



MabSelect

Affinity chromatography

Instructions for Use

MabSelect™ is a BioProcess™ affinity resin for capturing monoclonal antibodies from large volumes of feed by packed bed chromatography. 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.

MabSelect is designed to:

- Process more than 10 000 L feed from high expression fermentations in a single day.
- High volume flow rates under process conditions.
- Enhanced binding capacity for immunoglobulins due to oriented coupling of the ligand and optimized matrix.
- Straightforward scale-up in AxiChrom™, BPG, and Chromaflow™ columns.
- Withstand effective and rigorous CIP procedures.

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Important

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 Description

The recombinant protein A is produced in *Escherichia coli*. Fermentation and subsequent purification of the protein A are done in the absence of mammalian products. The recombinant protein has been specially engineered to favor an oriented coupling that gives an affinity resin with enhanced binding capacity for IgG.

The specificity of binding to the Fc region of IgG is similar to that of native protein A, and provides excellent purification in one step. The epoxy-based coupling chemistry ensures low ligand leakage. The high capacity, low ligand leakage, and specially developed base matrix make MabSelect ideal for the purification of monoclonal antibodies from lab to process-scale. The basic characteristics are summarized in Table 1.

The dynamic capacity of MabSelect is exemplified in Figure 1 where flow velocity/capacity dependence is shown for three mobile phase velocities. Figure 2 shows a pressure/flow velocity comparison in different columns.

Table 1. Characteristics of MabSelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v}¹	~ 85 μ m
Ligand	Recombinant protein A (<i>E. Coli</i>)
Coupling chemistry	Epoxy
Dynamic binding capacity, Q_{B10}²	~ 30 mg human IgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 10 mM NaOH (pH 12) 0.1 M sodium citrate/HCl (pH 3) 6 M Gua-HCl 8 M urea 20% ethanol 2% benzyl alcohol
pH stability, operational³	3 to 10 ⁴
pH stability, CIP⁵	3 to 12 ^{4, 6}
Recommended maximum operating flow velocity	500 cm/h ⁷
Temperature stability	2°C to 40°C
Delivery conditions	20% ethanol
Storage	20% ethanol, 2°C to 8°C

¹ Mean particle size of the cumulative volume distribution

² Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in an XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M NaH₂PO₄, pH 7.4

³ pH range where resin can be operated without significant change in function

⁴ pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands can hydrolyze at pH below 2.

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

⁶ Reducing agent, for example, 100 mM 1-thioglycerol followed by 15 mM NaOH, is among the most efficient CIP for MabSelect.

⁷ In a BGP 300 column with 30 cm diameter and a 20 cm bed height using buffers with the same viscosity as water at 25°C

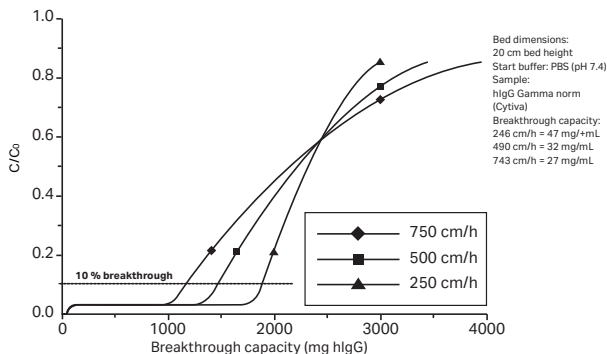


Fig 1. Example of flow velocity/capacity dependence for MabSelect. Breakthrough capacity for hlgG was determined at three different flow velocities. Breakthrough capacity is defined as mg hlgG applied per mL resin at the point where the concentration of hlgG in the column effluent reaches a value of 10% of the concentration in the sample.

