

Multimodal media

Capto™ adhere

Capto adhere is a multimodal BioProcess™ medium (resin) designed for post-protein A purification of monoclonal antibodies (MAbs) at process scale (Fig 1). The strong ion exchange multimodal ligand gives a different selectivity compared with traditional ion exchangers. Capto adhere can remove key impurities such as DNA, host cell proteins (HCP), leached protein A, aggregates, and viruses in a single step, allowing the design of a two-step process together with MabSelect SuRe™ protein A medium. If necessary, Capto adhere can be used in combination with anion or cation exchange chromatography for polishing, as a second or third step in any MAb purification platform.

Key performance benefits of Capto adhere are:

- High capacity and productivity, enabling time and cost savings
- Efficient viral clearance and removal of aggregates and other impurities in post-protein A purification steps
- Wide window of operation for pH and conductivity

Multimodal chromatography

Capto adhere is a multimodal anion exchanger. In multimodal chromatography, the ligand interacts with the target molecule through multiple types of interactions. Ionic interactions are commonly involved, but other interactions such as hydrogen bonding and hydrophobic interactions can be significant. The strength of these individual interactions often depends on the overall process conditions.

Multimodal chromatography media are characterized by selectivities that are different from those of “traditional” ligands, thereby opening up new opportunities for solving challenging purifications. At the same time, the higher complexity of multimodal media requires somewhat more process optimization to take full advantage of the outstanding potential of this technology. Having efficient high-throughput process development (HTPD) tools and methodology facilitates this optimization work.



Fig 1. Capto adhere allows time and cost savings through high capacity and productivity.

The multimodal ligand structure for Capto adhere is shown in Fig 2.

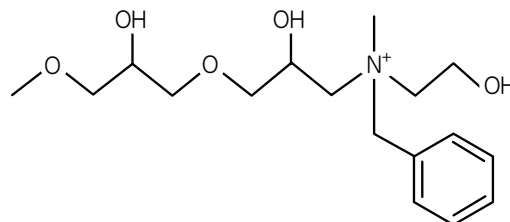


Fig 2. The Capto adhere ligand, N-benzyl-N-methylethanolamine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

Rigid matrix allows high fluid velocities

Capto adhere is based on a rigid agarose matrix that allows high fluid velocities to be used. The highly cross-linked agarose base matrix gives the medium high chemical and physical stability. High flow velocities increase the productivity of large-scale bioprocessing operations and allow large volumes to be processed in one working shift. Capto adhere is stable in conditions commonly used in process chromatography and cleaning procedures. The basic characteristics of Capto adhere are summarized in Table 1.

Removal of aggregates

High antibody titers tend to increase the generation of aggregates and other impurities in the feedstock. Capto adhere allows removal of aggregates to levels acceptable for formulation. For optimized performance of Capto adhere (i.e., to maximize the amount of impurities adsorbed to the medium while the monomeric MABs pass through the column), screening for optimal loading conditions is needed. Optimization is preferably done using design of experiments (DoE). For details about how to set up a DoE, see application notes 28907889 and 28950960.

In the application example described below, the sample used was a cell culture supernatant containing IgG₁ (BioInvent International, Sweden) that was first purified on MabSelect SuRe medium. The elution pool was frozen and thawed several times to force the formation of aggregates. The aggregate content of this pool was approximately 6% as determined by analytica size exclusion chromatography SEC using Superdex™ 200 medium.

Table 1. Characteristics of Capto adhere

Matrix	Highly cross-linked agarose
Functional group	Multimodal strong anion exchanger
Total ionic capacity	0.09 to 0.12 mmol Cl ⁻ /mL medium
Particle size*	75 µm (d _{50v})
Flow velocity†	At least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
CIP stability (short term)‡	2 to 14
pH stability (operational)§	3 to 12
Working temperature¶	4°C to 30°C
Chemical stability**	All commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide
Avoid	Oxidizing agents, anionic detergents

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

† The capacity for selective removal of some key impurities might decrease at high flow velocity.

‡ pH interval where the medium can be subjected to, cleaning or sanitization in place.

§ pH interval where the medium can be operated without significant change in function.

¶ Capto adhere can be used under cold-room conditions, but the capacity for some key impurities might decrease.

