Capto™ adhere

Capto adhere is a multimodal BioProcess™ resin for intermediate purification and polishing of monoclonal antibodies after capture on Protein A resin by packed bed chromatography. In combination with Protein A resin (i.e., MabSelect™ family), Capto adhere offers a robust chromatography platform for the development of monoclonal antibody manufacturing processes.

Capto adhere improves yield, productivity, and process economy with:

- High capacity in flow-through mode
- Contaminant removal to formulation levels in post Protein A purification:
    - Leached Protein A
    - Antibody dimers and aggregates
    - Host cell proteins
    - Nucleic acids
    - Viruses
- Wider operational window of pH and conductivity
- Savings in time and operating costs with a two steps chromatographic process

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Read these instructions carefully before using the product.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 BioProcess resins

BioProcess chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Properties of Capto adhere

Capto adhere is a strong anion exchanger with multimodal functionality (Fig 1). The multimodal functionality gives a different selectivity compared to traditional anion exchangers.

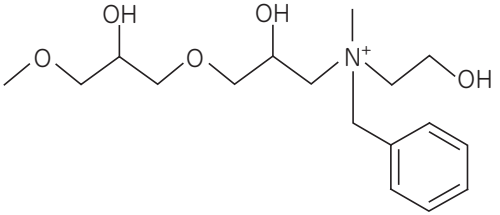

Fig 1. The Capto adhere ligand, N-Benzyl-N-methyl ethanol amine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

Capto adhere is designed for post Protein A purification of monoclonal antibodies. Removal of leached Protein A, aggregates, host cell proteins, nucleic acids, and viruses from monoclonal antibodies is performed in flow-through mode where the antibodies pass directly through the column while the contaminants are adsorbed.

The resin is based on a rigid high flow agarose matrix that allows high flow velocities to be used (Fig 2 and 3). The highly cross-linked agarose base matrix gives the resin high chemical and physical stability. Characteristics such as capacity, elution behavior, and

pressure/flow properties are unaffected by the solutions commonly used in process chromatography and cleaning procedures (Table 1).

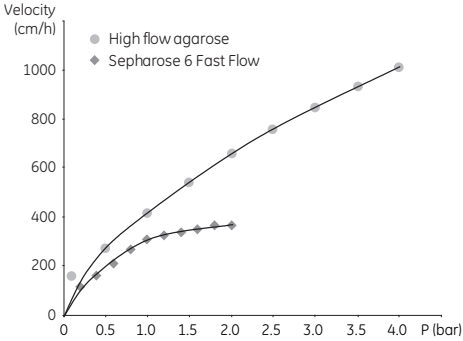

Fig 2. Pressure/flow characteristics of Capto adhere compared to Sepharose™ 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.) open bed at settled bed height of 20 cm, with water at 20°C

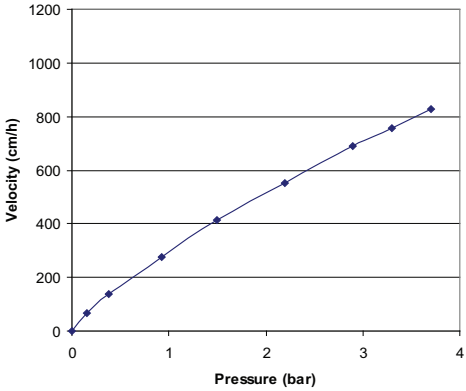

Fig 3. Pressure/flow characteristics of Capto adhere in a BPG 300, 20 cm packed bed with water at 20°C

Table 1. Characteristics of Capto adhere.

Matrix	Highly cross-linked agarose, spherical
Functional group	Multimodal strong anion exchanger
Ionic capacity	0.09 to 0.12 mmol Cl^-/mL resin
Particle size, d_{50v}[1]	~ 75 µm
Pressure/flow characteristics[2]	≥ 600 cm/h at ≤ 0.3 MPa in a 1 m diameter column and 20 cm bed height (at 20°C using process buffers with the same viscosity as water[3]
pH stability, operational[4]	3 to 12
pH stability, CIP[5]	2 to 14
pH ligand fully charged[6]	Entire pH range
Working temperature[7]	4°C to 30°C
Chemical stability	Stable to commonly used aqueous buffers, 1 M acetic acid, 1.0 M NaOH[8]
Avoid	Oxidizing agents, anionic detergents
Autoclavability	17 min at 121°C in 0.05 M phosphate buffer, pH 7, 10 cycles

[1] Median particle size of the cumulative volume distribution.

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

[3] The pressure/flow characteristics describes the relationship between pressure and flow under set circumstances. The pressure shall not be taken as the maximum pressure of the resin.