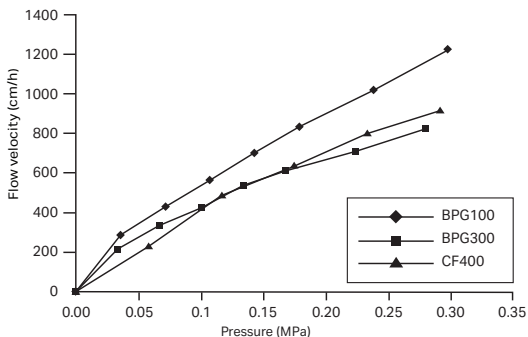


Fig 2. Pressure/flow curves for MabSelect packed to a bed height of 20 cm in three different columns. The pressure/flow data were determined in BPG 100 (i.d. 100 mm), BPG 300 (i.d. 300 mm), and Chromaflow 400 (i.d. 400 mm) using buffers with the same viscosity as water.

2 Method design and optimization

As with most affinity chromatography resins, MabSelect offers high selectivity which renders efficiency related parameters such as sample load, flow rate, particle size, and bed height less important for resolution.

The primary aim of method optimization is to establish the conditions that bind the highest amount of target molecule, in the shortest time and with the highest product recovery.

Specificity and affinity

The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass (Table 2). There might even be a substantial diversity in binding characteristics within a single subclass. This is an important consideration when developing the purification protocol. To achieve efficient capture of the target antibody it is often necessary to enhance the binding strength by formulation of the binding buffer in one of the following ways.

- By increasing pH, which titrates opposing histidyl residues in the binding sites of protein A and IgG.

This reduces electrostatic repulsion between protein A and IgG, allowing an uninhibited affinity interaction.

- By increasing salt concentration to reduce electrostatic repulsion and to increase hydrophobic interactions.
- By reducing the temperature reported, the dynamic binding capacity will most likely decrease.

Table 2. Affinity of protein A for selected classes of monoclonal antibodies. This table is compiled from a variety of sources. Comparisons are approximate since they are derived from runs conducted under a variety of conditions.

Antibody	Affinity	Binding pH	Binding pH
Human			
IgG1	very high	6.0 to 7.0	3.5 to 4.5
IgG2	very high	6.0 to 7.0	3.5 to 4.5
IgG3	low-none	8.0 to 9.0	≤ 7.0
IgG4	low-high	7.0 to 8.0	3.0 to 6.0
Mouse			
IgG1	low	8.0 to 9.0	4.5 to 6.0
IgG2a	moderate	7.0 to 8.0	3.5 to 5.5
IgG2b	high	≈ 7.0	3.0 to 4.0
IgG3	low-high	≈ 7.0	3.5 to 5.5

3 Method screening

Because the affinity of MabSelect for antibodies of different species, classes, and subclasses varies, conduct initial screening under conditions that bind the largest diversity of antibodies and reveal the relationship between the target antibody and possible contaminating antibodies.

An effective way of mapping antibody behavior on MabSelect is to bind them at high pH and high salt conditions, then elute them in a reducing linear salt/pH gradient.

It is important to make certain that the antibody is stable under the elution conditions. If there is any doubt about this, titrate the antibody fraction to neutrality immediately upon elution in order not to lose biological activity. Another frequent practice to reduce exposure of the antibody to harsh conditions is to reverse the direction of flow during elution. This also elutes the antibody in a more concentrated form.

Recommended screening conditions

Note: *To save material, screening can also be performed using PreDictor™ plates.*

Example of suitable buffers:

- Buffer A: 20 mM sodium phosphate, 150 mM NaCl, pH 7
- Buffer B: Either 50 mM sodium phosphate, pH 3.0, or 50 mM sodium citrate, pH 3.0

Experimental conditions:

- Equilibrate the column with 10 column volumes of buffer A
- Apply a small sample of antibody
- Wash the column with 5 column volumes of buffer A
- Elute the column with a linear gradient of 10 column volumes to 100% buffer B
- Collect fractions into titrating diluent (e.g., 1.0 M Tris-HCl, pH 8.0 so that the diluent volume equals 5% of the programmed fraction volume)
- Regenerate the column with 5 to 10 column volumes of 100% buffer B
- Re-equilibrate the column with buffer A

Conditions can be subsequently modified to provide the best purification performance. Due to the natural diversity of antibodies, binding, and elution conditions must be optimized for the antibody to be purified. The linear gradient used in the initial experiment will reveal the relative binding requirements of the target antibody versus any contaminating antibodies.

