GE Healthcare

Instructions 28-9064-05 AA **Multimodal media**

Capto adhere

Capto™ adhere is a multimodal BioProcess™ medium for intermediate purification and polishing of monoclonal antibodies after capture on Protein A medium by packed bed chromatography. In combination with Protein A medium (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

Capto

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1. BioProcess media

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

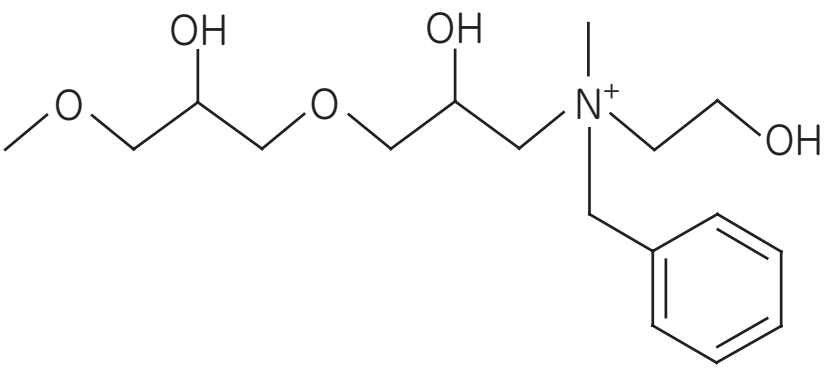

Fig 1. The Capto adhere ligand, N-Benzyl-N-methyl ethanol amine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding and hydrophobic interaction.

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 at where the antibodies pass directly through the column while the contaminants are adsorbed.

The medium is based on a rigid high flow agarose matrix that allows high flow velocities to be used (Fig 2). The highly cross-linked agarose base matrix gives the medium 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).

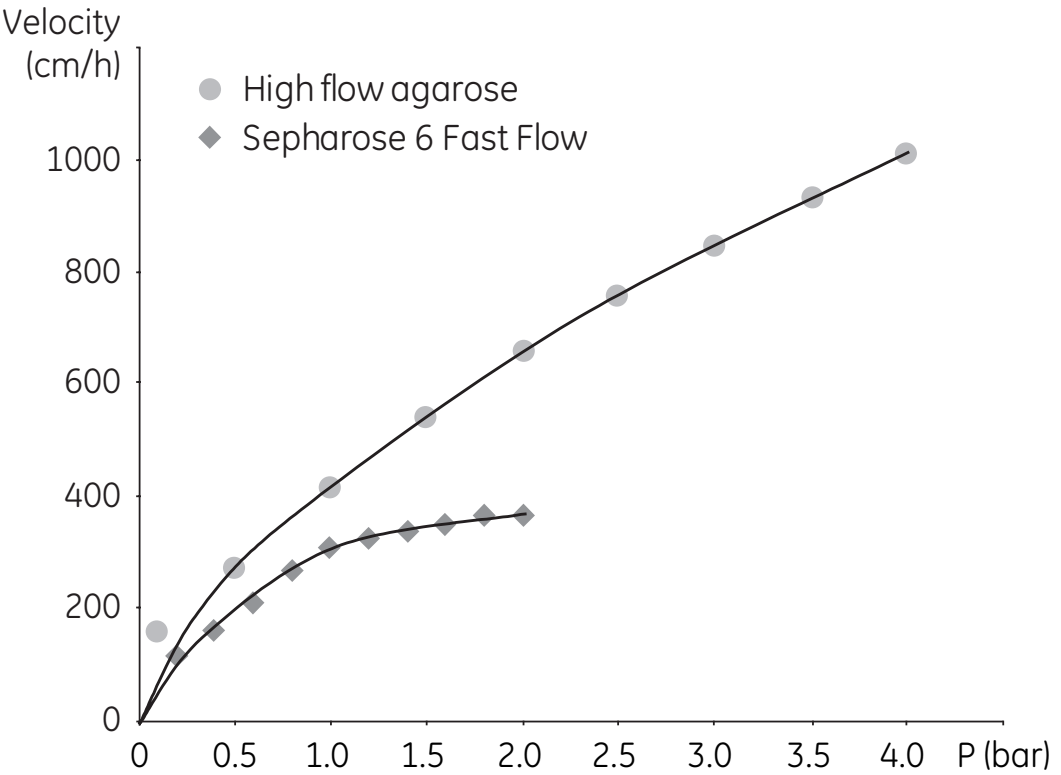

Fig 2. Pressure-flow properties for the high flow agarose base matrix used for Capto adhere compared to Sepharose™ 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.) open bed at settled bed height of 20 cm, with water at 20°C.