[4] pH range where resin can be operated without significant change in function.

[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

[6] pH range where ligand is fully charged.

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

[8] 1.0 M NaOH must only be used for cleaning purposes.

3 Method design and optimization

Capto adhere is a multimodal ion exchanger and is designed to be used as a second or third step in monoclonal antibody (mAb) purification processes, (i.e., after capture on a Protein A resin). Removal of leached Protein A, antibody dimers and aggregates (D/A), host cell proteins (HCP), viruses, and nucleic acids is preferably performed in flow-through mode where the antibodies passes directly through the column while the contaminants are adsorbed.

General purification protocol

- Adjust pH and conductivity of the Protein A pool to loading conditions for flow-through mode.
- Equilibrate the column with loading buffer of the same pH and conductivity as the sample.
- Apply sample onto the column. Collect the flowthrough fraction.
- Wash out unbound material with loading buffer and collect together with the flowthrough fraction.
- Regenerate column to elute bound material.
- Clean-In-Place.
- Re-equilibrate.

Sample preparation

Before sample loading, pH and conductivity of the sample must be adjusted to desired loading conditions. This is done either by buffer exchange or by direct adjustment of pH and conductivity. Buffers normally used for ion exchange chromatography can also be used for Capto adhere (Table 2).

Table 2. Recommended buffers

pH interval	Buffer[1,2]	Concentration[3]
4 to 5	Acetate	20 to 100 mM
4 to 6	Citrate	20 to 200 mM
5.5 to 6.5	Bis-TRIS	20 to 50 mM
6 to 7.5	Phosphate	50 to 200 mM
7.5 to 8.5	TRIS	20 to 50 mM
8.5 –	Glycin-NaOH	20 to 100 mM

[1] The choice of buffer systems and salts can influence both yield and contaminant clearance.

[2] Buffers in the interval 5.5 to 8 will normally be most efficient for contaminant removal.

[3] Conductivity can be adjusted by addition of salt or by varying the buffer concentration.

Buffer exchange

For preparation of well defined samples for optimization of loading conditions in lab scale, buffer exchange can be performed on HiPrep™ 26/10 desalting[1]. A typical chromatogram is shown in Figure 4

[1] Prepacked with Sephadex™ G-25 Fine to provide reliable and reproducible desalting and buffer exchange with sample sizes up to 15 mL per column.

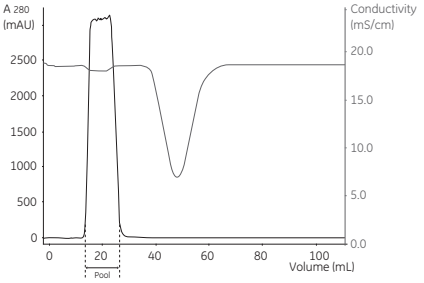

Fig 4. Buffer exchange on HiPrep 26/10 Desalting. Sample volume: 10 mL MabSelect SuRe™ eluate. Buffer exchange to 25 mM BIS-TRIS, 175 mM NaCl, pH 7.0 Flow rate: 15 mL/min. Pooled material: 13 mL.

Conductivity and pH adjustment

For larger volumes of feed, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity[2].

[2] Buffer exchange can result in reduction of HCP levels and improve column performance.

Initial screening of loading conditions

Product yield and loading conditions

Balancing product yield against product purity is the major consideration when optimizing a method. When running in flow-through mode, loading conditions will usually be a compromise between conditions favoring yield and conditions favoring contaminant clearance. By adjusting pH and conductivity of the sample as well as the sample load, conditions can be obtained where most contaminants are adsorbed while the monomeric antibodies pass through the column.

Optimization of loading conditions is preferably performed by using Design of Experiments (DoE). A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. To be able to define the center point and the variable ranges some initial experiments are required.

Initial experiments

To find conditions suitable for the DoE, initial experiments can be performed in binding mode, using a pH gradient for elution (Fig 5, left). The elution position, that is pH at peak maximum, defines the lower pH in the design. The upper pH in the design is normally about two pH units higher. Experiments can also be performed in flow-through mode, keeping the conductivity constant at a moderate level.

A comparison of chromatograms is shown in Figure 5. At high pH (i.e., close to pI for the antibodies) the breakthrough during sample load is delayed, the breakthrough and wash curves are shallow and significant amounts of mAb binds to the adsorbent. A decrease in pH (i.e., further from pI) results in weaker electrostatic

interaction between the antibodies and the adsorbent, steeper breakthrough and wash curves and increased yield.