** No significant change in ionic binding capacity and carbon content after storage for one week in 1 M NaOH at 40°C.

The pH, conductivity, and load were considered to influence binding capacity and were therefore varied in the DoE setup. The pH range for the DoE study was defined by initial experiments in binding mode using a pH gradient for elution. The elution position (pH at peak maximum) defines the lower pH in the design. The upper pH should be about 2 pH units higher. The results from the study are summarized in Table 2. For this antibody, the yield is affected by pH, but is independent of conductivity and load within the range of 100 to 200 mg/mL. A non-linear increase in yield is obtained when changing from high to low pH (Fig 3).

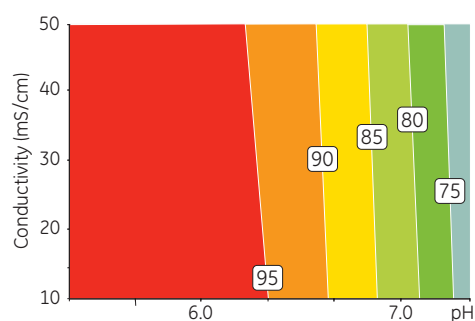


Fig 3. Response surface plot demonstrating the effect of pH and conductivity on the yield. Yield is expressed in percent (labels). Neither the load (data not shown) nor the conductivity significantly affected the yield. Low pH facilitates high yield.

Table 2. Experimental results from the DoE study

pH	Conductivity (mS/cm)	Load (mg IgG/mL medium)	Aggregates (% in flowthrough)	Yield (%)
5.5	10	100	0.77	94
5.5	10	200	0.98	100
5.5	50	100	0.3	94
5.5	50	200	0.52	99
6.25	30	150	0.29	93
6.25	30	150	0.25	95
7	10	100	0.13	47
7	10	200	0.29	76
7	50	100	0.24	74
7	50	200	0.35	68

Clearance of aggregates is influenced by pH, conductivity, and load (Fig 4). Higher pH, higher conductivity, and/or lower load results in higher clearance of aggregates. Using the results above, loading conditions were chosen to favor aggregate removal (i.e., pH 6.5 and conductivity 30 mS/cm). The chromatogram is shown in Figure 5 and a summary of how load affects the aggregate clearance is shown in Table 3. Total yield after a sample load of 265 mg/mL medium is 94%. At a sample load of approximately 120 mg/mL, the aggregate levels are reduced from 6% to 0.6%, a 10-fold reduction.

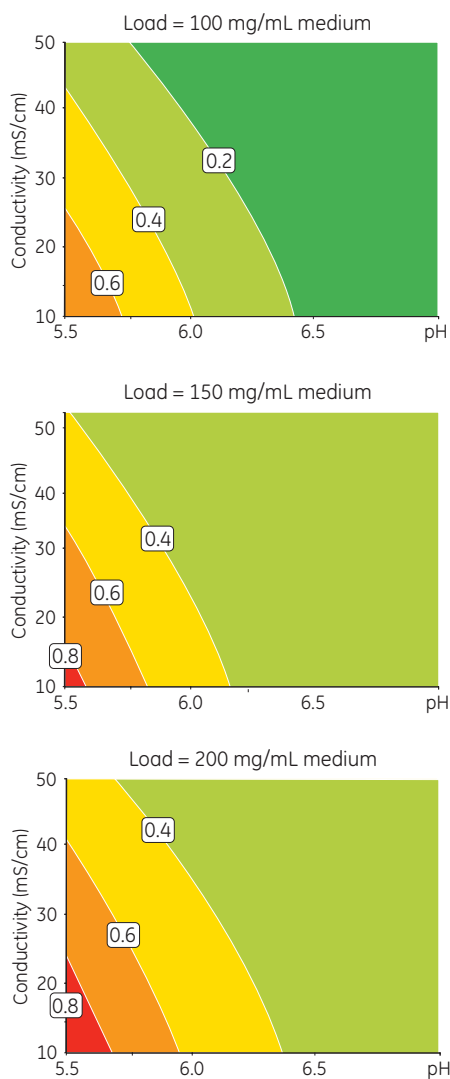


Fig 4. Response surface plots demonstrating the effect of pH, conductivity, and load on the clearance of aggregates. High pH, high conductivity, and low load gives the best reduction of aggregates in this study. Aggregate concentration in the flowthrough pool is expressed in percent (labels).