Table 1. Characteristics of Capto adhere.

Matrix	highly cross-linked agarose
Functional group	multimodal strong anion exchanger
Total ionic capacity	0.09 - 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 – 14
long term	3 – 12
Working temperature[4]	4 to 30°C
Chemical stability[5]	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 1 week storage in 1M NaOH at 40°C.

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 medium). 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 should 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 - 5	Acetate	20 - 100 mM
4 - 6	Citrate	20 - 200 mM
5.5 - 6.5	Bis-TRIS	20 - 50 mM
6 - 7.5	Phosphate	50 - 200 mM
7.5 - 8.5	TRIS	20 - 50 mM
8.5 -	Glycin-NaOH	20 - 100 mM

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

[2] Buffers in the interval 5.5 – 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 3.

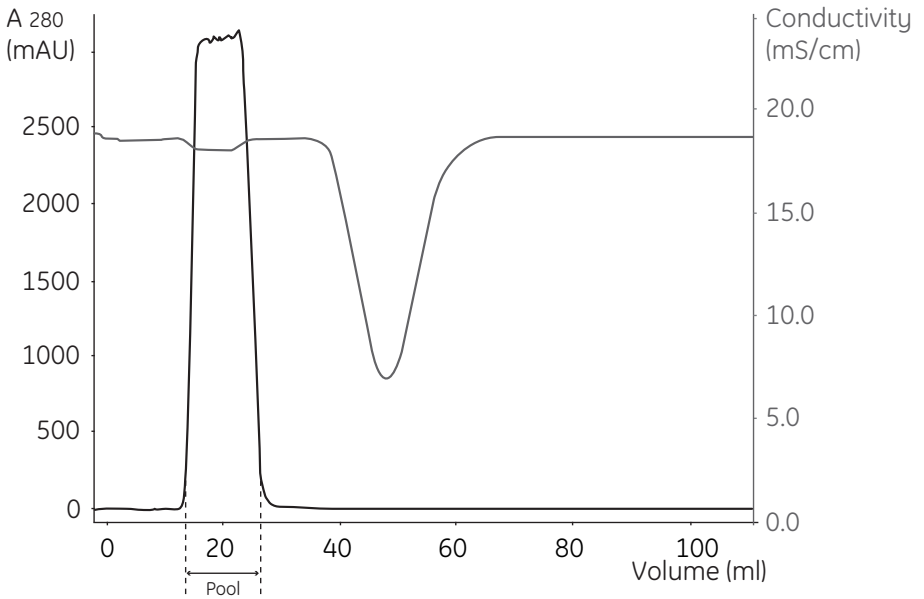

Fig 3. Buffer exchange on HiPrep 26/10 Desalting.
Sample volume: 10 ml MabSelect SuRe™ eluate. Buffer exchange to 25 mM BIS-TRIS, 175 mM NaCl, pH 7.0 Flow rate: 15 ml/min. Pooled material: 13 ml

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].

[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.

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

Initial screening of 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.

To find conditions suitable for the DoE, initial experiments can be performed in binding mode, using a pH gradient for elution (Fig 4, left). The elution position, that is pH at peak maximum, defines the lower pH in the design. The upper pH in the design should normally be 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 4. 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.

