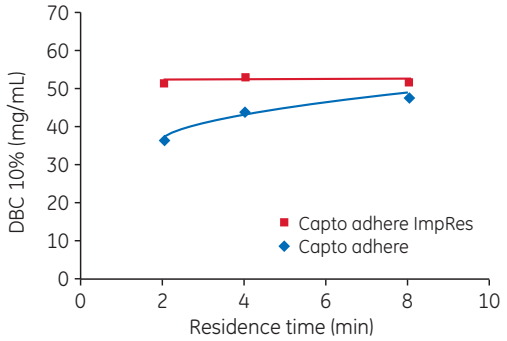

Fig 5. Dynamic binding capacity at 10% breakthrough as a function of residence time. Comparison of Capto adhere ImpRes with Capto adhere.

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 in B/E mode, the main peak, containing monomeric MAb, was divided into two peaks. The last peak (or shoulder) contained MAb aggregates (Fig 6A). 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 6B. 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 offers a higher resolution than Capto adhere, and this impacts the yield. Improvements in yield for different MAbs with Capto adhere ImpRes have been greater than 3% (up to 12%) compared with Capto adhere 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 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 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 (Table 6).

Column:	Tricorn 5/100, bed height ~ 10 cm, volume ~ 2 mL
Medium:	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:	ÄKTAexplorer 10

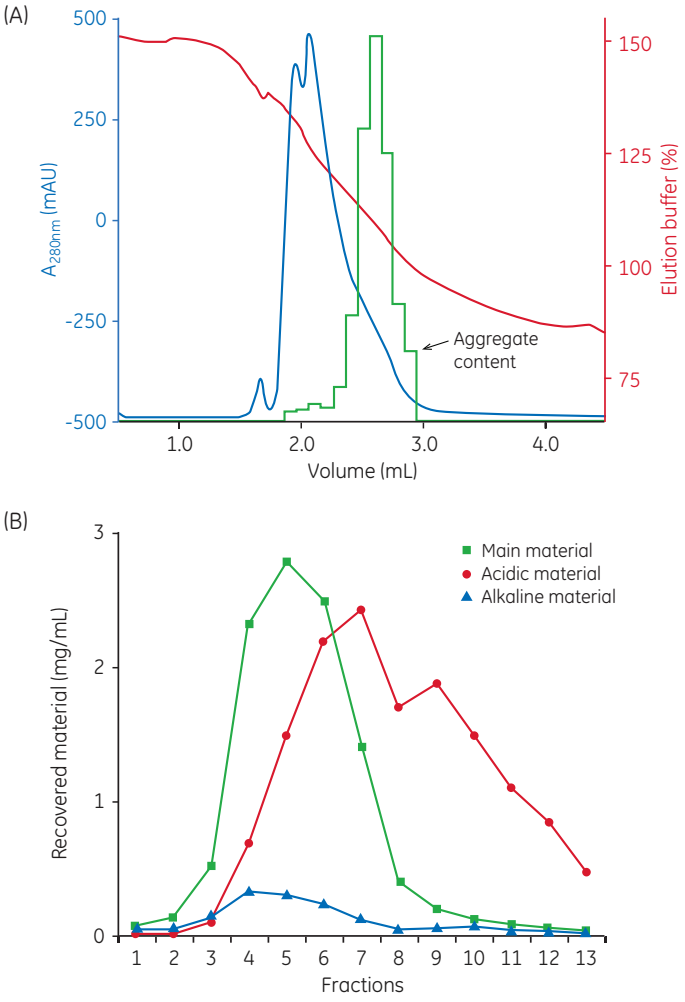

Fig 6. (A) Chromatogram showing gradient elution of MAb aggregates on Capto adhere ImpRes. (B) Relative recovery of main-, acidic-, and alkaline-charged variants in the pH gradient.

Table 5. Monomer yield from purification of three different MAbs on Capto adhere and Capto adhere ImpRes 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

	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

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 7 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, which is used 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 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 7 shows, the window of operation of Capto adhere ImpRes suits most needs both in terms of bed height and flow velocities.

