



MabSelect Xtra

Affinity chromatography

Instructions for Use

Abstract

- High dynamic binding capacity and suitable for high expression feedstocks
- Improved process economics through reduced raw materials costs and/or reduced number of cycles
- Improved process economics through reduced raw materials costs and/or reduced number of cycles
- Very low, unspecific binding due to high ligand selectivity and matrix hydrophilicity
- High capacity for many Fc-fusion proteins
- Simple scale-up to production-sized AxiChrom™, BPG and Chromaflow™ columns

MabSelect Xtra™ addresses the increasing levels of expression found in monoclonal antibody feedstocks. MabSelect Xtra lowers the overall cost of antibody production by allowing manufacturers to process concentrated feedstocks in fewer cycles or on smaller columns.

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1 Description

The recombinant protein A used in MabSelect Xtra is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of mammalian products. The recombinant protein has been specially engineered to favor an oriented coupling that gives an affinity medium 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. High capacity and the specially engineered base matrix, make MabSelect Xtra ideal for purifying monoclonal antibodies from lab to process scale. The dynamic binding capacity (DBC) of MabSelect Xtra is about 30% higher than conventional high capacity Protein A-based media such as MabSelect™. Its maximum flow rate is high due to the use of the new highly rigid agarose base matrix.

The medium's basic characteristics are summarized in Table 1.

Figure 1 shows dynamic binding capacity for MabSelect Xtra and MabSelect. A pressure/flow curve is shown in Figure 2.

Table 1. Characteristics of MabSelect Xtra.

Composition	highly cross-linked agarose
Average particle size (d_{50v}) ¹	75 μm
Ligand	recombinant protein A (<i>E. coli</i>)
Coupling chemistry	Epoxy
Dynamic binding capacity ²	≈ 40 mg human IgG/mL medium(MabSelect: ≈ 30 mg human IgG/mL medium)
Chemical stability ³	stable in all aqueous buffers commonly used in protein A chromatography – 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 50 mM NaOH in 0.5 M Na_2SO_4 , 0.1 M sodiumcitrate/HCl (pH 3), 6 M GuHCl, 6M urea, 20% ethanol, 2% benzyl alcohol
Recommended pH	
Working range	3 to 10
Cleaning-In-Place (CIP)	2 to 12
Maximum operational nominal	
Flow velocity ⁴	300 cm/h (MabSelect 500 cm/h)
Temperature stability ⁵	2°C to 40°C
Delivery conditions	20% ethanol
Regulatory support	Regulatory support file No material of animal origin is used in the manufacturing process

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

² Determined at 10% breakthrough by frontal analysis at a nominal flow velocity of 250 cm/h in a column with a bed height of 10 cm, i.e. residence time is 2.4 min. Residence time is equal to bed height (cm) divided by nominal flow velocity (cm/h) applied during sample loading. Nominal flow velocity is equal to volumetric flow rate (ml/h) divided by column cross sectional area (cm²).

³ No significant change in chromatographic performance after 1 week storage or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 hours (100 mM H_3PO_4 ; 10 mM NaOH, 50 mM NaOH in 0.5 M Na_2SO_4).

⁴ Packed bed height 20 cm, operating pressure < 1.5 bar, with water at 20°C.

⁵ Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

DBC at 10% breakthrough
(mg hlgG/ml gel)

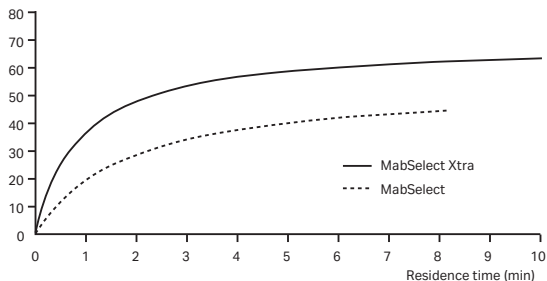


Fig 1. Relation between dynamic binding capacity and residence time for MabSelect Xtra. Dynamic binding capacity is defined as mg human polyclonal IgG bound per ml medium at the point where the concentration of hlgG in the column effluent reaches a value of 10% of the concentration in the sample. Sample concentration: 1.1 mg hlgG/ml.

