HiScreen™ Capto™ adhere
HiTrap™ Capto adhere

Capto adhere is a multimodal BioProcess™ resin for polishing of monoclonal antibodies samples purified from Protein A resin (for example, MabSelect™), using column chromatography.

Capto adhere improves the yield, productivity and process economy by:

- Providing high capacity in the flow-through mode
- Removing contaminants to formulation levels in post Protein A purification:
  - Leached Protein A
  - Antibody dimers and aggregates
  - Host cell proteins
  - Nucleic acids
  - Viruses
- Allowing a wide pH and conductivity operational window
- Savings time and operating costs via a two steps chromatographic process

Table of Contents

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

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

1 Product description

HiScreen column characteristics

HiScreen Capto adhere is a prepacked 4.7 mL column for method parameters optimization for examples, selectivity, binding and elution conditions. The column is also used for robustness testing when developing a new purification process. The small column volume, 4.7 mL, and the 10 cm bed height makes it possible to perform scalable experiments.

The column is suitable for liquid chromatography using systems such as ÄKTA™.

HiScreen columns are made of biocompatible polypropylene that does not interact with the biomolecules. The arrow on the column label shows the recommended flow direction.

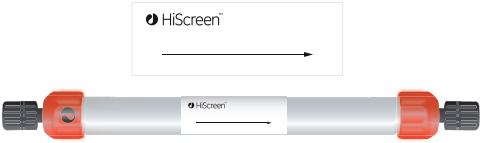

Fig 1. HiScreen column

When a higher bed height is required, two columns can be connected in series to give a 20 cm bed height, see *Section 6 Scaling up, on page 27*.

Note: *Do not open or refill HiScreen columns.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	0.8 MPa (8 bar, 115 psi) [1]

[1] The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/liquid viscosity, and the column tubing used.

HiTrap column characteristics

HiTrap columns are made of biocompatible polypropylene that does not interact with biomolecules.

The prepacked 1 mL and 5 mL columns are designed for method scouting and screening of loading and elution conditions. The columns provide fast and reproducible separations in a convenient format. The columns are delivered with a stopper at the inlet and a snapoff end at the outlet. HiTrap Capto adhere are best used with liquid chromatography systems such as ÄKTA, but can be operated with a syringe or peristaltic pump.

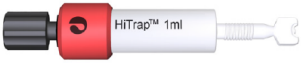

Fig 2. HiTrap 1mL

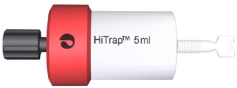

Fig 3. HiTrap 5mL

Note: *Do not open or refill HiTrap columns.*

Note: *Check that the connector is tightened to prevent leakage.*

Table 2. Characteristics of HiTrap columns

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	0. 5 MPa (5 bar, 72 psi)[1]	0.5 MPa (5 bar, 72 psi)[1]

[1] The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/liquid viscosity, and the column tubing used.

Supplied Connector kit with HiTrap column	**No. supplied**
Union 1/16" male/ luer female for Connection of syringe to HiTrap column	1
Stop plug female, 1/16" For Sealing bottom of HiTrap column	2,5 or 7

Properties of Capto adhere

Capto adhere is a strong anion exchanger with multimodal functionality. The multimodal functionality gives a different selectivity compared to traditional anion exchangers. The multimodal functionality gives a different selectivity compared to traditional anion exchangers. 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 which the antibodies pass directly through the column while the contaminants are adsorbed.

The ligand (Fig 4) exhibits many pronounced functionalities such as the ionic interaction, hydrogen bonding and hydrophobic interaction.

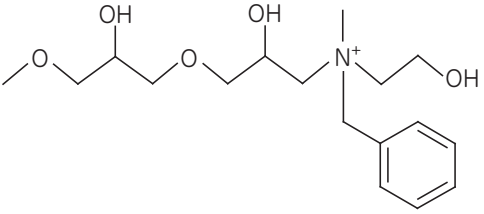

Fig 4. The Capto adhere ligand, N-Benzyl-N-methyl ethanol amine

The ligand is coupled to a chemically modified, high flow agarose matrix. The agarose matrix provides particle rigidity without compromising the pore size. The properties of the agarose matrix allow fast mass transfer, that results in a high dynamic binding capacities of Capto adhere at high flow rates. This makes the resin suitable for high volume process scale applications.