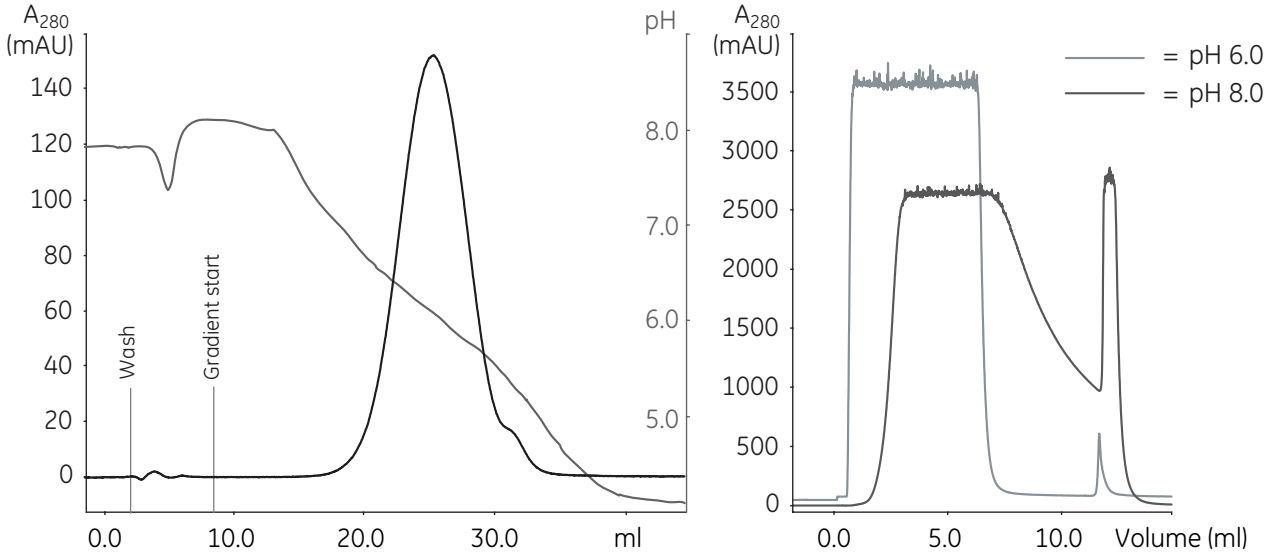

Fig 4. Sample: rProtein A elution pool, after buffer exchange on HiPrep 26/10 Desalting.
Binding mode (left): Sample loading at pH 7.8. Load: 1 mg/ml medium. Elution performed in a pH gradient from 7.8 to 4 .0.
Flow-through mode (right): Comparison of chromatograms obtained at different pH. Load 75 mg MAb/ml medium. 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 following is an example of how to set up a full factorial DoE with three parameters.

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.
   Limiting values, high and low, should be used of each parameter. The high and low values should be combined in a way that makes the parameters independent of each other to be able to separate effects. For further information, see www.umetrics.com.

Example of design of experiments

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

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

Parameters affecting the yield

The parameters that affect the yield are shown in the coefficient plot[1] (Fig 5). 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%.

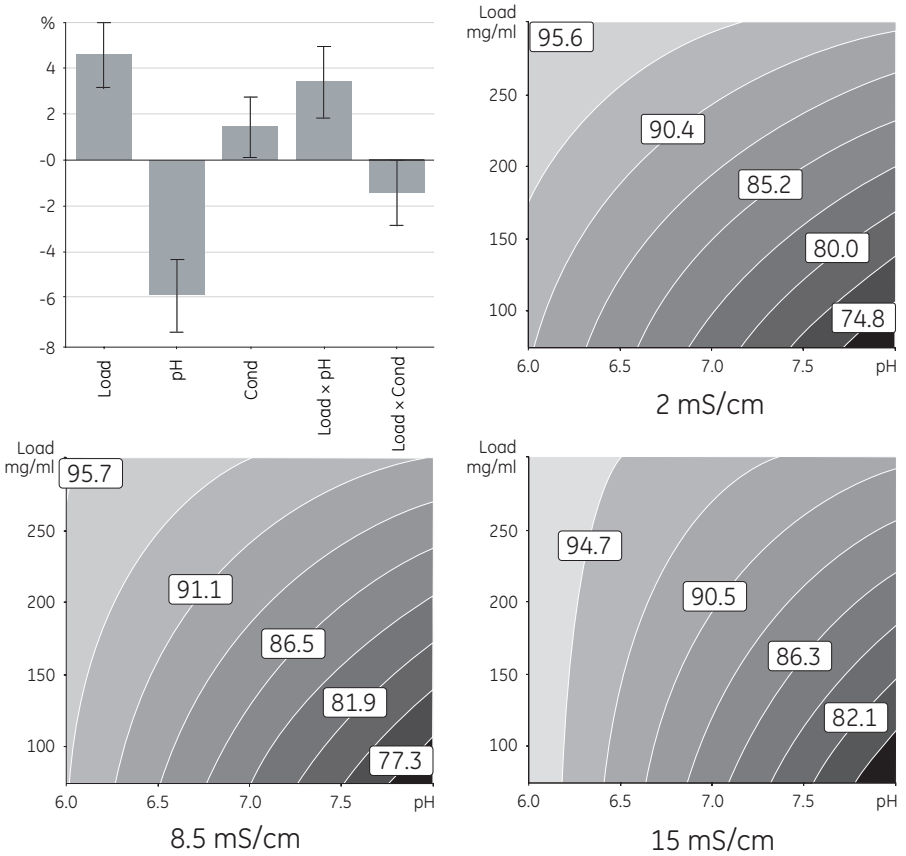

Fig 5. Coefficient plot and response surfaces for the yield model. Load *versus* pH at different conductivities.

[1] 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.

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 6). 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.