In the DoE, pH, conductivity and load must be included. It is important to include conditions at the higher pH range resulting in lower yield and higher purity as well as conditions at lower pH range resulting in higher yield and lower purity. An example is given below.

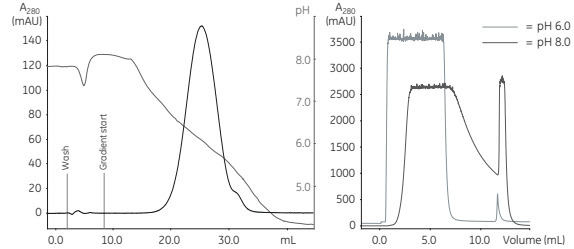

Fig 5. Sample: rProtein A elution pool, after buffer exchange on HiPrep 26/10 Desalting. Binding mode (left): Sample loading at pH 7.8. Load: 1 mg/mL resin. Elution performed in a pH gradient from 7.8 to 4.0. Flow-through mode (right): Comparison of chromatograms obtained at different pH. Load 75 mg mAb/mL resin. Conditions: pH 8.0, 2 mS/cm and pH 6.0, 2 mS/cm.

Design of Experiments for optimization of loading conditions

Design of Experiments (DoE) is recommended for optimization of loading conditions. By systematically varying important parameters (i.e., pH, conductivity and sample load) response surfaces can be obtained for yield and for clearance of key contaminants.

The table below describes a procedure for setting up a full factorial DoE with three parameters:

Step	Purpose	Description
1	Work prior to actual setup of the design.	Perform initial loading experiments at varying pH, as described above. Choose parameters to include and define parameter ranges and responses.
2	Choose design for screening or optimization.	Full factorial design is commonly used in both screening and optimization. A full factorial DoE in 3 parameters will give $2^3 = 8$ experiments + center points.
3	Choose center points for the design.	Center points are important in DoE because they give an indication if there is curvature in the data. Three replicated center points are recommended. A full factorial design in three parameters with three center points gives a total of 11 experiments.
4	Systematic variation of the parameters.	Use limiting values, high and low, of each parameter. To be able to separate effects, combine the high and low values in a way that makes the parameters independent of each other. For further information, see www.umetrics.com.

Note: *A module for planning and evaluation of DoE is included in UNICORN™ 6.*

Example of high-throughput screening optimization

As an alternative to running small columns, high-throughput process development (HTPD) tools like PreDictor™ 96-well plates can be used for process optimization. An example of this is described in application note *High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process* (28950960).

Description

The application note describes the development and optimization of a polishing step for the purification of a monoclonal antibody on Capto adhere. In the initial part of this study, PreDictor 96-well plates prefilled with Capto adhere were used to screen a large experimental space quickly. Promising results from the plate study were further optimized with HiScreen columns and DoE approach to establish the final process conditions.

Result

Application of the optimized protocol led to a reduction in aggregate levels from 12.6% to < 0.5% in a single step with a monomer yield of 87%. Host cell protein (HCP) and ligand leakage were reduced to negligible amounts. In total, 192 conditions (flowthrough and selective elution experiments) were screened in approximately 4 h and analyzed in 48 h.

Example of design of experiments performed in columns

Sample

Start material: Monoclonal IgG1 expressed in CHO cell culture supernatant, initially purified on rProtein A resin.

Sample characteristics: pI ≈ 9, leached Protein A 36 ppm, D/A 3.3%[1] and HCP 210 ppm.

Experimental setup

The experimental setup was a full factorial design[2, 3] in three variables, load, pH and conductivity, with additional points to resolve curvature effects (Table 3). In all, the number of experiments included in the model was 14, and the measured responses were yield (%) and Protein A (ppm), dimer/ aggregate (%) and HCP (ppm) in the flowthrough pool. For each response a separate model was calculated. The models were fitted to MLR (multiple linear regression) and are well explained and show good stability to cross validation.

[1] As determined by analytical size exclusion chromatography on Superdex™ 200/ 300 GL.

[2] Experiments were designed and evaluated using Modde 7.0 software (Umetrics, Sweden).

[3] www.umetrics.com

Table 3. Design setup[1]

Load (mg mAb/mL)	pH	Conductivity (mS/cm)
75	6	2
300	6	2
75	8	2
300	8	2
75	6	15
300	6	15
75	8	15
300	8	15
187.5	**7**	**8.5**
187.5	**7**	**8.5**
75	7	15
300	7	15
187.5	7	2
187.5	7	15

[1] The design setup includes 2 center points (bold) and 4 additional points at pH 7 to resolve curvature effects.