Table 3. Aggregate content in starting material, fractions and eluate during sample loading

Load mg IgG/mL medium	Aggregates	
	%	Reduction
Starting material	6	Not applicable
60	0.7	8.8
120	0.6	10.3
150	0.9	6.4
180	1.2	4.9
265	2.2	2.7
Pooled fractions	1.3	4.8
Eluate	~ 60	Not applicable

Viral clearance

Capto adhere viral clearance was tested with two representative viruses, minute virus of mice (MVM) and murine leukemia virus (MuLV). Monoclonal IgG₁ was purified from Chinese hamster ovary (CHO) cell supernatant on MabSelect SuRe medium. Buffer concentration and pH of the elution pool were adjusted to typical process conditions. The conductivity was adjusted to 10 and 30 mS/cm by addition of NaCl. The samples were spiked with virus stock solution and applied in flow-through mode on Capto adhere. The log₁₀ reduction factor at 10 mS/cm was 5.8 and 4.5 logs for MVM and MuLV, respectively. Even at high conductivity (30 mS/cm), where traditional ion exchangers do not work, the log reduction factor was 5.9 for MVM and 3.6 for MuLV (Table 4).

Table 4. Viral clearance using Capto adhere at 22°C and pH 6.75

Virus	Conductivity (mS/cm)	Log ₁₀ Reduction Factor ± 95% confidence limit
MVM	10	5.8 ± 0.3
MVM	30	5.9 ± 0.3
MuLV	10	4.5 ± 0.4
MuLV	30	3.6 ± 0.4

Experiments performed in duplicate (NewLab BioQuality AG, Germany).

Column: Tricorn™ 5/50, bed height 3 cm
Sample: MabSelect SuRe elution pool
Sample load: 265 mg of MAb/mL medium
Loading buffer: 20 mM citrate, 300 mM NaCl, pH 6.5 (conductivity 30 mS/cm)
Elution buffer: 0.1 M acetic acid, pH 3.0
Residence time: 2 min
System: ÄKTA chromatography system

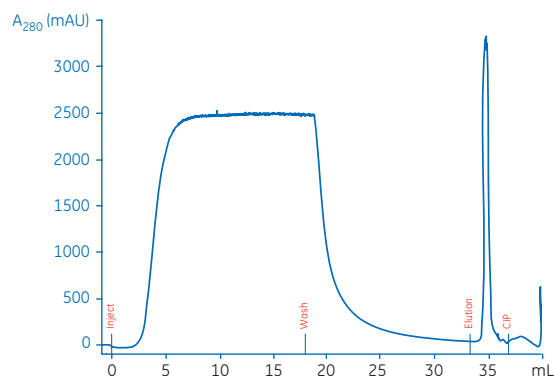


Fig 5. Purification of an IgG₁ MAb: polishing on Capto adhere.

Two-step process for MAb purification

Processes for large-scale purification of MAbs have usually consisted of three chromatographic steps. First, the feed is affinity-purified on a protein A column, which gives a product with a high purity, typically 99%. This product is further polished, often by cation and anion exchange chromatography and sometimes in combination with hydrophobic interaction chromatography (HIC), to remove aggregates and other impurities. The GE Healthcare chromatography medium toolbox simplifies this process. The high purity obtained after capture on protein A medium, in combination with the multimodal functionality of Capto adhere, makes it possible to design a two-step process for purification of MAbs (Fig 6). To verify this concept, a cell culture supernatant containing IgG₁ (Polymun Scientific, Austria) was first purified on MabSelect SuRe medium and then further polished on Capto adhere medium.