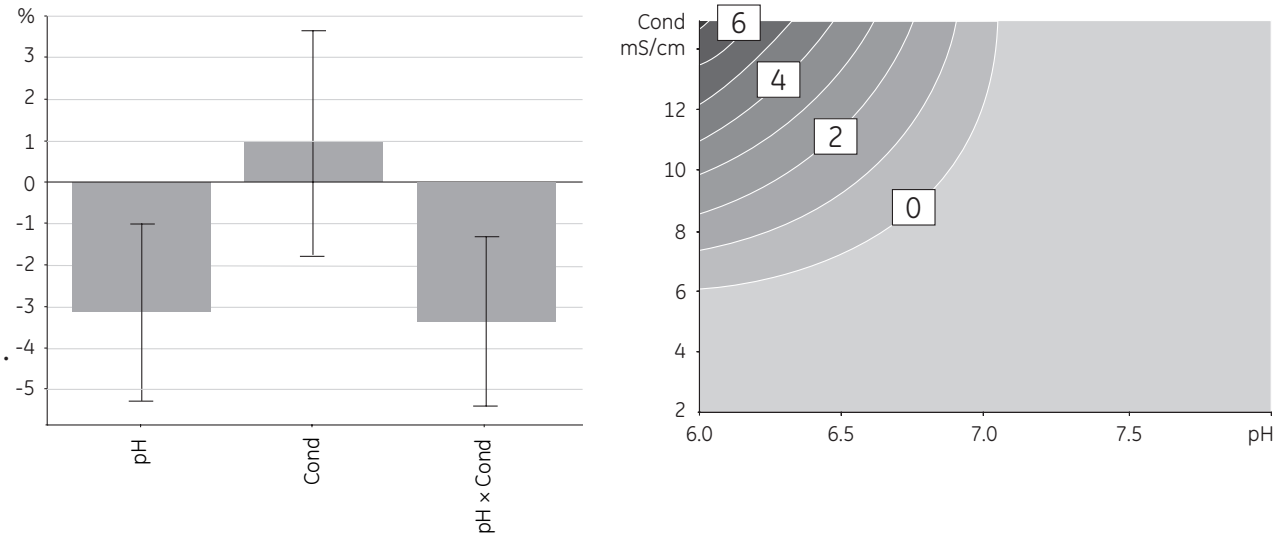

Fig 6. Coefficient plot and response surfaces for the Protein A model. Conductivity *versus* pH. Protein A concentration expressed in ppm.

Parameters affecting dimer/aggregate clearance

The coefficient plot (Fig 7) 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. The load should be low to give 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.

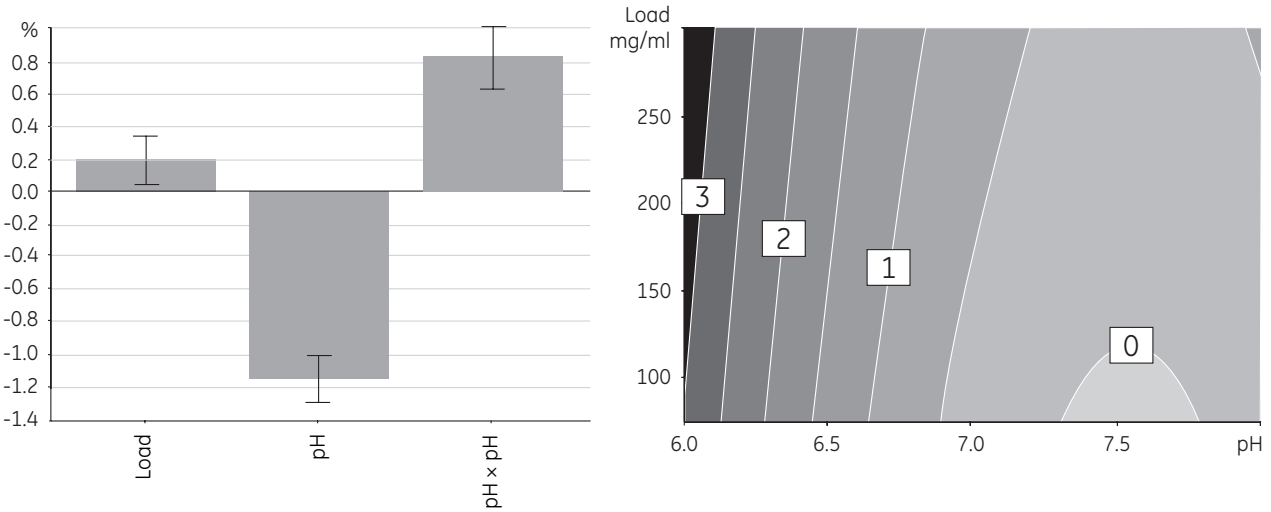

Fig 7. Coefficient plot and responce surface for the D/A model. Load *versus* pH. D/A concentration expressed in %.