Result

Parameters affecting the yield

The parameters that affect the yield are shown in the coefficient plot[1] (Fig 6). The plot shows that high sample load, low pH, and high conductivity results in high yield. The interaction effects (load × pH and load × conductivity) are also significant for the yield response. The response surfaces also show that higher loads will give larger pH window with yield > 90%.

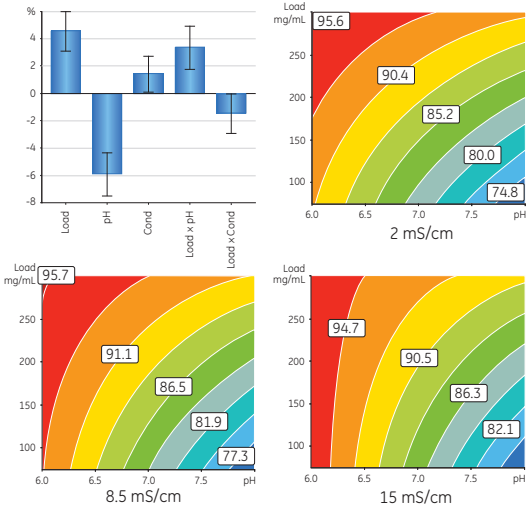

Fig 6. Coefficient plot and response surfaces for the yield model. Load versus pH at different conductivities.

The coefficient plot describes the impact on the response from the investigated parameters. In the case above it can be seen that load is positively correlated to the response, meaning that a higher load will give a higher value on the response, pH is negatively correlated to the response, meaning that a lower pH will give a higher value on the response and conductivity is positively correlated to the response, but smaller, meaning that a higher conductivity will also

give a higher response value, but not to the same extent as the effect of load.

One way to further improve yield is to perform selective elution of monomers. For further information, see Application note *High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process* (28950960).

The interaction effects that are present in the coefficient plot (load × pH and load × conductivity) means that if, for example pH is changed the response will not only be changed with the effect of pH but also with the effect of load at that specific pH. The same goes for the load × conductivity interaction.

Parameters affecting the Protein A clearance

The coefficient plot shows that high pH will give good Protein A clearance. The conductivity by itself did not significantly affect the response, but there is a significant interaction effect for pH × conductivity (Fig 7). If this term is high, the Protein A clearance will be low. Load was not a significant factor for this response.

The response surfaces show that high pH and low conductivity will give high Protein A clearance.

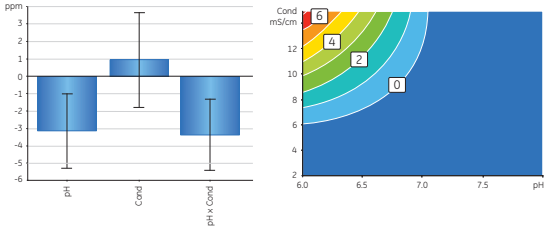

Fig 7. Coefficient plot and response surfaces for the Protein A model. Conductivity versus pH. Protein A concentration expressed in ppm.

Parameters affecting dimer/aggregate clearance

The coefficient plot (Fig 8) shows that pH is the most important parameter and that high pH will give low D/A concentration in the flowthrough pool. The load parameter is also significant, but very small. A low load gives high D/A-clearance. There is also a significant curvature effect assigned to pH. If pH is too high or too low the D/A response will increase. The conductivity did not significantly affect D/A-clearance. The response curve shows that the load has only small affect on D/A-clearance, so only pH has to be considered.

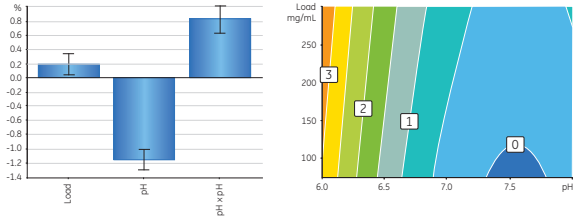

Fig 8. Coefficient plot and response surface for the D/A model. Load versus pH. D/A concentration expressed in percent.

Parameters affecting host cell protein (HCP) clearance

The coefficient plot (Fig 9) and response curves show that low sample load, low conductivity, and high pH will give low HCP values.

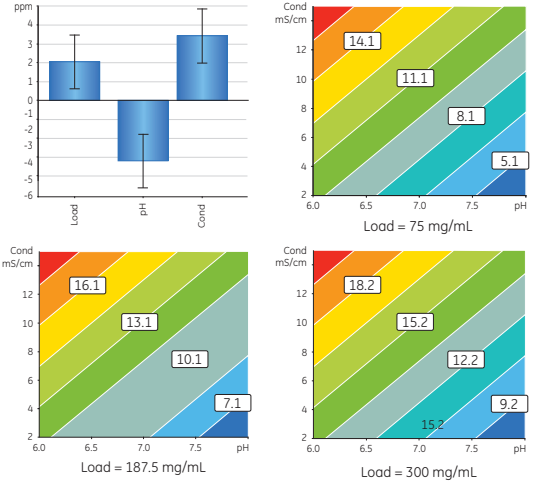

Fig 9. Coefficient plot and response surfaces for the HCP model. Conductivity versus pH. HCP concentration expressed in ppm.