Table 3. Characteristics of Capto adhere

Matrix	Highly cross-linked agarose, spherical
Ion exchange type	Multimodal strong anion exchanger
Ionic capacity	0.09 to 0.12 mmol Cl^-/mL resin
Particle size (d_{50v}) [1]	~ 75 µm
Recommended Operating flow rate [2]	2 to 4.7 mL/min (HiScreen) 1 to 4 mL/min (HiTrap 1 mL) 5 to 20 mL/min (HiTrap 5 mL)
Pressure/flow characteristics (large scale)	At least 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] **pH stability, CIP** [5] **pH ligand fully charged** [6]	3 to 12 2 to 14 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]
Storage	20% ethanol, 4°C to 30°C

1 Median particle size of the cumulative volume distribution.

2 At room temperature, using buffers with the same viscosity as water. See also tables 8-10.

3 The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given 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 ligand fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

7 Capto adhere can be used in cold-room conditions, but the capacity of removal of some key contaminants may decrease.

8 1.0 M NaOH should only be used for cleaning purposes. Avoid oxidizing agents and anionic detergents.

2 Optimization

Aim

The aim of designing and optimizing a method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. For optimization of binding conditions, pH and conductivity (salt concentration) should be screened.

Screening

When using Capto adhere, protein binding can be expected at pH values lower than those used with traditional ion exchangers due to the contribution from non-electrostatic interactions. The multimodal nature of the ligand results in different interaction modes, electrostatic *versus* hydrophobic, depending on buffer conditions and target protein characteristics. A thorough screening of conditions is therefore strongly recommended on Capto adhere in order to optimize conditions for binding and elution.

Workflow

The recommended workflow is described in the figure below. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.

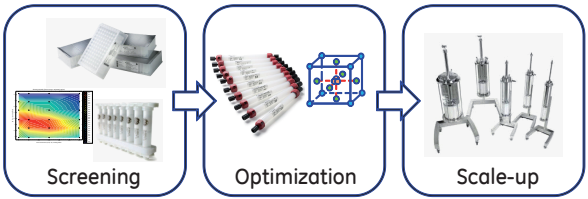

Fig 5. Recommended workflow during process development.

3 Design of Experiment

Introduction

The major consideration when optimizing a purification process is to achieve a high product yield while maintaining the purity.

Optimized conditions where most contaminants are adsorbed while the monomeric antibodies pass through the column can be obtained by adjusting the pH and conductivity of the sample. Optimization of loading conditions is preferably performed using Design of Experiment.

Design of Experiments (DoE) is used for robustness testing, method parameter screening and optimization of a purification process.

Initial experiments

In the DoE, pH, conductivity, and load must be included. The following initial experiments are then performed:

- Binding mode, using a pH gradient for elution. The elution position, that is pH at peak maximum, defines the lower pH in the design. The upper pH in the design should be about two pH units higher.

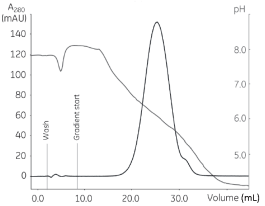

Fig 6. Binding mode: Sample load: 1 mg/mL resin at pH 7.8. Elution: in a pH gradient from 7.8 to 4.0.

- Flow-through mode, where the conductivity is kept constant at a moderate level and the pH is altered.

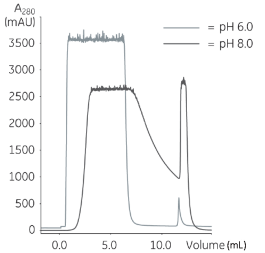

Fig 7. Flow-through mode: Comparison of chromatograms obtained at different pH. Load: 75 mg mAb/mL resin. Conditions: pH 8.0, 2 mS/cm and pH 6.0, 2 mS/cm.

Altering the pH

In the DoE, it is important to include conditions at higher and lower pH range. The higher pH range results in a lower yield and higher purity, while a lower pH range results in a higher yield but lower purity.

At higher pH, close to the pI of the antibodies:

- The breakthrough during sample load is delayed.
- The breakthrough and wash curves are shallow.
- Significant amounts of mAb bind to the resin.

At lower pH, far from the pI of the antibodies:

- The breakthrough of wash curves is steep.
- The yield is increased.
- The electrostatic interaction between the antibodies and the resin is weakened.