Parameters affecting host cell protein (HCP) clearance

The coefficient plot (Fig 8) and response curves show that low sample load, low conductivity and high pH will give low HCP values.

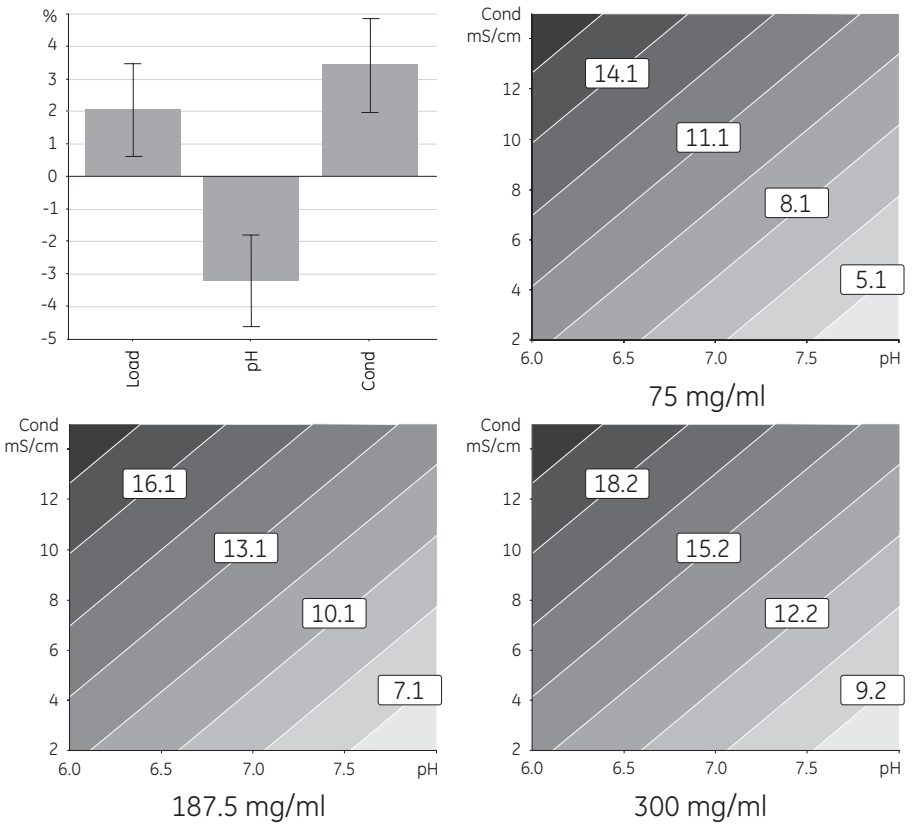

Fig 8. Coefficient plot and response surfaces for the HCP model. Conductivity *versus* pH. HCP concentration expressed in ppm.

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 may 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 9).

- For best yield load should be high, the pH low and conductivity high.
- For the best D/A-clearance the pH should be high, while load and conductivity should be low. D/A-clearance is often less affected by conductivity than Protein A and HCP clearance.
- For the best Protein A- and HCP-clearance the pH should be high and conductivity low.