Binding

High salt concentration and high pH will often increase dynamic binding capacity, even for antibodies that bind fairly well to protein A. On the other hand, by decreasing salt concentration and/or pH during binding it is possible to avoid binding contaminating antibodies. This can also increase the dynamic binding capacity since more binding sites will be available for the target antibody.

It will also increase selectivity in the system. The balance between selectivity and capacity must be defined with respect to the nature of the feed, that is, presence of contaminating antibodies and the purity requirement in the eluted product.

With some antibodies, good binding can be achieved without enhancing binding strength. For other antibodies, for example, mouse IgG1, it is usually necessary to add up to 4 M NaCl to the binding buffer and feed material to achieve efficient binding.

Elution

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column. This will prevent denaturation of sensitive antibodies due to exposure to low pH values. Stepwise elution (Figure 3) is often preferred in large-scale applications since it is technically simpler than elution with continuous gradients. It also allows the target monoclonal antibody to be eluted in a more concentrated form and provides decreased buffer consumption and shorter cycle times. Linear gradient elution can be feasible for scale-up. Its main advantage is that it provides the best and most reproducible fractionation from contaminating antibodies.

Example

An example of a purification of monoclonal antibody is shown in Figure 3. IgG was purified from a clarified supernatant using MabSelect as a capture separation step. The IgG originated from a large-scale culture of Chinese hamster ovary (CHO) cells. The sample load was 24 mg IgG/mL bed volume and the recovery was 99% of highly purified antibody.

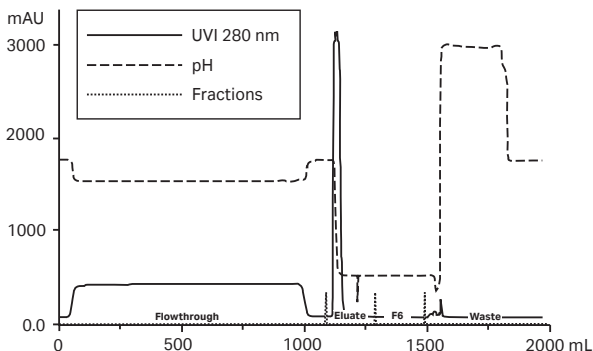


Fig 3. Purification of monoclonal antibody from a large-scale culture of CHO cells on MabSelect.

4 Optimization of throughput

When optimizing for highest throughput and productivity it is necessary to define the highest sample load over the shortest sample application time with the most acceptable loss in product recovery. The dynamic binding capacity for the target antibody must be determined by frontal analysis using real process feedstock. Since the dynamic binding capacity is a function of the linear flow rate applied during sample application, the breakthrough capacity must be defined over a range of different flow rates. The optimal flow rate is that which gives the highest throughput in terms of amount of antibody processed per time unit and volume of resin. Example of breakthrough profiles at different flow rates are shown in Figure 1.

Removal of leached protein A from final product

Leakage of protein A from MabSelect is generally low. However, in many monoclonal applications it is a requirement that leached protein A is eliminated from the final product.

There are a number of chromatographic solutions, such as cation and anion exchange chromatography, or multimodal anion exchange chromatography, which can be used to remove leached ligand.