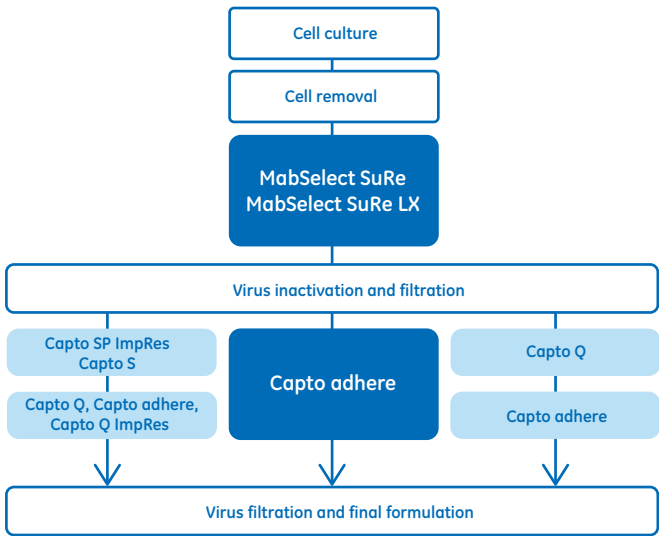


Fig 6. GE Healthcare chromatography media toolbox.

The yield for the Capto adhere step was 92%. The HCP concentration was reduced from 250 to 20 ng/mL (7.5 ppm) and the protein A content was below detection limit. The aggregate concentration in the MabSelect SuRe pool was low (< 0.7%) and below detection limit after the polishing step (Fig 7, red line). The Capto adhere elution pool contained approximately 5% aggregates (and other low-molecular weight impurities), demonstrating that aggregates remaining after the capture step were efficiently adsorbed to Capto adhere (Fig 7, blue line).

A comparison of the MabSelect SuRe/Capto adhere two-step process with a three-step process based on MabSelect SuRe, Capto S and Capto Q media demonstrates that both alternatives give similar yields with impurity levels acceptable for formulation (i.e. HCP content < 10 ppm, protein A below detection limit and aggregate concentration less than 0.1% (Table 5). A process consisting of two chromatographic steps can help improve productivity with shorter process time and lower operating cost compared with typical three-step purification processes.

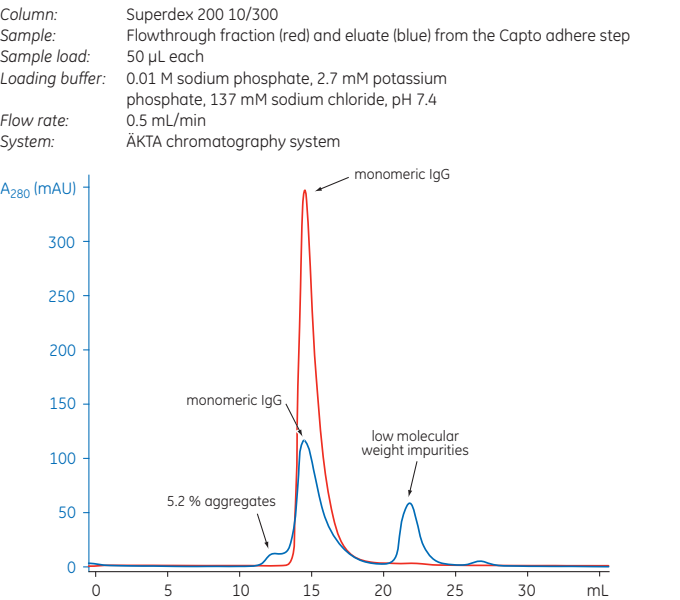


Fig 7. Flowthrough and elution pools from Capto adhere analyzed by analytical SEC chromatography. Flowthrough (red): monomeric IgG. Elution pool (blue): 5.2% aggregates, monomeric IgG, low molecular weight impurities.

Table 5. Comparison of yield and purity of a MAb purified with a two-step process using MabSelect SuRe and Capto adhere and a three-step process using MabSelect SuRe, Capto S, and Capto Q

Final result	Two-step process	Three-step process
Total yield (%)	90	90
Dimers/aggregates (%)	< 0.1	< 0.1
Protein A (ng/mL)	< 5	< 5
Protein A (ppm)	n.q.	n.q.
HCP (ng/mL)	20	20
HCP (ppm)	7.5	3

n.q. = not quantifiable

Three-step process for MAb purification

If necessary, Capto adhere can be used in combination with anion or cation exchange chromatography, or HIC, for polishing, as a second or third step in a MAb purification platform (Fig 6).

A Capto Q step was added to the two-step model (data not shown). The yield in this step was 99.7% and the HCP content was further reduced by 50%.