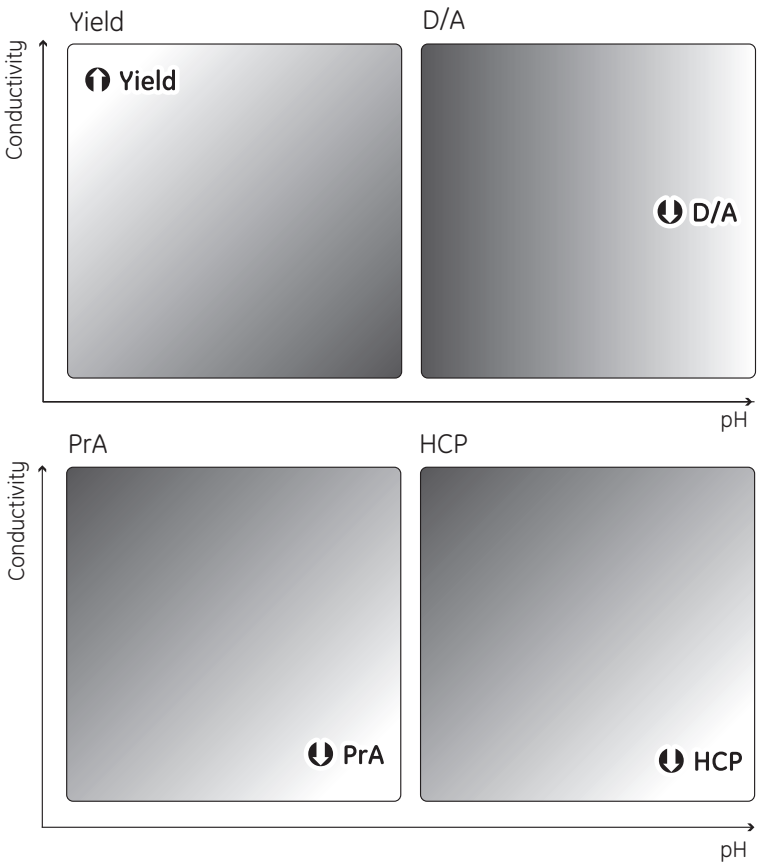

Fig 9. General trends with respect to loading conditions for yield, D/A-, Protein A- and HCP-clearance.

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, all three parameters should be varied in the same experimental series.

In Table 4 optimal loading conditions for five different antibodies are shown. As can be seen, pH should 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 - 8.9	5.5	3
MAb 3	7.5 - 8.4	6.0	2
MAb 4	7.7 - 8.0	7.0	20
MAb 5	6.5 - 9.0	7.7	20

Bed height, flow velocity and residence time

In production scale bed heights of 10 - 20 cm are recommended. Recommended flow velocity is between 150 – 600 cm/h. The residence time,[1] should be 2 minutes[2] or more. Longer residence time may result in more efficient contaminant removal.

As the loading capacity in flow-through mode is relatively high (100 - 300 mg MAb/ml medium) lower bed heights can by advantage be used in lab scale. However, the flow velocity should 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] 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.

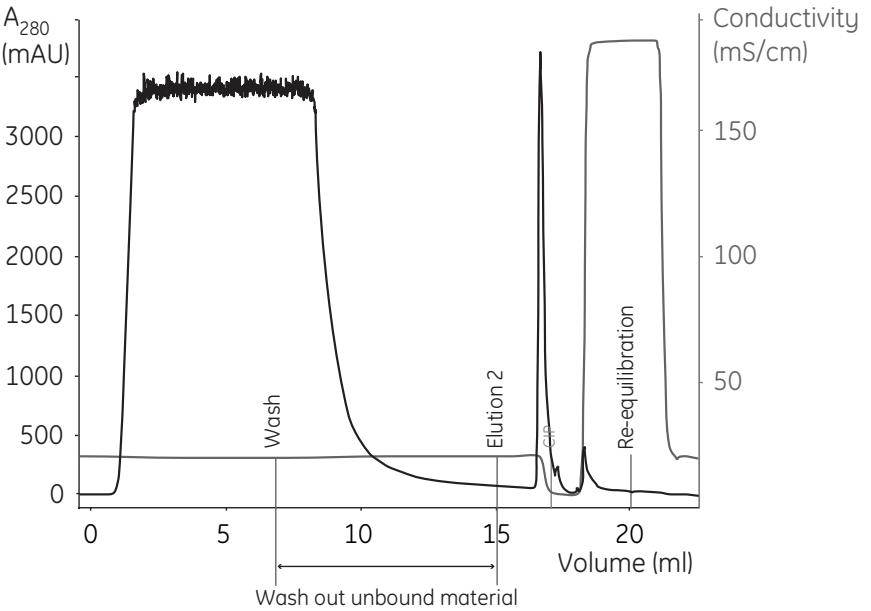

Fig 10. Example of wash after sample application

Regeneration, cleaning in place and re-equilibration

After wash out of unbound material, regeneration should be performed at low pH as described in Chapter 6, Regeneration.

Depending on the nature and the condition of the feedstock, cleaning-in-place (CIP) is recommended after 1-5 cycles. A standard protocol for CIP is to wash with 1 M NaOH, using a contact time of 15 minutes. For further details, see Chapter 6, Cleaning-in-place.

At the end of each purification cycle, and after regeneration and cleaning-in-place, the column should be re-equilibrated with 5 bed volumes of loading buffer, or until the column effluent shows stable conductivity and pH values.

4. Column packing

Packing process-scale columns

For information, please contact your local GE healthcare representative.

Packing lab-scale columns

The following instructions are for packing lab-scale columns: Tricorn™ 5/100, Tricorn 10/100, XK 16/20 with 10 cm bed height and XK 16/40 with 20 cm bed height. For further details see column manuals.

Packing Tricorn 5/100 and 10/100 columns

Materials needed

Capto adhere
Glass filter funnel
Plastic spoon
Filtering flask
Measuring cylinder
20% ethanol

Amount of medium

The amount of Capto adhere medium needed can be calculated by: column cross sectional area (cm^2) × bed height (cm) × compression factor (sedimented medium bed height/packed medium bed height). The compression factor is approximately 1.15 for Capto adhere.