Velocity (cm/h)

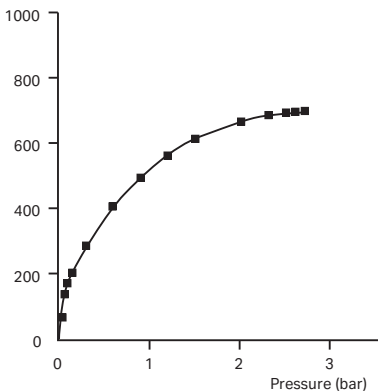


Fig 2. Pressure/flow profile for MabSelect Xtra in an open bed (initial bed height 23 cm) in BPG 300 column (i.d. 300 mm).

In a packed bed, the maximum operating velocity for MabSelect Xtra with water at 20°C is 300 cm/h at 20 cm bed height. Specifications are set to ensure this is valid for any column size. Increasing bed height and/or viscosity leads to a reduction in maximum operating velocity.

2 Process development

For initial studies of MabSelect Xtra in small-scale columns, we recommend HiScreen™ columns HiScale™ 16 columns with 10 cm bed height. Choose a residence time that fulfills your demand on dynamic binding capacity and nominal flow velocity according to the Figure below. Make sure the chosen bed height and velocity do not conflict with the large-scale pressure/flow limitations (see the Figure below).

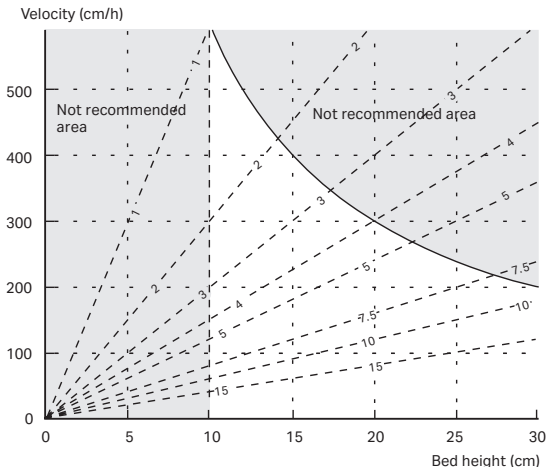


Fig 3. Recommended operating window for MabSelect Xtra. Choosing bed height and operating velocity in terms of residence time, pressure restrictions and large-scale column packing challenges.

The Figure above shows the possible combinations of bed height and operational nominal flow velocity for MabSelect Xtra. The Figure also displays the residence time in minutes (1–15 min) for any bed height and velocity. Included are also pressure drop limitations and packing limitations at large scale. The solid curved line shows the large-scale column pressure flow restriction. The dashed vertical line indicates that operating at below 10 cm bed height is not favorable.

The reason for this is that large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.

Figure 3 can be used as a guide when determining suitable bed height and operating velocity in terms of residence time and thus capacity and pressure drop.

Recommended screening conditions

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

Examples of suitable buffers:

- Buffer A: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2
- Buffer B: 0.1 M sodium citrate, pH 3.0–3.6.

Experimental conditions:

- Equilibrate the column with 5 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 5–10 column volumes of 100% buffer B.
- Re-equilibrate the column with buffer A.

Washing

To minimize the use of buffer, we recommend optimizing the washing procedure with respect to residence time, volumes, pH and conductivity.

Elution

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column. This will prevent denaturing sensitive antibodies due to exposure to low pH. Step-wise elution (see Figure 4) is often preferred in large-scale applications since it is simpler than elution with continuous gradients.

It also allows the target monoclonal antibody to be eluted in a more concentrated form and thus decreases buffer consumption and shortens cycle times. It might be necessary to decrease the flow velocity due to the high concentrations of protein in the eluate.

Example

The Figure below shows an example of a monoclonal antibody purification. IgG was purified from clarified supernatant using MabSelect Xtra as the capture step. The IgG originated from a large-scale culture of mouse myeloma lymphoblastoid (NS0)-like cells. Sample load was 14 mg IgG/ml bed volume and the yield was 94% of highly purified antibody.