Loading conditions: general trends for Capto adhere

Based on a DoE study performed with several different antibodies, some general trends have been identified (Fig 8).

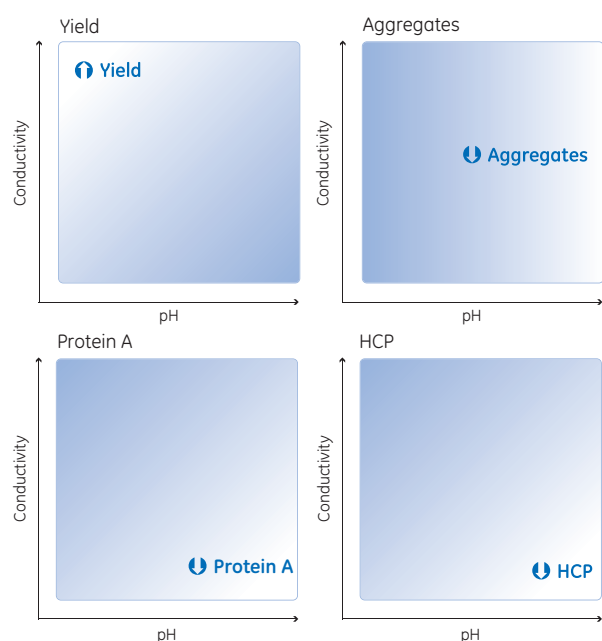
- For high yield, load and conductivity should be high and pH low.
- For optimized clearance of aggregates, pH should be high, while load and conductivity should be low. Aggregate clearance is often less affected by conductivity than protein A and HCP clearance.
- For optimized protein A and HCP clearance, pH should be high, conductivity low, and sample load low.

Table 6. Loading conditions for different MABs for high yield and clearance of aggregates, HCP, and protein A

MAB	pI	pH	Conductivity (mS/cm)	Yield (%)	Aggregates (%)	Protein A (ppm)	HCP (ppm)
1	~ 9	7	8	90	0.5	n.q.	< 15
2	8.3–8.9	5.5	3	95	0.6	n.q.	2
3	7.5–8.4	6	2	95	0.8	n.q.	9
4	7.7–8.0	7	20	91	0.2	n.q.	30
5	6.5–9.0	7.5	20	92	< 0.1	n.q.	7.5

n.q. = not quantifiable

Even though the optimal conditions obtained for each response are not the same, there is still a large area where acceptable values can be obtained for all four responses.

**Fig 8.** General trends with respect to loading conditions (conductivity and pH) for yield, clearance of aggregates, protein A, and HCP.

Loading conditions for five MABs, together with yield and impurity clearance obtained in a two-step process (including protein A and Capto adhere), are shown in Table 6. While optimal conductivity is hard to predict, pH should normally be well below the isoelectric point.

Operation

Bind-Elute mode

As a first option, Capto adhere is recommended to be operated in non-binding mode, as this gives higher throughput. Nevertheless, in cases where low molecular weight impurities such as antibody fragments are present, Capto adhere in bind-elute mode might give higher purity. Figure 9A gives an example, where a MAB of the IgG₁ subclass was purified (post-protein A capture step) by Capto adhere in bind-elute mode. Binding at pH 7.5 and elution using lower pH and higher sodium chloride concentration resulted in a highly purified MAB.

