Capto adhere

Capto™ adhere is a multimodal BioProcess™ medium designed for post-protein A purification of monoclonal antibodies (MAbs) at process scale (Fig 1). The strong multimodal ligand (Fig 2) gives a different selectivity compared to traditional ion exchangers. Capto adhere can remove key contaminants such as DNA, host cell proteins (HCP), leached protein A, dimers and larger aggregates, and viruses in a single step, allowing the design of a two-step process together with MabSelect SuRe™. 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
- Contaminant removal to formulation levels in post protein A purification
- Wide operational window of pH and conductivity
- Savings in time and operating costs with a two-step chromatographic process

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. General characteristics of the base matrix are described in Data File 11-0035-45, Capto MMC. Capto adhere is stable in conditions commonly used in process chromatography and cleaning procedures (Table 1).

Fig 1. Capto adhere allows the design of a MabSelect SuRe/Capto adhere two-step process for large-scale purification of MAbs.

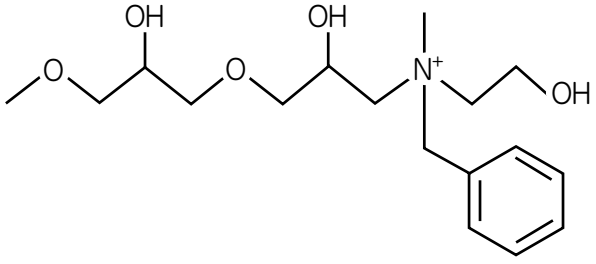

Fig 2. The Capto adhere ligand, N-Benzyl-N-methyl ethanolamine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding and hydrophobic interaction.

Removal of aggregates

High antibody titers tend to increase the generation of aggregates and contaminants in the feedstock. Capto adhere allows removal of aggregates to target values acceptable for formulation. The sample was a cell culture supernatant containing IgG_1 (BioInvent International, Sweden) that was first purified on MabSelect SuRe. The elution pool was frozen and thawed several times to force the formation of aggregates. The aggregate content of this pool contained approximately 6% as determined by analytical gel filtration chromatography using Superdex™ 200.

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[1]	75 µm (d_{50v})
Flow velocity[2]	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)
pH-stability[3]	
short-term	2 to 14
long-term	3 to 12
Working temperature[4]	4°C to 30°C
Chemical stability5	All commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide
Avoid	Oxidizing agents, anionic detergents

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

2 The capacity for selective removal of some key contaminants may decrease at high flow velocity.

3 *Short term pH:* pH interval where the medium can be subjected to, for cleaning- or sanitization-in-place.

Long term pH: pH interval where the medium can be operated without significant change in function.

4 Capto adhere can be used under cold-room conditions, but the capacity for some key contaminants may decrease.

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

To achieve the best performance of Capto adhere, that is as much as possible of the contaminants adsorbed to the medium while the monomeric MAbs pass through the column, screening for optimal loading conditions is needed. Optimization is preferably done with design of experiments (DoE). For details about how to set up a DoE, see Application Note 28-9078-89.

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 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 experiments are summarized in Table 2.

For this antibody, the yield is controlled 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 making the transition from high to low pH, (Fig 3).

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. The total yield after sample application of 265 mg/ml 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.

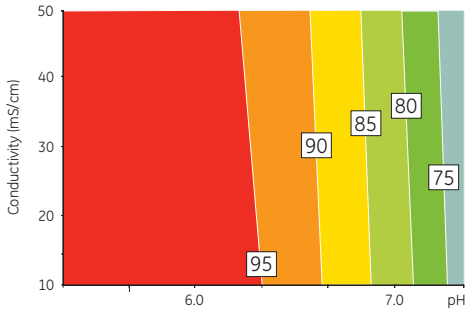

Fig 3. Response surface plot demonstrating the effect of pH on the yield. Neither the load nor the conductivity significantly affected the yield. Low pH facilitates high yield. Yield expressed in percent (labels).