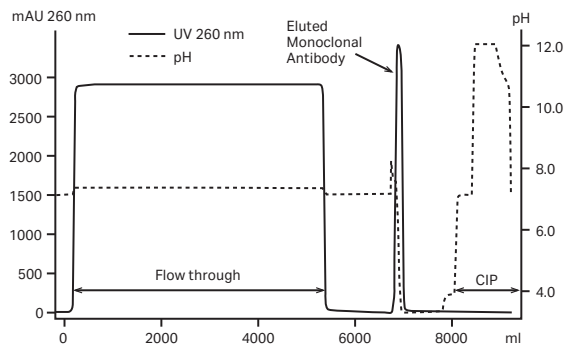


Fig 4. Purification of a monoclonal antibody from a large-scale culture of NS0 cells on MabSelect Xtra.

Dynamic binding capacity

The dynamic binding capacity for the target antibody should be determined by frontal analysis using real process feedstock. Since dynamic binding capacity is a function of the nominal flow velocity applied during sample application, the breakthrough capacity must be defined over a range of different velocities. Choose a flow velocity that fulfills your requirements regarding volume throughput and dynamic binding capacity.

3 Removal of leached protein A from final product

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

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.

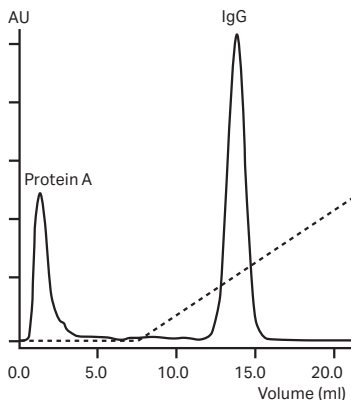
For more details about removal of leached ligand and antibody aggregates, see the application note *Two step purification of monoclonal IgG1 from CHO cell culture supernatant* (28-9078-92).

Note: *The low protein A levels in real eluates mean that the protein A-containing peak cannot be visualized in the UV-trace. In order to demonstrate the removal efficiency in the examples below, IgG samples have been spiked with high amounts of protein A.*

- **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 (see Figure 5).
- **Size exclusion chromatography** can be applied for removing protein A-IgG aggregates by conducting the separation under neutral pH conditions. The large IgG-protein A complexes that are formed will elute early from the column (see Figure 6).
- **Anion exchange chromatography** can also be used to reduce leached protein A contamination. It is best suited for 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 non-complex antibodies (see Figure 7). These complexes do not generally form separate peaks, but often exhibit a tailing shoulder. To determine the ability of anion exchange chromatography to remove protein A complexes,

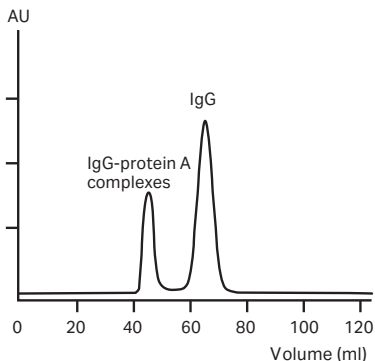
equilibrate the column to 20 mM Tris-HCl, pH 8.5, apply sample and elute in a linear gradient ending at 0.25 M NaCl (20 mM Tris-HCl, pH 8.5). Collect fractions across the antibody peak and screen for protein A using a sensitive assay.

- **Multimodal anion exchange chromatography** See Data File *Capto adhere* (28-9078-88) and application note *Selective removal of aggregates with Capto adhere* (28-9078-93).



Column:	HiTrap SP HP (1 mL)
Sample:	Purified antibody (0.61 mg) spiked with recombinant protein A (1.8 mg)
Buffer A:	20 mM sodium citrate, pH 5.2
Buffer B:	20 mM sodium citrate, 1.0 M NaCl, pH 5.2
Flow velocity:	150 cm/h
Gradient:	0–45% B; 15 column volumes

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



Column: HiLoad™ 16/60 Superdex™ 200 prep grade,
bed height 60 cm (120 mL)

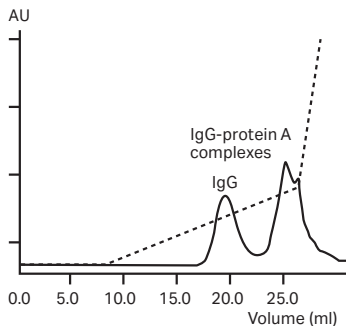
Sample: Purified antibody (14 mg) spiked with
recombinant protein A (0.36 mg)

Sample volume: 4.8 mL

Flow velocity: 60 cm/h

Buffer: 20 mM sodium phosphate,
0.15 M NaCl, pH 7.0

Fig 6. 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}.