- **Size exclusion chromatography** can be applied for removal of protein A-IgG aggregates by conducting the separation under moderate pH conditions. The large IgG-protein A complexes that are formed will elute early from the column (Figure 4).
- **Cation exchange chromatography** is an effective tool for removing residual protein A, especially when the particular monoclonal has strong cation exchange binding characteristics. The run is conducted at a pH in which the antibody is known to dissociate from protein A. Protein A binds poorly to cation exchangers and will pass unretained or elute early in the gradient (Figure 5).
- **Anion exchange chromatography** can also be used to reduce leached protein A contamination. It is best suited to antibodies that are weakly retained on anion exchangers. Because of the strong anion exchange binding characteristics of protein A, protein A-IgG complexes tend to be more strongly retained than noncomplex antibodies (Figure 6). These complexes do not generally form separate peaks, but often exhibit a trailing shoulder. To determine the ability of anion exchange chromatography to remove complex protein A, equilibrate the column to 20 mM Tris-HCl, pH 8.5, apply sample and elute in a linear gradient ending at 0.25 M NaCl (20 mM Tris-HCl, pH 8.5). Collect fractions across the antibody peak and screen for protein A.
- **Multimodal anion exchange chromatography** See Data File *Capto adhere* (28907888) and application note *Selective removal of aggregates with Capto adhere* (28907893).

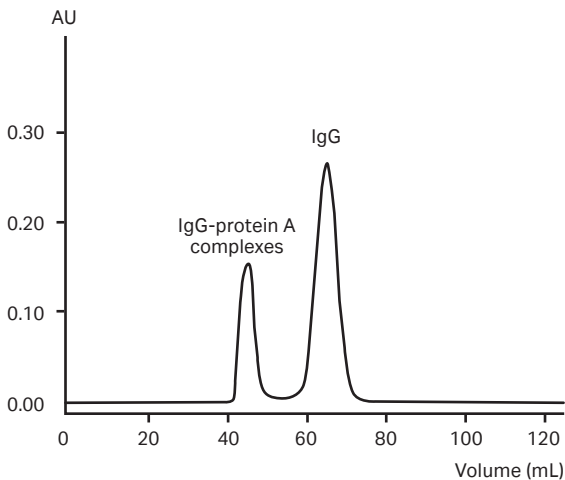


Fig 4. Removal of IgG-protein A complex from mouse IgG_{2a} by size exclusion chromatography on Superdex™ 200 prep grade. Recombinant protein A was spiked into mouse IgG_{2a}.

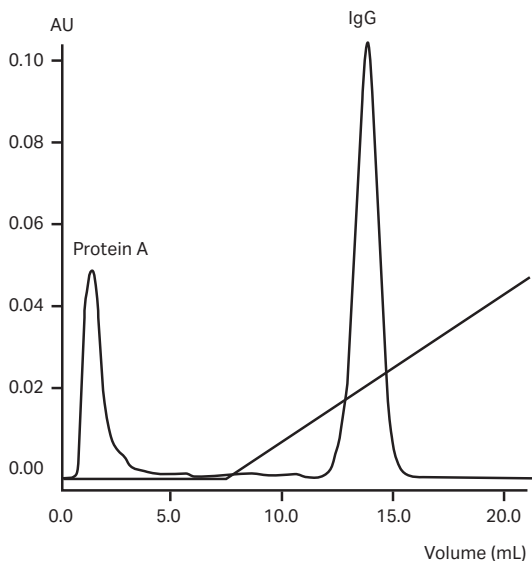


Fig 5. Removal of protein A from mouse IgG_{2b} by cation exchange chromatography on HiTrap™ SP HP. Recombinant protein 0.005

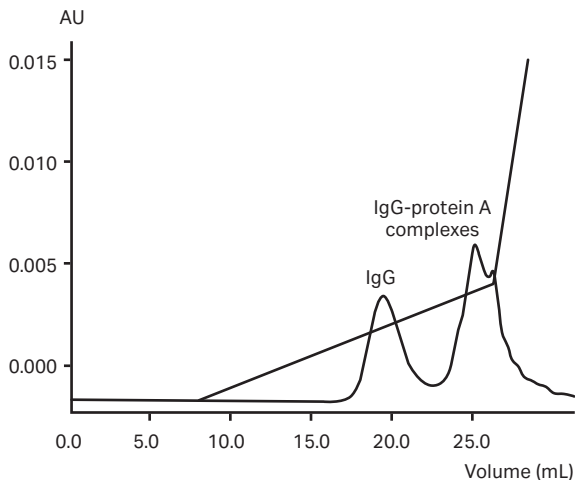


Fig 6. Removal of IgG-protein A complex from mouse IgG_{2a} by anion exchange chromatography on HiTrap Q HP. Recombinant protein A was spiked into IgG_{2a}.