Setting up a full factorial DoE

By systematically varying pH, conductivity and sample load, response surfaces can be obtained for the yield and clearance of the key contaminants. The following is an example of how to set up a full factorial DoE with the three parameters.

1 Perform initial loading experiments at varying pH see Section *Initial Experiments*.

2 Choose design for screening or optimization.

Note: *A full factorial DoE with 3 parameters gives 8 experiments + center points.*

3 Choose parameters to include and define parameter ranges and responses.

4 Choose center points for the design. Center points are important in DoE, because they give an indication of possible curvature in the data. Three replicated center points are recommended.

5 Make systematic variation of the parameters.

Note: *High and low values for each parameter should be used independently to separate the effects.*

Example of a DoE

The experimental setup in this example is a full factorial design with three variables, load, pH and conductivity, 2 center points and 4 additional points to resolve the curvature effects (Table 4). The measured responses in the flowthrough pool are:

- Yield (%)
- Protein A (ppm)
- S-dimer/aggregate (D/A %)
- Host cell proteins (HCP ppm)

For each response, a separate model is calculated. The models are fitted to multiple linear regression (MLR).

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

Sample characteristics: pI ~ 9, leached protein A 36 ppm, D/A 3.3 % and HCP 210 ppm.

Note: *D/A is determined by size exclusion chromatography using Superdex™ 200 10/300 GL.*

Note: *Experiments were designed and evaluated using Modde 7.0 software (Umetrics, Sweden), see www.umetrics.com.*

Table 4. The design set up

Experiment	Load (mg mAb/mL)	pH	Conductivity (mS/cm)
1	75	6	2
2	300	6	2
3	75	8	2
4	300	8	2
5	75	6	15
6	300	6	15
7	75	8	15
8	300	8	15
9	**187.5**	**7**	**8.5**
10	**187.5**	**7**	**8.5**
11	75	7	15
12	300	7	15
13	187.5	7	2
14	187.5	7	15

Note: *The experiments are performed randomly.*

Parameters affecting the yield

The coefficient plot shows (Fig 8) that the load is positively correlated to the response, (i.e., a higher load gives a higher response).

The response surfaces show that the higher loads give larger pH window with a yield > 90%.

The interaction effects that are present in the coefficient plot means that if the pH is changed, the response is changed with the effect of pH, and the load at that specific pH. The same is valid for the load × conductivity interaction.

The pH is negatively correlated to the response, (i.e., a lower pH gives a higher response value).

The conductivity is positively correlated to the response, (i.e., a higher conductivity gives a higher response value) but less than the effect of the Load.

The high sample load, low pH and high conductivity result in a high yield. The interaction effects of the factors are significant for the yield response.

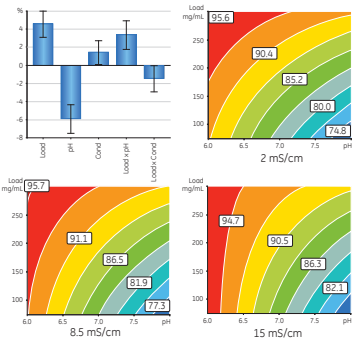

Fig 8. Coefficient plot and response surfaces for the yield model. Load *versus* pH at different conductivities.

Parameters affecting protein A clearance

The coefficient plot shows that a high pH gives a good protein A clearance.

The response surfaces show that high pH and low conductivity give a high protein A clearance.

The conductivity alone does not significantly affect the responses, but there is a significant interaction effect of pH × conductivity (Fig 9).

If the interaction effect of the pH is high, the protein A clearance is low. The load is not a significant factor for this response.

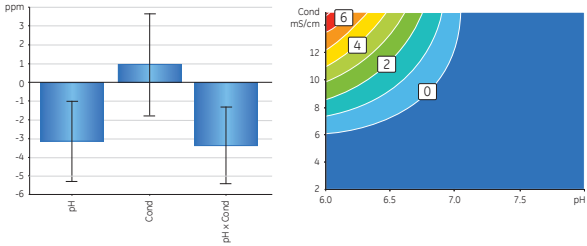

Fig 9. Coefficient plot and response surfaces for the protein A model. Conductivity *versus* pH. The response surfaces show protein A concentration in ppm.