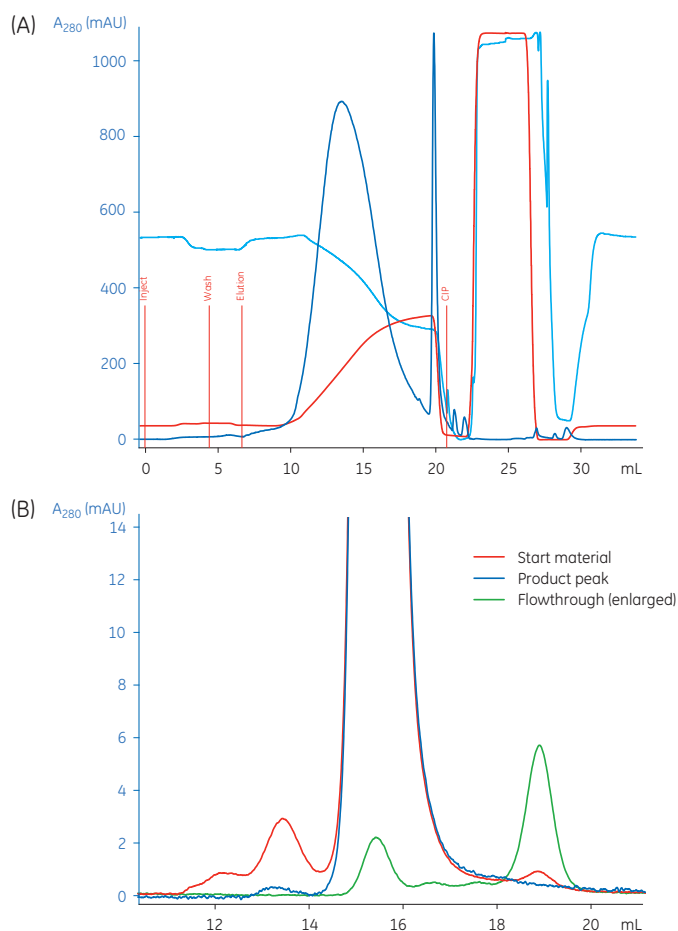
Optimization of binding and elution conditions was done using a DoE approach. Both gradient and step-wise elution (not shown) were successfully evaluated. Analysis by SEC showed that low-molecular weight impurities ended up in the flowthrough and could not be removed using non-binding conditions (Fig 9B).

Capto adhere step (A)

Column: Tricorn 5/30, packed with Capto adhere
Sample: IgG1 MAB, elution pool from Protein A capture step
Sample load: 25 mg/mL medium
Start buffer: 25 mM sodium phosphate, pH 7.5
Elution buffer: 50 mM sodium acetate, 300 mM NaCl, pH 5.5 (gradient)

SEC analysis (B)

Column: Superdex 200 10/300 GL
Sample volume: 50 µL
Flow rate: 0.5 mL/min
Running buffer: phosphate buffered saline (PBS)

**Fig 9.** (A) Purification of an IgG₁ MAB post-protein A using Capto adhere in bind-elute mode. (B) SEC analysis shows that low-molecular weight impurities ended up in the flowthrough (green curve).

Using gradient elution, the aggregate content was reduced from > 2.6% to 0.21% (12.5-fold reduction) and the protein A content was reduced from almost 900 ppm to < 3 ppm in a single step (300-fold reduction). The monomer yield of 91% is acceptable but could be further improved to 95% with stepwise elution, while only moderately sacrificing purity.

High-Throughput Process Development

HTPD shortens development time and increases the amount of information available during early process development. In multimodal chromatography, the availability of HTPD tools are especially valuable as the experimental conditions should be carefully screened to fully exploit the potential of the multimodal chromatography media. Capto adhere is available in PreDicator™ 96-well filter plates and PreDicator RoboColumn™ format as well as HiScreen™ and HiTrap™ columns.

PreDicator plates and PreDicator RoboColumn units are suitable for early chromatographic screening experiments. These tools can be used for the initial screening of process conditions, or for a more thorough investigation of a defined space as basis for detailed process understanding. To facilitate HTPD using PreDicator plates, Assist software was developed. The software supports experimental setup and data evaluation. After scouting and screening in the PreDicator formats, verification and fine-tuning can be performed with ÄKTA chromatography systems such as the ÄKTA avant system with a DoE functionality. Prepacked columns such as HiScreen columns are recommended, or HiTrap columns if sample volumes are low. The UNICORN™ control software for ÄKTA systems makes it simple to transfer the optimized method to a production-scale process system.

Fully scalable

Capto adhere belongs to the BioProcess range of media that are developed for production-scale chromatography. These media are supported with validated manufacturing methods, security of supply and regulatory support files (RSF) to assist process validation and submission to regulatory authorities. Process scale-up is typically performed by keeping bed height and linear flow velocity (cm/h) constant (i.e. constant residence time) while increasing column bed diameter and flow rate (L/min). Yield and clearance of critical impurities can change when column bed height or residence time is changed and should be validated using the final bed height.