5 Packing columns

MabSelect is supplied as suspension in 20% ethanol. Decant the 20% ethanol solution and replace it with packing buffer before use.

Recommended columns

Table 3. Recommended columns for MabSelect

Column	Inner diameter (mm)	Bed volume ¹	Bed height (cm)
Lab-scale			
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 ²	50 to 200	0.2 to 12.5 L	max 30
AxiChrom	300 to 1000	7 to 314 L	max 30
BPG ³	100 to 300	1 to 28 L	max 40
Chromaflo standard ^{4,5}	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 Intelligent Packing method according to MabSelect can be used.

³ The pressure rating of BPG 450 is too low to use with MabSelect resins.

⁴ See Application note: *Methods for packing MabSelect resins in production-scale columns* (11000752).

⁵ Larger pack stations might be required at larger diameters.

All large-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). For information on packing of process-scale columns, contact your local Cytiva representative.

Packing HiScale columns

Packing preparations

Materials needed

MabSelect

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, 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 $CF = L_{\text{settled}}/L_{\text{packed}}$
PF	Packing factor $PF = L_{\text{cons}}/L_{\text{packed}}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{\text{packed}} \times A_C$
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 with HiScale columns.

Washing the resin

Attach 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 4. 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	1.06
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	10	10	10
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	25	15	8.6

Table 5. 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	1.13	1.10
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	27	27	27
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	66	40	23

Table 6. Main features of the packing method for HiScale 50/20 and HiScale 50/40

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	1.10	1.06
Packing velocity (cm/h)	300	300	300
Packing flow rate (mL/min)	100	100	100
Flow condition (cm/h)	750	450	260
Flow condition (mL/min)	250	150	86

Packing procedure

- 1 Assemble the column according to the column instructions (*HiScale columns (16, 26, 50) and accessories, 28967470*).
- 2 Put 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** Attach 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** Put 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** Put 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 4, 5, and 6.
- 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 buildup 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.
$$L_{\text{packed}} = L_{\text{cons}} / \text{PF}$$
- 14** Turn off the flow and put a stop plug in the bottom.
- 15** Remove the top adapter from the packing tube.
- 16** Over a beaker or a sink, detach the packing tube from the column.
- 17** Put the top adapter in the column tube again. 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 4, 5, and 6.
- 21** Let the flow run for about 10 column volumes.
The column is ready to be tested.

6 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:

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

L = bed height (cm)

N = number of theoretical plates

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

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

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:

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

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

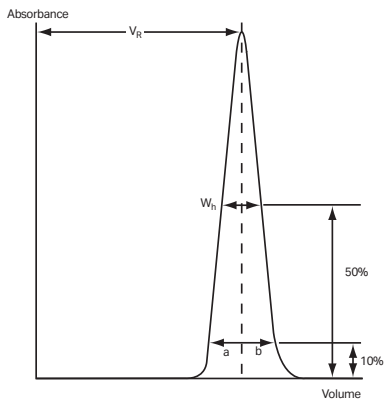


Fig 7. A typical test chromatogram showing the parameters used for HETP and A_s calculations

7 Cleaning-In-Place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the purification system. If such contaminants are allowed to accumulate they can affect the chromatographic properties of the resin. If the fouling is severe, it can block the resin, increase back pressure, and reduce flow rate.

Regular CIP prevents the buildup of these contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of MabSelect.

CIP protocols

Use these CIP protocols as guidelines for formulating a cleaning protocol specific for the feed material applied to the column. Frequency of use will depend on the nature of the feed material but we recommend using a CIP procedure at least every 5 cycles during normal use. Depending on the nature of the contaminants, different protocols maybe have to be combined. If fouling is severe, the protocols maybe have to be further optimized.