Parameters affecting dimer/aggregate clearance

The coefficient plot (Fig 10) shows that pH is the most important parameter that affects the dimer and aggregate clearance. High pH gives low D/A concentration in the flow-through pool. The load parameter is also significant, but very small.

There is a significant curvature effect assigned to pH. If pH is too high or too low the D/A response is increased. The conductivity does not significantly affect D/A-clearance.

The response curve shows that the load has only small effect on the D/A-clearance.

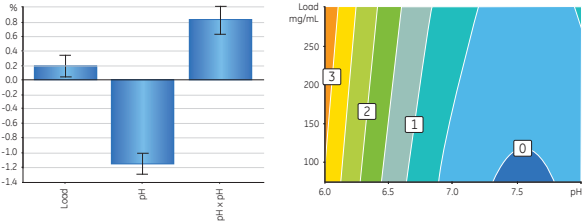

Fig 10. Coefficient plot and response surfaces for the D/A model. Load *versus* pH. D/A concentration expressed in percentage.

Parameters affecting host cell proteins clearance

The coefficient plot (Fig 11) and response curves shows that low sample load, low conductivity and high pH give low HCP values.

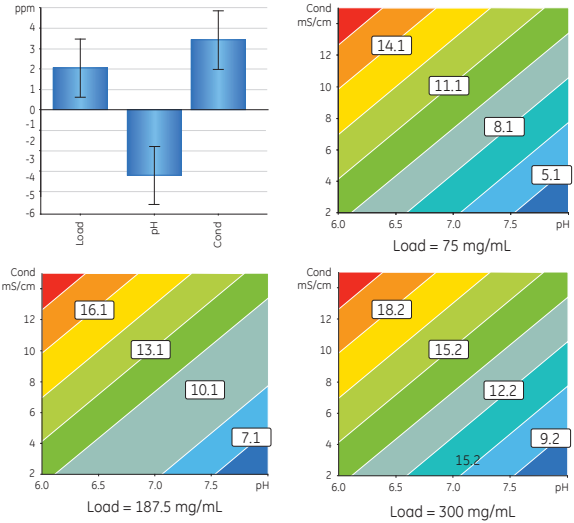

Fig 11. Coefficient plot and response surfaces for the HCP model. Conductivity *versus* pH. HCP concentration expressed in ppm.

Summary of the parameters effects

Based on the DoE results of the tested factors, the suggested loading condition for this mAb type is a sample load of 200 mg/mL, pH 7, at 8.5 mS/cm conductivity.

Predictions for loading condition

Each monoclonal antibody is unique, and the level of contaminants varies between the different cell lines and in previous purification steps. Based on the results of several DoE performed with the different antibodies, some general trends are identified (Fig 12).

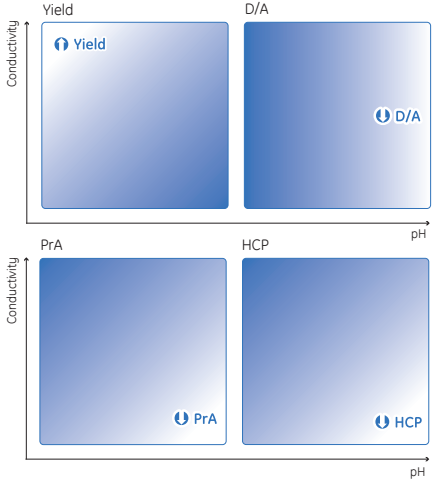

Fig 12. General trends with respect to loading conditions for yield, D/A protein A, and HCP-clearance.

For the best:

- **Yield:** the load should be high, pH low and the conductivity high.
- **D/A-clearance**: the pH should be high, while load and conductivity should be low.
- **Protein A and HCP-clearance**: the pH should be high and the conductivity low.

Loading conditions are usually a compromise between conditions favoring yield and those favoring contaminant clearance.

An optimal loading condition is a balance between load, pH and conductivity. For optimization of the loading condition, all the three parameters should be varied in the same experimental series.

Table 5 shows the optimal loading condition for five different antibodies. According to the results, the pH should be below the isoelectric point. The optimal conductivity is difficult to predict.

Table 5. 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

Optimizing washing conditions

For an efficient removal of contaminants in the flow-through mode, residence time should be 2 minutes or more. Longer residence time may result in more efficient contaminant removal.