Conclusions

The response surfaces above show the influence of sample load, pH, and conductivity on four different responses (yield of monomeric mAb and clearance of Protein A, dimer/aggregates and HCP respectively), and how to reach desired values for each of them.

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. Suggested loading conditions for this mAb could be a sample load of 200 mg/mL, pH 7, and conductivity 8.5 mS/cm.

General trends

Each monoclonal antibody is unique, and the level of contaminants varies between different cell lines and differences in previous purification steps. This implies that it can be difficult to predict optimal loading conditions.

However, based on design of experiments performed with several different antibodies some general trends have been identified (Fig 10).

- Best yield: High load, low pH, and high conductivity
- Best D/A-clearance: High pH, low load, and low conductivity. D/A-clearance is often less affected by conductivity than Protein A- and HCP-clearance.
- Best Protein A- and HCP-clearance: High pH and low conductivity

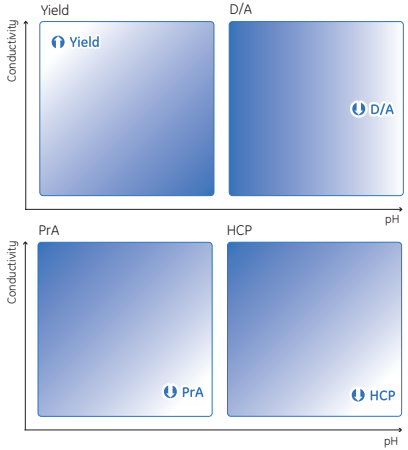

Fig 10. General trends with respect to loading conditions for yield, D/A-, Protein A- and HCP-clearance.

Loading conditions will therefore be a compromise between conditions favoring yield and conditions favoring contaminant clearance. Optimal loading conditions will be a balance between load, pH, and conductivity. Consequently, for optimization of the loading step, vary all three parameters in the same experimental series.

In Table 4, optimal loading conditions for five different antibodies are shown. As can be seen, pH is normally be below the isoelectric point, while optimal conductivity is harder to predict.

Table 4. Optimal loading conditions for different mAbs

mAb	pI	pH	Conductivity (mS/cm)
mAb 1	≈ 9	7.0	8
mAb 2	8.3 to 8.9	5.5	3
mAb 3	7.5 to 8.4	6.0	2
mAb 4	7.7 to 8.0	7.0	20
mAb 5	6.5 to 9.0	7.7	20

Bed height, flow velocity, and residence time

Typical bed height range in production scale is 10 to 20 cm. Recommended flow velocity is between 150 and 600 cm/h[1], and the residence time[2] 2 minutes[3], or more. Longer residence time can result in more efficient contaminant removal.

As the loading capacity in flow-through mode is relatively high (100 to 300 mg mAb/mL resin), lower bed heights can by advantage be used in lab scale. However, the flow velocity must be adapted to a suitable residence time. Prepacked 1 mL and 5 mL HiTrap™ Capto adhere columns are available for screening of loading and elution conditions, and for method scouting.

[1] 1 m diameter, 20 cm bed height at 20°C using buffer with the same viscosity as water.

[2] Residence time, $\tau = L/u$, where L is the bed height in cm and u is the linear velocity (cm/h).

[2] Corresponding to 600 cm/h at 20 cm bed height.

Wash out unbound material

After sample load, continue to wash out unbound mAb with loading buffer until the UV-curve starts to level off. The wash fractions can normally be pooled together with the flowthrough fractions. How much of the wash fractions that is collected is a balance between yield and dilution of the pooled material.

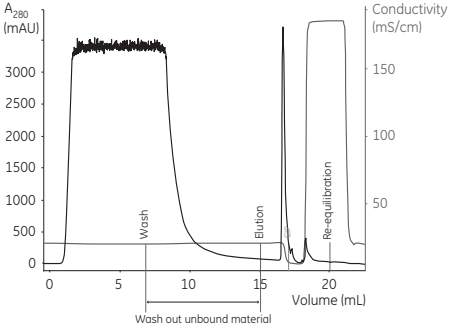

Fig 11. Example of wash after sample application

Regeneration, Cleaning-In-Place, and re-equilibration

After wash out of unbound material, perform regeneration at low pH as described in Chapter *Maintenance*.