Two-step sequence with reducing agent

Wash with 2 column volumes of 100 mM 1-Thioglycerol pH 8.5 followed by CIP with 2 column volumes of 15 mM NaOH. Use a contact time of 15 min for each step. Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7 to 8. Reversed flow direction.

One-step protocol

Wash with 2 column volumes of 50 mM NaOH. Contact time for CIP must be at least 10 minutes. Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7 to 8. Reversed flow direction.

This solution might reduce the lifetime of the resin. Addition of salt (e.g., NaCl and Na₂SO₄) to the caustic CIP solution can increase the rProtein A stability but might decrease the cleaning efficiency. Lower NaOH concentrations (10 to 30 mM) are not efficient for cleaning.

As an alternative to sodium hydroxide, 6 M guanidine hydrochloride at contact times of 30 to 60 minutes, can be used

Protocol for hydrophobically bound substances

If fouling is caused by hydrophobically bound substances, solvents such as 1-propanol or isopropanol can be used. Typical concentrations are: 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.

Reference

Grönberg, A. et al. Automated HTPD Technology for Design of Cleaning-In-Place (CIP) Protocols for Chromatography Resins, Poster at 1st HTPD International Conference. Krakow, Poland (2010).

8 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum. Equilibrate the column with a solution consisting of 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours, then wash with at least 5 column volumes of sterile binding buffer.

or

Equilibrate the column with a solution consisting of 0.1 M acetic acid and 20% ethanol. Allow to stand for 1 hour, then wash with at least 5 column volumes of sterile binding buffer.

or

Equilibrate the column with 70% ethanol. Allow to stand for 12 hours, then wash with at least 5 column volumes of sterile binding buffer.

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

9 Scaling up

After optimizing the antibody fractionation at laboratory-scale, the process can be scaled up. For this, some parameters will change while others remain constant.

- Select bed volume according to required binding capacity.
- Select column diameter to obtain a bed height of approximately 20 cm so that high flow rates and high dynamic capacity can be used. (See Figure 2, pressure/flow curve. Maximum flow rate is approximately inversely proportional to the bed height. Expect to operate at no more than 70% of the maximum flow rate.)
- Define linear flow rate during sample application to ensure that residence time is equal to that established in the small-scale experiments. The residence time is equal to the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.
- Keep sample concentration and gradient slope constant.

The larger equipment needed when scaling up can cause some deviations from the optimized method at small scale. In such cases, check the buffer delivery system and monitoring system for time delays or volume changes.

Different lengths and diameters of outlet pipes can cause zone spreading on larger systems.

10 Storage

Unused resin can be stored in the container at a temperature of 2°C to 8°C. Ensure that the screw top is fully tightened.

Packed columns must be equilibrated in binding buffer containing 20% ethanol to prevent microbial growth.

After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

11 Ordering information

Product	Pack size	Product code
MabSelect	25 mL	17519901
	200 mL	17519902
	1 L	17519903
	5 L	17519904
	10L	17519906
MabSelect in BnOH	5 L	17519921
	10 L	17519922

Related product	Quantity	Product code
HiTrap™ MabSelect	5 × 1 mL	28408253
	1 × 5 mL	28408255
	5 × 5 mL	28408256
HiScreen™ MabSelect	1 × 4.7 mL	28926973
PreDicator MabSelect, 6 µL	4 × 96-well filter plates	28925820
PreDicator MabSelect, 20 µL	4 × 96-well filter plates	28925821
PreDicator MabSelect, 50 µL	4 × 96-well filter plates	28925822
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
Data Files	MabSelect	18114994
	AxiChrom Columns	28929041
	BPG columns	18111523
	Chromaflow columns	18113892
Application note	MabSelect – Column packing	11000752
	High-throughput process development for design of cleaning-in-place protocols	28984564

For additional information, including Data File, contact your local Cytiva representative.

For technical support,
visit: [cytiva.com/techsupport](https://www.cytiva.com/techsupport)



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