After the sample load, continue to wash out the unbound mAb with the start buffer until the UV-curve reaches the baseline. The required wash fractions can then be pooled with the flow-through fractions.

Note: *Pooling the wash fractions results in dilution of the sample and thereby affects the yield.*

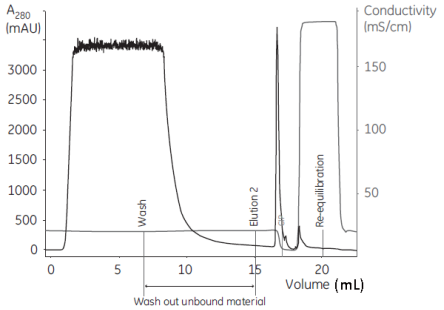

Fig 13. Example of the wash after the sample loading.

4 Operation

Prepare buffers

The buffer type and concentration are important for reproducible and robust methods. Table 6 shows suitable buffers for anion exchangers and suggested start concentrations.

Users of the ÄKTA systems with ***BufferPrep*** functionality can select from a range of buffer recipes to examine the resin at different pH and elution conditions.

Table 6. Recommended buffers for anion exchange chromatography

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 may influence both yield and the contaminant clearance.

2 Buffers in the interval 5.5 to 8 are normally the most efficient for contaminant removal.

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

Note: *The water and chemicals used for the buffer preparation should be of high purity. Filter the buffers through a 0.22 or 0.45 µm filter before use.*

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of the following methods: • Dilute the sample with the start buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see table below).
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading the sample to the column. This prevents clogging and increases the life time of the column specially when loading large sample volumes.

Note: *Buffer exchange may result in reduction of HCP levels and improve the column performance.*

Note: *For larger volumes of feed, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity.*

Prepacked columns for desalting

The prepacked columns described in the Table 7 are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).

Table 7. Prepacked columns.

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting [1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting [2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting [3]	1.0 to 2.5 mL [4] 1.75 to 2.5 mL [5]	3.5 mL Up to 2.5 mL
PD MiniTrap G-25	0.1 to 2.5 mL[4] 0.2 to 0.5 mL[5]	1.0 mL Up to 0.5 mL
PD MidiTrap G-25	0.5 to 1 mL[4] 0.75 to 1 m[5]	1.5 mL Up to mL

1 Prepacked with Sephadex™ G-25™ Fine and requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 Volumes with centrifugation.

Recommended flow rates

The pressure-flow specification for the Capto adhere resin in large columns is ≥ 600 cm/h in a 20 cm bed height at 3 bar and a buffer viscosity comparable to the one of water. The residence time used should however match those appropriate for the final intended scale of the process and this should be considered when running HiScreen and HiTrap columns. Recommended flow rates for different operations are found in the tables below.

Table 8. Recommended flow rates using HiScreen Capto adhere.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration[1]	≤ 4.7	≤ 600	≥ 1
Wash	≤ 4.7	≤ 600	≥ 1
Sample load	1.2 to 2.3	150 to 300	2 to 4
Cleaning-in-place[2]	≤ 0.78	≤ 100	≥ 6

Table 9. Recommended flow rates using HiTrap Capto adhere, 1mL.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration[1]	≤ 4.0	≤ 600	≥ 0.25
Wash	≤ 4.0	≤ 600	≥ 0.25
Sample load	0.25 to 0.5	35.5 to 75	2 to 4
Cleaning-in-place[2]	≤ 0.16	≤ 25	≥ 6

Table 10. Recommended flow rates using HiTrap Capto adhere, 5mL.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration [1]	≤ 20	≤ 600	≥ 0.25
Wash	≤ 20	≤ 600	≥ 0.25
Sample load	1.2 to 2.5	35.5 to 75	2 to 4
Cleaning-in-place [2]	≤ 0.84	≤ 25	≥ 6

[1] The flow rates are for buffers with the same viscosityas water at 20°C. For solutions with higher viscosities, (e.g, 20% ethanol), lower flow rates should be used.

[2] The Cleaning-In-Place should be done in at least 3 column volumes with a total contact time of at least 15 minutes. See also Chapter *Cleaning-in-place (CIP).*

Purification

Removal of leached protein A, antibody dimers and aggregates, host cell proteins, viruses, and nucleic acids is preferably performed in flow-through mode where the antibodies pass directly through the column while the contaminants are adsorbed.