Table 2. Experimental results from the DoE

pH	Cond (mS/cm)	Load (mg IgG/ml)	D/A (% 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

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

Load mg IgG/ml	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 2 representative viruses, Minute Virus of Mice (MVM) and Murine Leukemia Virus (MuLV). Monoclonal IgG_1 was purified from CHO cell supernatant on MabSelect SuRe. 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 were then applied in flowthrough mode on Capto adhere. The log_{10} 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 of 5.9 for MVM and 3.6 logs for MuLV (Table 4).

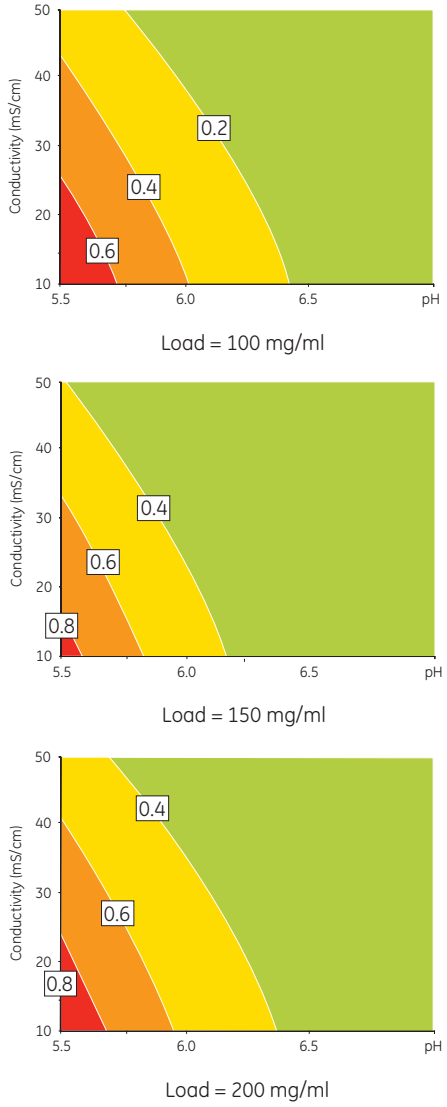

Fig 4. Response surface plots demonstrating the effect of pH, conductivity and load on the clearance of aggregates. High pH, high conductivity and low load gives the best reduction of aggregates. Aggregate concentration in the flowthrough pool is expressed in percent (labels).

Table 4. Capto adhere viral clearance study performed at NewLab BioQuality AG, Germany. Test conditions were: pH 6.75, temperature 22°C, experiments performed in duplicates.

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

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: ÄKTAexplorer™100

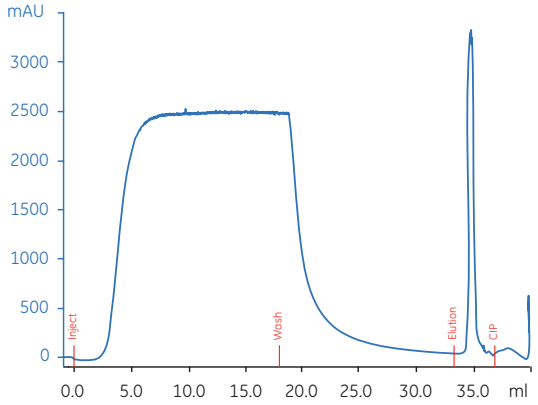

Fig 5. Purification of an IgG_1 MAb; polishing on Capto adhere.

Two-step process for MAb purification

Processes for large-scale purification of MAbs usually consist 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 then further polished, often with cation and anion exchange chromatography, and sometimes in combinations with hydrophobic interaction chromatography (HIC), to remove aggregates and other contaminants. The GE Healtcare chromatography media toolbox simplifies this process.

The high purity obtained after capture on protein A medium and the multimodal functionality of Capto adhere make it possible to design a two-step process based on MabSelect SuRe and Capto adhere for the purification of MAbs (Fig 6). To verify this concept, a cell culture supernatant containing IgG_1 (Polymun Scientific, Austria) was first purified on MabSelect SuRe and then further polished on Capto adhere.