Depending on the nature and the condition of the feedstock, Cleaning-In-Place (CIP) is recommended after 1 to 5 cycles. A standard protocol for CIP is to wash with 1.0 M NaOH, using a contact time of 15 minutes. For further details, see Chapter *Maintenance*.

At the end of each purification cycle, and after regeneration and Cleaning-In-Place, re-equilibrate the column with 5 bed volumes of loading buffer, or until the column effluent shows stable conductivity and pH values.

4 Column packing

Recommended columns

Table 5. Recommended columns for Capto adhere

Column	Inner diameter (mm)	Bed volume[1]	Bed height (cm)
Lab scale			
Tricorn™ 5/100	5	2 mL	10
Tricorn 10/100	10	8 mL	10
HiScale™ 16/20	16	20 to 40 mL	max 20
HiScale 16/40	16	20 to 70 mL	max 35
HiScale 26/20	26	53 to 106 mL	max 20
HiScale 26/40	26	53 to 186 mL	max 35
HiScale 50/20	50	196 to 393 mL	max 20
HiScale 50/40	50	196 to 687 mL	max 35
Production scale			
AxiChrom™	50 to 200	0.2 to 12.5 L	max 40
AxiChrom	300 to 1000	7 to 314 L	max 50
BPG[2]	100 to 300	1 to 28 L	max 40
Chromaflow™ standard[3]	400 to 800	12 to 151 L	max 30 cm

[1] Bed volume range calculated from 10 cm bed height to maximum bed height.
[2] The pressure rating of BPG 450 is too low to use with Capto resins.
[3] Larger pack stations might be required at larger diameters.

All production scale columns can be supplied as variable bed height columns. Do not choose large diameter columns if the bed height is low.

For more details about packing HiScale columns, see instructions *HiScale™ columns (16, 26, 50) and accessories* (28967470).

Packing Tricorn 5/100 and 10/100 columns

Materials needed

Capto adhere

Glass filter funnel

Plastic spoon

Filtering flask

Measuring cylinder

10 mM NaCl in purified water

Calculating amount of resin

The amount of Capto adhere resin needed can be calculated by: column cross sectional area (cm^2) × bed height (cm) × compression factor (settled resin bed height/packed resin bed height).

Washing the resin

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the resin into the funnel and wash with approximately 5 to 10 mL 10 mM NaCl per mL resin.

Preparing the packing slurry

The slurry concentration must be 40 to 60% in 10 mM NaCl, measured in a measuring cylinder after settling overnight.

Equipment needed

An ÄKTA design system or a stand-alone pump that can deliver 40 mL/min is used. The pump filter unit and the flow restrictor must be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure.

For packing Tricorn 5/100 column

Tricorn 5/100 column, Tricorn 5/100 glass tube (used as a packing reservoir), packing connector 5-5, and bottom unit.

For packing Tricorn 10/100 column

Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector, Tricorn 10/100 glass tube (used as a packing reservoir), and bottom unit with filter holder, cap, and stop plug.

When working with large sample volumes or when repeatedly loading sample, the Tricorn coarse filter kit is recommended to reduce the risk of clogging. Use Tricorn 5 Coarse filter kit (11001253) or Tricorn 10 Coarse filter kit 10 (11001254).

Column packing procedure

To pack the column, use 10 mM NaCl and proceed as follows:

1. Assemble the column according to the column instruction (Tricorn empty 28409488).
2. Pour the chromatography resin into the top of the packing tube filling both column tube and packing reservoir. Remove all air bubbles.
3. Attach a bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column and connect a pump to the top of the column, remove the stop plug from the bottom of the column tube.
4. Settle the resin at 0.25 mL/min (Tricorn 5/100) or 1 mL/min (Tricorn 10/100) for 10 minutes or until the bed has settled.
5. After the bed has settled, raise to packing flow –10 mL/min (Tricorn 5/100) or 40 mL/min (Tricorn 10/100) for 2 minutes.
6. When the resin is packed, switch off the pump, attach the stop plug into bottom of the column tube, disconnect the column and remove the packing reservoir and packing connector. Let the liquid in the packing reservoir pour down the column and into the beaker. If necessary, carefully remove excess resin by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula.
7. Top off the column with the same fluid as used for packing the column.
8. Place a pre-wet filter on top of the fluid in the column.

   Note: *The top coarse filter is inserted by another procedure. See separate instruction included in the coarse filter kit.*

9 Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then back 1.5 turns.

10 Wet the O-ring on the adapter unit by dipping it into water, packing buffer or 20% ethanol.

11 Screw the adapter unit onto the column tube, making sure that the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there are no air bubbles.