For efficient removal of the contaminants:

- Residence time should be 2 minutes or more. Longer residence time may result in more efficient contaminant removal.
- Flow rate: See the recommended flow rates, see Table 8-10.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 mm).

Note: *A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.*

Step	Action
1	Remove the stoppers and connect the column to the system. Avoid introducing air into the column. ***Note:*** *To prevent leakage, check that the connectors are tight. Use fingertight 1/16" connector (28401081).*
2	Wash with 5 column volumes (CV) of ultra pure water. *Result*: The ethanol is removed and thereby the precipitation of buffer salts upon exposure to ethanol is avoided. ***Note:*** *The step can be omitted if precipitation is not likely to be a problem.*
3	Equilibrate the column with at least 5 CV start buffer or until the UV baseline, eluent pH and the conductivity are stable.
4	Adjust the sample to the starting pH and conductivity and load on the column. Collect the flowthrough fraction.
5	Continue to wash out the unbound protein with start buffer until the UV-curve reaches the baseline. The wash fractions can be pooled with the flowthrough fractions. ***Note:*** *Pooling the wash fractions results in dilution of the sample and thereby affects the yield.*
6	To elute any reversibly bound material, regenerate the resin at low pH (e.g., 0.1 M acetate pH 3.0) by washing the column until the effluent shows stable pH and conductivity values.
7	Perform a CIP to clean the column.
8	Re-equilibrate with 5 to 10 CV start buffer or until the UV-signal, eluent pH, and conductivity reach the required values.

Note: *Do not exceed the maximum recommended flow rate and limit the back pressure according to the column specification.*

Note: *A higher flow rate may be applied during the regeneration and re-equilibration steps.*

5 Cleaning-In-Place (CIP)

General description

CIP removes very tightly bound, precipitated or denatured substances/proteins from the resin. The accumulated contaminants can affect the chromatographic properties of the prepacked column, reduce the capacity, or contaminate the subsequent runs.

CIP must be performed regularly to prevent the enrichment of the contaminants and to maintain the capacity, flow properties, and general performance of the prepacked columns.

It is recommended to perform a CIP:

- After every run with real feed.
- When an increase in the back pressure is noticed.
- If a reduced column performance is observed.
- Before first time use or after a long term storage.
- To prevent possible cross-contamination, when the same column is used for purification of different proteins.

CIP protocol

The nature of the sample ultimately determines the final CIP protocol. The contact time, frequency and NaOH concentration, are the main parameters to vary during the CIP optimization. The CIP procedure below describes removal of the most common contaminant.

Flow rate: For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during the purification, see Tables 8-10.

Step	Action
1	Wash the column with at least 2 column volumes (CV) of 2 M NaCl.
2	Wash with at least 3 CV 1 M NaOH and with at least 15 minutes contact time.
3	Wash with at least 2 CV 2 M NaCl.
4	Wash with at least 2 CV ultra pure water.
5	Wash with the 5 CV start buffer or until the eluent pH and conductivity reach the required values.

6 Scaling up

After optimizing the method at laboratory-scale, the process is ready for scaling up. For quick small scale-up of purification, either two HiTrap columns, or two HiScreen columns can easy be connected in series with a union (Product code: 18112093) to give increased bed height.

Note: *The back pressure is increased with an increased bed height. Decrease the flow rate to adjust the back pressure.*

Other factors, such as clearance of the impurities may change, when the column bed height is modified. The factors must then be validated with the final bed height.

Scaling up is otherwise typically performed by keeping bed height and linear flow velocity (cm/h) constant while increasing bed diameter and volumetric flow rate (mL/min or L/h). Bulk BioProcess resin is available for further scaling up, see *Ordering information*.

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.

A general description of the scaling up procedure is described below:

Step	Action
1	Select bed volume according to the required sample load. Keep sample concentration constant.
2	Select column diameter to obtain the desired bed height. ***Note:*** *The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.*
3	Check the buffer delivery and monitoring systems for time delays or volume changes if the larger equipment used when scaling up causes some deviations.