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 contents was below the detection limit. The aggregate concentration in MabSelect SuRe pool was already low (< 0.7%) and after the polishing step below the

detection limit (Fig 7, red line). The adsorbed material, eluted from Capto adhere, contained approximately 5% aggregates (and other low molecular weight impurities), which proves 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 (Fig 6) demonstrates that both alternatives give similar yields with impurity target levels acceptable for formulation (i.e., HCP content < 10 ppm, protein A below the detection limit, and aggregate concentration less than 0.1%, Table 5). A process consisting of two chromatographic steps shows improved productivity with shorter process times and lower operating costs compared to typical three-step purification processes.

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 any 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 contents were reduced by 50% from an already low level.

Loading conditions – general trends for Capto adhere

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

- For best yield, load should be high, pH low, and conductivity high.
- For the best 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 the best protein A- and HCP-clearance, pH should be high, conductivity low, and sample load low.

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

Optimal loading conditions for five MAbs together with yield and contaminant clearance results from two step process, including Protein A medium and Capto adhere are shown in Table 6. As can be seen, pH should normally be well below the isoelectric point, while optimal conductivity is harder to predict.

Operation

Fast method development

Fast method development can easily be achieved on ÄKTAexplorer with quick initial screening of selectivity and process conditions using prepacked HiTrap™ columns.

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
D/A (%)	< 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

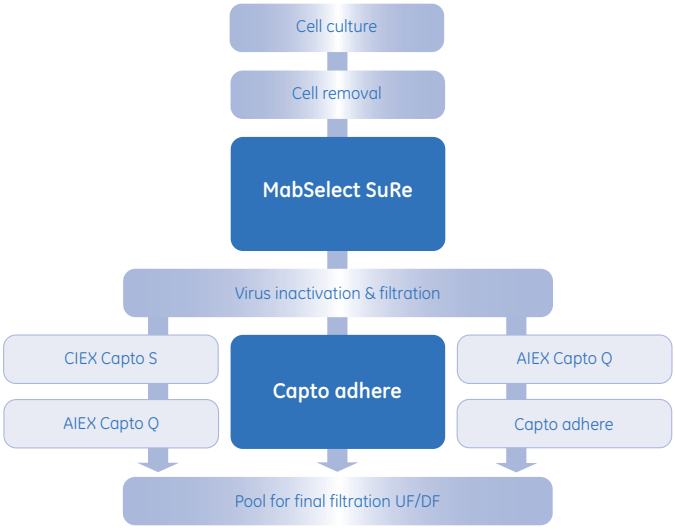

Fig 6. GE Healthcare chromatography media toolbox.

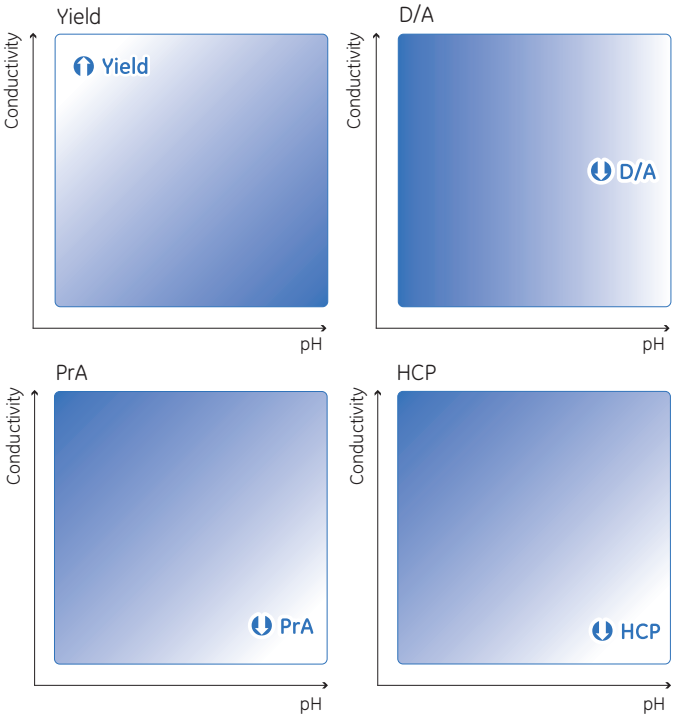

Fig 8. General trends with respect to loading conditions (conductivity and pH) for yield, clearance of aggregates, protein A and HCP.