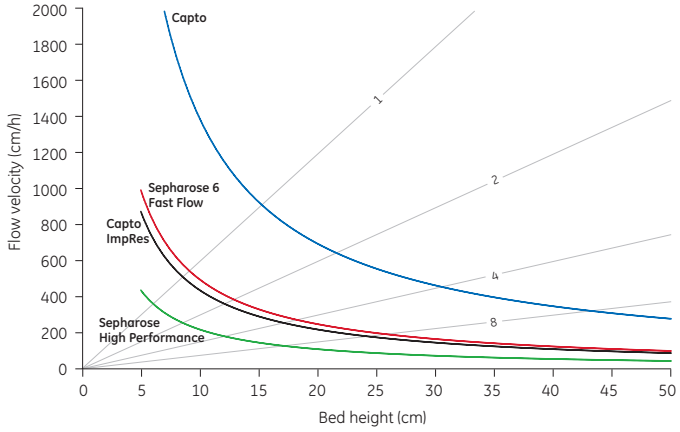

Fig 7. The window of operation (area below the curves) of different chromatography media from GE. Data correspond to a process diameter column at 20°C and viscosity equivalent to water. Gray contours give the residence time in the column in minutes.

Columns

Capto adhere ImpRes can be used with most modern chromatography equipment from laboratory to production scale. Due to the higher rigidity of Capto adhere ImpRes, packing procedures in pilot- and process-scale columns differ slightly compared with 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 8 lists suitable empty columns from GE.

Table 8. GE column families for packing with Capto adhere 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 adhere ImpRes is available in 96-well PreDictor™ plates, which support high-throughput process development (HTPD) by allowing 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 BioProcess media for HTPD using robotic liquid handling workstations. Capto adhere 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, prepacked HiTrap and HiScreen columns are convenient to use when developing an efficient and robust separation 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 adhere 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 adhere 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 adhere ImpRes medium lifetime study, the DBC remained stable over 300 cycles of CIP using 1 M NaOH. The results 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.

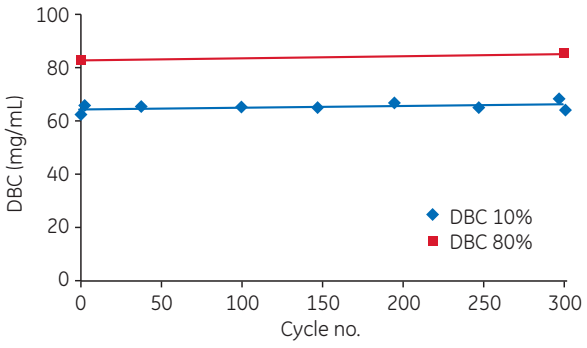

Fig 8. Medium lifetime study of dynamic binding capacity over multiple CIP cycles.

BioProcess chromatography media

Capto adhere 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. The Fast Trak Training & Education team provide high level, hands-on training for all key aspects of BioProcess development and manufacturing.

Ordering Information

Products	Quantity	Product code
Capto adhere ImpRes	25 mL	17371501
Capto adhere ImpRes	100 mL	17371502
Capto adhere ImpRes	1 L	17371503
Capto adhere ImpRes	5 L	17371504
Capto adhere ImpRes	10 L	17371505
PreDictor Capto adhere ImpRes, 6 µL	4 × 96-well filter plates	17371530
PreDictor Capto adhere ImpRes, 20 µL	4 × 96-well filter plates	17371531
PreDictor RoboColumn Capto adhere ImpRes, 200 µL	One row of 8 columns	17371540
PreDictor RoboColumn Capto adhere ImpRes, 600 µL	One row of 8 columns	17371541
HiTrap Capto adhere ImpRes	5 × 1 mL	17371510
HiScreen Capto adhere ImpRes	1 × 4.7 mL	17371520

Related literature

Application notes	
Polishing of monoclonal antibodies using Capto adhere ImpRes in bind and elute mode	29027338
Polishing of monoclonal antibodies using Capto MMC ImpRes in bind and elute mode	29027349
Data files	
Capto MMC ImpRes	29035674
Capto adhere	28907888
Capto MMC	11003545
Capto SP ImpRes, Capto Q ImpRes	28983763
PreDictor 96-well filter plates and Assist software	28925839
PreDictor RoboColumn	28988634
HiScreen prepacked columns	28930581
HiScale columns	28975523
AxiChrom columns	28929041
BPG columns	18111523
Handbook	
Multimodal Chromatography Handbook	29054808
Selection guide	
Prepacked columns for ÄKTA start and ÄKTAprime plus systems	29090697
White paper	
Navigate the road MAb	29094443

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