Cleaning and sanitization

Cleaning in place (CIP) is a procedure that removes impurities such as lipids, endotoxins, nucleic acids and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the accumulation of impurities in the medium bed and helps to maintain the capacity, flow properties and general performance of Capto adhere.

CIP is normally recommended after each cycle. Prior to CIP, Capto adhere should be stripped with an acidic buffer (e.g., 50 mM Na-phosphate, pH 3, or 100 mM acetic acid) to efficiently remove bound proteins that could foul the medium if a CIP solution was to be applied directly. A specific CIP protocol should be designed for each process according to the type of impurities present. The frequency of CIP depends on the nature and the condition of the feedstock.

Capto adhere withstands standard CIP procedures, for example, 1 M NaOH, 2 M NaCl, or 70% ethanol.

Storage

Store used medium in the container at a temperature of 4°C to 30°C. Packed columns should be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least five bed volumes of loading buffer before use.

Equipment

Capto adhere can be used together with most equipment available for chromatography, from laboratory scale to production scale. In process scale, the preferred packing technique for Capto media is axial compression. The optimal approach is to use AxiChrom™ columns, with Intelligent Packing functionality and pre-set packing methods for all Capto media. Appropriate columns from GE Healthcare are shown in Table 7.

Table 7. Appropriate columns for Capto adhere

Column family range	Inner diameter (mm)
Lab scale:	
Tricorn	5, 10
HiScale™	16, 26, 50
Pilot and production scale:	
AxiChrom	50–1000
BPG	100–300 [†]
Chromaflo	400–800 [‡]

[†] The pressure rating of BPG 450 is too low to use it with Capto media.
[‡] Larger pack stations might be required at larger diameters.

All Capto media are also available in the ReadyToProcess™ format, comprising prepacked, prequalified and presanitized columns in the size range from 1 to 20 L and designed for purification of biopharmaceuticals for preclinical as well as clinical phase I and II studies. If the scale allows, these columns can also be used for commercial production.

Ordering information

Product	Pack size	Product code	Application notes	Product code
Capto adhere	25 mL	17544410	Optimization of loading conditions on Capto adhere using design of experiments	28907889
	100 mL	17544401		
	1 L	17544403	Selective removal of aggregates with Capto adhere	28907893
	5 L	17544404	Two-step purification of monoclonal IgG ₁ from CHO cell supernatant	28907892
	10 L	17544405		
HiTrap Capto adhere	60 L	17544460	Purification of a monoclonal antibody using ReadyToProcess columns	28919856
	5 × 1 mL	28405844	Two-step purification of monoclonal IgG ₁ from CHO cell culture supernatant	28907892
	5 × 5 mL	28405846		
PreDicator ALEX screening plate, 20 µL	4 × 96 well filter plate	28943289	High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process	28950960
PreDicator ALEX screening plate, 2 µL/6 µL	4 × 96 well filter plate	28943288		
PreDicator Capto adhere, 6 µL	4 × 96 well filter plate	28925817		
PreDicator Capto adhere, 20 µL	4 × 96 well filter plate	28925818		
PreDicator Capto adhere, 50 µL	4 × 96 well filter plate	28925819		
PreDicator RoboColumn Capto adhere, 200 µl	one row of eight columns	28986085		
PreDicator RoboColumn Capto adhere, 600 µl	one row of eight columns	28986179		
HiScreen Capto adhere	1 × 4.7 mL	28926981		
ReadyToProcess Capto adhere	1 L	28951109		
ReadyToProcess Capto adhere	2.5 L	28901714		
ReadyToProcess Capto adhere	10 L	28901715		
ReadyToProcess Capto adhere	20 L	28901716		

Capto adhere bulk medium products are supplied in suspension in 20% ethanol. For more information, contact your local GE Healthcare representative.

Data files	Product code
PreDicator 96-well filter plates and Assist software	28925839
PreDicator RoboColumn	28988634
HiScreen prepacked columns	28930581
ReadyToProcess columns	28915987
AxiChrom columns	28929041
HiScale columns	28975523
MabSelect SuRe	11001165
MabSelect SuRe LX	28987062
Capto S, Capto Q and Capto DEAE	11002576
Capto SP ImpRes, Capto Q ImpRes	28983763

Handbooks	Product code
Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11000421
High-throughput process development with PreDicator plates	28940358

Acknowledgements

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