Preparing the packing slurry

The slurry concentration should be approximately 60% in 20% ethanol, measured in a measuring cylinder after settling overnight or after centrifugation at 3000 rpm for 3 minutes, wait for 5 minutes before reading.

Equipment needed

Tricorn 5/100 or 10/100 column, Tricorn 5/100 or 10/100 glass tube (used as a packing tube) and a Tricorn packing connector 5-5 or 10-10. When working with large volumes or when repeatedly loading, the Tricorn coarse filter kit is recommended to reduce the risk of clogging. Use Tricorn coarse filter kit 5 (11-0012-53) or Tricorn coarse filter kit 10 (11-0012-54).

ÄKTAdesign™ 100 system for Tricorn 10, ÄKTAdesign 10 system for Tricorn 5 or a stand-alone pump such as P-901.

Column packing procedure

To pack the column, use 20% ethanol and proceed as follows:

1. Assemble the column according to the column instruction (Tricorn empty 56-3154-71).
2. Pour the chromatography medium into the top of the packing tube filling both column tube and packing tube. Remove all air bubbles.
3. Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker underneath the column tube and connect a pump to the top of the packing unit, remove the stop plug from the bottom of the column tube.
4. Settle the media at 0.2 ml/min (Tricorn 5/100) or 0.4 ml/min (Tricorn 10/100) for 10 minutes.
5. After the bed is settled, raise to packing flow – 10 ml/min (Tricorn 5/100) or 14 ml/min (Tricorn 10/100) for 2 minutes.
6. When the medium is packed, switch off the pump and attach the stop plug into bottom of the column tube. Disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess medium 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 turn it back 1.5 turns.
10. Wet the O-ring on the adapter unit by dipping it into water, buffer or 20% ethanol.
11. Screw the adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there is no air trapped below the filter.
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 cap of the column tube.
14. Pack the column at 10 ml/min (Tricorn 5/100) or 14 ml/min (Tricorn 10/100) for 2 additional minutes.
15. Before switching off the pump mark the position of the medium 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 pump. Screw the adapter unit down to slightly below 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 or storage.

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 5 *"Evaluation of column packing"*.

Packing XK 16/20 and XK 16/40 columns

Materials needed

Capto adhere
Glass filter funnel
Plastic spoon
Filtering flask
Measuring cylinder
20% ethanol

Amount of medium

The amount of Capto adhere medium needed can be calculated by: column cross sectional area (cm2) × bed height (cm) × compression factor (sedimented medium bed height/packed medium bed height). The compression factor is approximately 1.15 for Capto adhere.

Preparing the packing slurry

The slurry concentration should be approximately 60% in 20% ethanol, measured in a measuring cylinder after settling overnight or after centrifugation at 3000 rpm for 3 minutes, wait for 5 minutes before reading.

Equipment needed

XK 16/20 is used for 10 cm bed heights and XK 16/40 for 20 cm bed height. To obtain 15 cm bed height an XK 16/40 with two flow adapters can be used.

Use ÄKTAdesign 100 system or a stand-alone pump such as P-901 that can deliver 20 ml/min. The pump filter unit should be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure. Mount a pressure gauge at the inlet of the column to make sure that the pressure does not exceed the operating pressure limit for the column.

XK 16/20 and XK 16/40 packing procedure

To pack the column, use 20% ethanol and proceed as follows:

1. Wet the bottom filter with 20% ethanol, with the aid of a syringe mounted on the outlet tubing. After the filter is wetted mount a stop screw on the outlet.
2. Mount the bottom piece in the column tube.
3. Wet the adapter filter with 20% ethanol, with the aid of a syringe mounted on the inlet tubing. After the filter is wetted mount a stop screw on the inlet.
4. Keep the adapter in a beaker with 20% ethanol.
5. Mount the column vertically on a stand.
6. Fill the glass tube with 60% medium slurry up to the upper edge of the glass tube.
7. Mount the adapter in the upper part of the glass tube with no air trapped below the filter.
8. Tighten the sealing-ring and connect the column inlet to the system outlet.
9. Pack the column at 40 ml/min for 10 min.

Note: Do not exceed the operating pressure limit for the columns (5 bar for XK 16 and XK 26).

10. Mark the position of the medium surface with a pen on the column.
11. Stop the pump and mount a stop screw on the outlet.
12. Adjust the adapter quickly down to the marked medium surface and then a further 15 mm into the medium bed.

Testing the packed column

See Chapter 5 *"Evaluation of column packing"*.