Table 6. Optimal loading conditions for different MAbs with regard to yield and clearance of aggregates (D/A), HCP, and Protein A

MAb	pI	pH	Conductivity (mS/cm)	Yield %	D/A %	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

Column: Superdex 200 10/100
Sample: Flowthrough fraction (red) and eluate (blue) from the Capto adhere step
Sample load: 50 µl each
Loading buffer: 0.01 M sodium phosphate, 2.7 mM potassium phosphate, 137 mM sodium chloride, pH 7.4
Flow rate: 0.5 ml/min
System: ÄKTAexplorer

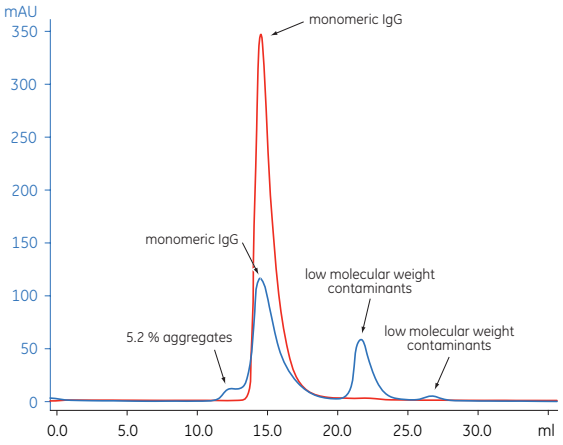

Fig 7. Flowthrough and elution pools from Capto adhere analyzed by analytical gel filtration chromatography. Flowthrough pool (red): monomeric IgG. Elution pool (blue): 5.2% aggregates, monomeric IgG, low molecular weight contaminants.

Further optimization and method development using Tricorn or XK columns allow straightforward scale-up. UNICORN™ software on ÄKTAdesign™ systems makes it simple to transfer the optimized method to a production-scale process system.

Fully scalable

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 may 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 contaminants 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 contaminants 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. 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.

Capto adhere withstands all 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. Ensure that the screw top is fully tightened. Packed columns should be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 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. Appropriate columns from GE Healthcare are shown in Table 7.

BioProcess media for production-scale chromatography

Capto adhere belongs to the BioProcess range of media that are developed and supported for production-scale chromatography. This includes validated manufacturing methods, secure supply, and Regulatory Support Files (RSF) to assist process validation and submission to regulatory authorities.

Table 7. Appropriate columns for packing with Capto adhere

Column family	Range (inner diameters)
Tricorn	5 mm, 10 mm
XK	16 mm, 26 mm
FineLINE™	35 to 350 mm
BPG™	100 to 300 mm[1]
BioProcess LPLC	100 to 1200 mm
Chromaflow™	400 to 2000 mm

[1] The pressure rating of BPG 450 is too low to use with Capto adhere

Ordering information

Product	Pack size	Code No.
Capto adhere	25 ml	17-5444-10
	100 ml	17-5444-01
	1 l	17-5444-03
	5 l	17-5444-04
	10 l	17-5444-05
	60 l	17-5444-60
HiTrap Capto adhere	5 × 1 ml	28-4058-44
	5 × 5 ml	28-4058-46

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

Application notes	Code No.
Optimization of loading conditions on Capto adhere using design of experiments	28-9078-89
Selective removal of aggregates with Capto adhere	28-9078-93
Two-step purification of monoclonal IgG_1 from CHO cell supernatant	28-9078-92

Other literature

Data file MabSelect SuRe	11-0011-65
Data file Capto Q Capto S	11-0025-76

Acknowledgements

Filtered NSØ cell line feedstock was supplied by BioInvent International AB, Lund, Sweden. Filtered CHO cell line supernatant was supplied by Polymun Scientific Immunbiologische Forschung GmbH, Nassdorfer Lindell, 1190 Vienna, Austria. Viral clearance studies were performed by NewLab BioQuality AG, Erkrath, Germany.

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