12 Screw the adapter down to approximately 1 mm above the surface.

13 Connect the pump to the adapter unit. Remove the stop plug from the bottom of the column tube.

14 Pack the column at 10 mL/min (Tricorn 5/100) or 40 mL/min (Tricorn 10/100) for 2 additional minutes.

15 Before switching off the pump mark the position of the resin surface with a pen on the column.

16 Switch off the pump, attach the stop plug into bottom of the column tube and disconnect the column from the pump. Screw the adapter unit down to the marking.

17 Press the adapter lock down into the locked position.

18 Screw a stop plug into the adapter unit. The column is now ready for use.

Note: *Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.*

Testing the packed column

See Chapter *Evaluation of column packing*.

Packing HiScale columns

Packing preparations

Materials needed

Capto adhere

HiScale column

HiScale packing tube (depending on bed height)

Plastic spoon or spatula

Glass filter G3

Vacuum suction equipment

Filter flask

Measuring cylinder

20% ethanol with 0.4 M NaCl

Equipment

ÄKTA™ system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at a given flow (consolidated). Therefore, the compression factor (CF) has to be separated from the packing factor (PF).

$L_{settled}$	Bed height measured after settling by gravity.
L_{cons}	Consolidated bed height Bed height measured after settling the resin at a given flow velocity
L_{packed}	Packed bed height
CF	Compression factor $CF = L_{settled}/L_{packed}$
PF	Packing factor $PF = L_{cons}/L_{packed}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28925932. This method can also be used for HiScale columns.

Washing the resin

Mount a glass filter funnel onto a filtering flask. Suspend the resin by shaking and pour into the funnel and wash according to the following instructions:

- 5 times with 5 mL 20% ethanol with 0.4 M NaCl/mL resin
- Gently stir with a spatula between additions.
- Move the washed resin from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.

Packing the column

Table 6. Main features of the packing method for HiScale 16/20 and HiScale 16/40

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.10	1.10	1.02
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	25	25	25
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	25	25	14

Table 7. Main features of the packing method for HiScale 26/20 and HiScale 26/40

Column	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.10	1.03
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	66	66	66
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	66	66	37

Table 8. Main features of the packing method for HiScale 26/20 and HiScale 26/40

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.15	1.03
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	250	250	250
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	66	250	140

Packing procedure

1. Assemble the column according to the column instructions (HiScale columns (16, 26, 50) and accessories, code no 28967470).
2. Mount the column tube in a stand.
3. Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry but if air is trapped under the net it can be removed by a light suction with a syringe.
4. Mount the bottom adapter unit in the bottom of the column tube and tighten the O-ring.
5. Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet.
6. Mount the packing tube on top of the column tube.
7. Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing the roof as this is done. If air is trapped under the net it can be removed by a light suction with a syringe.
8. Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
9. Mount the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.
10. Start a downward flow with packing velocity according to Table 6, 7 and 8.
11. Let the flow run until the bed has consolidated.
12. Use the scale on the column to measure the bed height. There might be a build up of resin at the column wall after the bed is consolidated and to easier see where the top of the bed is, a light source can be used.
13. Calculate the final bed height by dividing the consolidated bed height with the desired packing factor.
    $L_{packed} = L_{cons}/PF$. See Table 6, 7, and 8.
14. Turn off the flow and put a stop plug in the bottom.

15 Dismount the top adapter from the packing tube.

16 Over a beaker or a sink, detach the packing tube from the column.

17 Remount the top adapter in the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.

18 Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.

19 Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the resin.

20 Start a downward flow to flow condition the bed. The flow rate is shown in Table 6, 7 and 8.

21 Let the flow run for about 10 column volumes. The column is ready to be tested.

Testing the packed column

See Chapter *Evaluation of column packing*.

5 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing must be done after packing, at regular intervals during the working life of the column or when separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28937207).

Note: *The calculated number of plates will vary according to the test conditions and must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum 2.5% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible
(A typical acceptable range could be $0.7 < A_S < 1.3$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 12 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

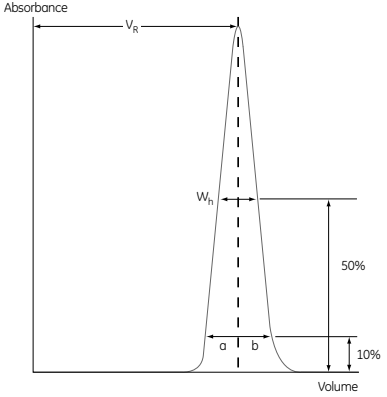

Fig 12. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

6 Maintenance

For best performance for Capto adhere over a long working life time, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with loading buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Regeneration

After each step, elute any reversibly bound material with low pH (e.g., 0.1 M acetate pH 3.0). Regenerate the resin by washing until the column effluent shows stable conductivity and pH values.