5. Evaluation of column packing

Test column efficiency to check the quality of the packing. Tests should be made directly after packing and at regular intervals during the working life of the column plus when separation performance is seen to deteriorate. 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 (As). 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 2.0 M NaCl in water with 0.5 M NaCl in water as eluent.

Note: The calculated plate number will vary according to the test conditions and it should 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.

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow velocity between 15 and 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:

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

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

where:

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 is calculated as follows:

$$\frac{HETP}{d}$$

where:

d is the diameter of the bead

As a guideline, a value of <3 is normally acceptable.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (values between 0.8–1.5 are usually acceptable).

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

Peak asymmetry factor calculation:

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

where:

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height.

Figure 11 shows a UV trace for acetone in a typical test chromatogram in which the HETP and A_s values are calculated.

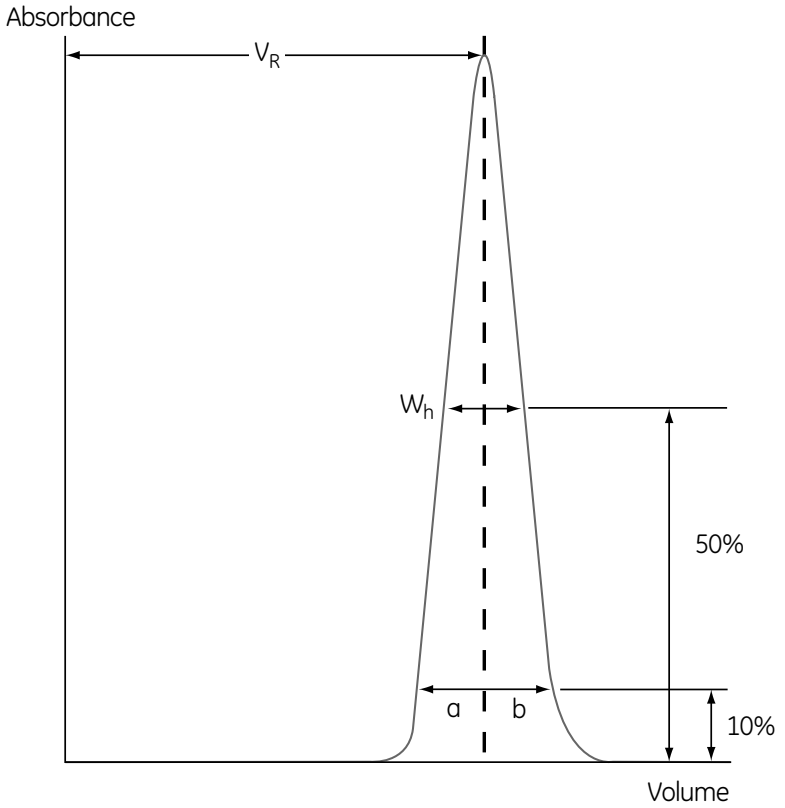

Fig 11. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

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 medium 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 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.

CIP protocols	
Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH at 150 cm/h with reversed flow direction. Contact time 15-30 minutes..
Ionically bound proteins	Wash with 0.5–2 column volumes of 2 M NaCl with reversed flow direction.
Lipids and very hydrophobic proteins	Wash with 2–4 column volumes of up to 70% ethanol[1] or 30% iso-propanol with reversed flow direction. Contact time 1-2 hours.
Nucleic acids	Wash with 0.1 M acetate pH 3 for 2-5 column volumes followed by equilibration buffer at neutral pH for 1-2 column volumes and wash 1 M NaOH at 150 cm/h with reversed flow direction. Contact time 15-30 minutes.

[1] Specific regulations may apply when using 70% ethanol since it can require the use of explosion-proof areas and equipment.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5–1 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 medium effectively.

Storage

Store used medium in the container at a temperature of 4 to 30ºC. 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.

7. 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

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

Related products

Product	Quantity	Code No
HiTrap Capto adhere	5 x 1 ml	28-4058-44
HiTrap Capto adhere	5 x 5 ml	28-4058-46
HiTrap MabSelect SuRe	5 x 1 ml	11-0034-93
HiTrap MabSelect SuRe	5 x 5 ml	11-0034-95
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02
Tricorn 5/100 column	1	18-1163-10
Tricorn 10/100 column	1	18-1163-15
XK 16/20 column	1	18-8773-01

Literature

Product	Code No
Data file Capto adhere	28-9078-88
Application note: Optimization of loading conditions on Capto adhere using design of experiments	28-9078-89
Application note: Two step purification of monoclonal IgG1 from CHO cell supernatant	28-9078-92
Application note: Selective removal of aggregates with Capto adhere	28-9078-93

8. Further information

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

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