7 Adjusting the pressure limits

The pressure in chromatography system is generated by the flow through a column. The pressure affects the packed bed and the column hardware. The pressure is increased during the run if the:

- Flow rate is high
- Buffer or sample is viscous
- Temperature is low
- System has a flow restrictor

Note: *Exceeding the flow rate limit can damage the column, see the recommended flow rates in Table 3.*

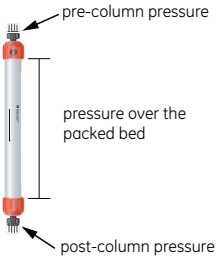

Fig 14. Pressure over the column

ÄKTA avant and ÄKTA pure

The systems automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The column hardware pressure limit is the same as the pre-column pressure limit (see Table 1 and Table 2).

The maximum pressure the packed bed maximum pressure can withstand depends on resin characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with a pressure sensor in the pump

To obtain a optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with a pressure sensor in the pump, the pressure limit can be set in the software as follows:

Step	Action
1	• Replace the column with a piece of tubing. • Run the pump at the maximum intended flow rate. • Record the pressure as total system pressure, P1.
2	• Disconnect the tubing and run the pump at the same flow rate as in step 1. *Result*: There will be a drip from the column valve. • Record the pressure as P2.
3	• Calculate the new pressure limit as, a sum of P2 and the column hardware pressure limit specification (see Table 1). • Set the pressure limit in the software with the calculated value. *Result:* The actual pressure over the packed bed (Δp) during the run is equal to the total system pressure (P1).

Note: *Repeat the procedure each time the parameters are changed.*

8 Storage

Step	Action
1	Wash the column with 2 column volumes (CV) of ultra pure water.
2	Wash the column with 2 CV 20% ethanol.
3	Store the column at 4°C to 30°C. Do not freeze.
4	Check that the column is tightly sealed to stop the column from drying out.

9 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Use a lower flow rate.
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	If possible, degas the sample using a vacuum degasser.
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual decrease in yield	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High back pressure during CIP	Proteins are precipitated in the column.	• Optimize elution conditions and/or run high salt wash before CIP. • Use a lower flow rate.
Reduced column performance despite optimized elution and CIP	Column longevity, which depends mainly on the sample type and sample preparation.	Change to a new column.

10 Ordering information

Product	Quantity	Product code
HiScreen Capto adhere	1 × 4.7 mL	28926981
HiTrap Capto adhere	5 × 1 mL	28405844
	5 × 5 mL	28405846
Capto adhere	25 mL	17544410
	100 mL	17544401
	1 L [1]	17544403
HiTrap™ Desalting	5 × 5 mL	17140801
HiPrep™ 26/10 Desalting	1 × 53 mL	17507801
	4 × 53 mL	17508702
PD MidiTrap™ G-25	50 Columns	28918008
PD MiniTrap™ G-25	50 Columns	28918007
PD-10 Desalting	30 Columns	17085101

[1] Process-scale quantities are available. Please contact your local representative.

Accessories	Quantity	Product code
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28401081
1/16" male/luer female (For connection of syringe to top of HiTrap column)	2	18111251
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18112093
Tubing connector flangeless/M6 female (For connection of tubing to bottom of HiTrap column)	2	18100368
Tubing connector flangeless/M6 male (For connection of tubing to top of HiTrap column)	2	18101798
Union 1/16" female/M6 male (For connection to original FPLC System through bottom of HiTrap column)	6	18111257

Accessories	Quantity	Product code
Union M6 female /1/16" male (For connection to original FPLC System through top of HiTrap column)	5	18385801
Union luerlock female/M6 female	2	18102712
Stop plug female, 1/16" (For sealing bottom of HiTrap column)	5	11000464
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11000355

[1] One fingertight stop plug is connected to the inlet and outlet of each HiScreen column at delivery.

Related literature	Product code
Handbook: Ion Exchange Chromatography, Principles and Methods	11000421
Selection Guide: Ion Exchange Columns and Media	18112731
Selection Guide: Prepacked chromatography columns for ÄKTA systems	28931778
Data file: HiScreen prepacked columns Capto adhere	28930581 28907888
Application Note: Optimization of loading conditions on Capto adhere using design of experiments - Process-scale antibody purification	28907889
Application Note: Two-step purification of monoclonal IgG1 from CHO cell culture supernatant	28907892
Application Note: Selective removal of aggregates with Capto adhere	28907893

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