Cleaning-In-Place

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 building-up of contaminants in the resin 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 must 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.

CIP protocols

Precipitated hydrophobically bound proteins or lipoproteins	Wash with 1.0 M NaOH at 150 cm/h with reversed flow direction. Contact time 15 to 30 minutes.
Ionically bound proteins	Wash with 0.5 to 2 column volumes of 2 M NaCl with reversed flow direction.
Lipids and very hydrophobic proteins	Wash with 1-propanol 1 to 5% or isopropanol 5 to 30%. 1-propanol has a higher flash point and might be preferred in an industrial environment.
Nucleic acids	Wash with 0.1 M acetate pH 3 for 2 to 5 column volumes followed by equilibration buffer at neutral pH for 1 to 2 column volumes and wash 1.0 M NaOH at 150 cm/h with reversed flow direction. Contact time 15 to 30 minutes.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 M NaOH with a contact time of 1 hour is recommended. The CIP protocol for precipitated, hydrophobic bound proteins or lipoproteins removes bound contaminants and sanitizes the resin effectively.

Storage

Store used resin in the container at a temperature of 4°C to 30°C. Make sure that the screw top is fully tightened. Packed columns must be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of loading buffer before use.

7 Ordering information

Product	Quantity	Product Code
Capto adhere	25 mL	17544410
	100 mL	17544401
	1 L	17544403
	5 L	17544404
	10 L	17544405
	60 L[1]	17544460

[1] Pack sizes available upon request

All bulk resin products are supplied in suspension in 20% ethanol. For additional information, including data file, contact your local GE representative.

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Related product	Quantity	Product Code
PreDictor Capto adhere, 6 µL	4 x 96-well filter plates	28925817
PreDictor Capto adhere, 20 µL	4 x 96-well filter plates	28925818
PreDictor Capto adhere, 50 µL	4 x 96-well filter plates	28925819
PreDictor Capto adhere Isotherm (2, 4, 6, 8, 20, and 50 µL, different in different wells within the same plate)		28943282
PreDictor RoboColumn Capto adhere, 200 µL	One row of eight columns	28986085
PreDictor RoboColumn Capto adhere, 600 µL	One row of eight columns	28986179
HiTrap Capto adhere	5 × 1 mL	28405844
HiTrap Capto adhere	5 × 5 mL	28405846
HiScreen Capto adhere	1 × 4.7 mL	28926981
ReadyToProcess Capto adhere 1 L		28951109
ReadyToProcess Capto adhere 5 L		29146144
ReadyToProcess Capto adhere 10 L		28901714

Related product	Quantity	Product Code
ReadyToProcess Capto adhere 20 L		28901716
ReadyToProcess Capto adhere 32 L		29212477
HiTrap MabSelect SuRe	5 × 1 mL	11003493
HiTrap MabSelect SuRe	5 × 5 mL	11003495
HiPrep 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/10 Desalting	4 × 53 mL	17508702
Tricorn 5/100 column	1	28406410
Tricorn 10/100 column	1	28406415
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Related literature		Product Code
Handbook	Multimodal Chromatography Handbook	29054808
Data file	Capto adhere	28907888
	HiScreen prepacked columns	28930581
	PreDictor 96-well filter plates and Assist Software	28925839
Application note	Optimization of loading conditions on Capto adhere using design of experiments	28907889
	Efficient purification of the pertussis antigens toxin, filamentous haemagglutinin, and pertactin in chromatography workflows	29227789
	Two step purification of monoclonal IgG1 from CHO cell supernatant	28907892
	Selective removal of aggregates with Capto adhere	28907893

Related literature		Product Code
	High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process	28950960
	Purification of a monoclonal antibody using ReadyToProcess columns	28919856
Instructions	Determine the compression factor and slurry concentration. Method description and practical example	29001351
	Determine the compression factor and slurry concentration: a brief overview	29001794
	Packing Capto adhere and Capto MMC using verified methods	28925933
Poster	K. Eriksson et al. Post protein A removal of contaminants from monoclonal antibodies with multimodal anion exchanger. *BioProcess International* (Feb. 2009).	

8 Further information

For the latest news, more product information and our handbooks, visit: www.gehealthcare.com/protein-purification
www.gelifesciences.com

For technical support,
visit: www.gelifesciences.com/techsupport

For local office contact information, visit
www.gelifesciences.com/contact

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