Sample: Purified antibody (0.15 mg) spiked with recombinant protein A (0.009 mg)

Buffer A: 20 mM Tris-HCl, pH 8.5

Buffer A: 20 mM Tris-HCl, pH 8.5

Flow velocity: 300 cm/h

Gradient: 0–25% B; 20 column volumes

Fig 7. 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}.

4 Packing columns

Recommended columns

Table 2. Recommended columns for MabSelect Xtra

Column	Inner diameter (mm)	Bed volume ¹	Bed height (cm)
Lab scale			
HiScale 16/20	16	20–40 ml	max 20
HiScale 16/40	16	20–70 ml	max 35
HiScale 26/20	26	53–106 ml	max 20
HiScale 26/40	26	53–186 ml	max 35
HiScale 50/20	50	196–393 ml	max 20
HiScale 50/40	50	196–687 ml	max 35
Production scale			
AxiChrom ²	50–200	0.2–12.5 l	max 30
AxiChrom ²	300–1000	7–314 l	max 30
BPG ³	100–300	1–28 l	max 40
Chromaflo standard ^{4,5}	400–800	12–151 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 Xtra can be used.

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

⁴ See Application note; *Methods for packing MabSelect media in production scale columns*. (11-0007-52)

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 practical instructions in good packing techniques, see the CD-ROM *Column Packing - The Movie* (18-1165-33).

For more details about packing HiScale columns, see instructions *HiScale™ columns (16, 26, 50) and accessories* (28-9674-70).

For information on packing of process scale columns, please contact your local Cytiva representative.

Packing HiScale columns

Packing preparations

Materials needed

MabSelect Xtra
HiScale column
HiScale packing tube (depending on bed height)
Plastic spoon or spatula
Glass filter G3
Vacuum suction equipment
Filter flask
Measuring cylinder
20% ethanol with 0.4 M NaCl

Equipment

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

Equilibrate all materials to room temperature.

Definitions

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

L_{settled}	Bed height measured after settling by gravity.
L_{cons}	Consolidated bed height Bed height measured after settling the medium 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 media settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28-9259-32. This method can also be used with HiScale columns.

Washing the medium

Mount a glass filter funnel onto a filtering flask. Suspend the medium 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 medium
- Gently stir with a spatula between additions.
- Move the washed medium 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 3. 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 4. 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 5. 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)	27	27	27
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, Product code 28-9674-70).
- 2** Mount the column tube in a stand.
- 3** Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry but if air is trapped under the net it can be removed by a light suction with a syringe.
- 4** Mount the bottom adapter unit in the bottom of the column tube and tighten the o-ring.
- 5** Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet.
- 6** Mount the packing tube on top of the column tube.
- 7** Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing the roof as this is done. If air is trapped under the net it can be removed by a light suction with a syringe.
- 8** Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
- 9** Mount the top adapter unit on top of the packing tube. Tighten the o-ring firmly and remove the bottom stop plug.
- 10** Start a downward flow with packing velocity according to Table 3, 4 and 5.
- 11** Let the flow run until the bed has consolidated.
- 12** Use the scale on the column to measure the bed height. There might be a build up of media 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** Dismount the top adapter from the packing tube.
- 16** Over a beaker or a sink, detach the packing tube from the column.
- 17** Remount the top adapter in the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.
- 18** Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.
- 19** Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the media.
- 20** Start a downward flow to flow condition the bed. The flow rate is shown in Table 3, 4 and 5.
- 21** Let the flow run for about 10 column volumes.
The column is ready to be tested.

5 Evaluation of column packing

Test the column efficiency to check the quality of packing. Testing should be done after packing, at regular intervals during the working life of the column or 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 (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* (28-9372-07).

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 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} = mean diameter of the beads (cm)

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

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible

(A typical acceptable range could be $0.8 < A_s < 1.8$).

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

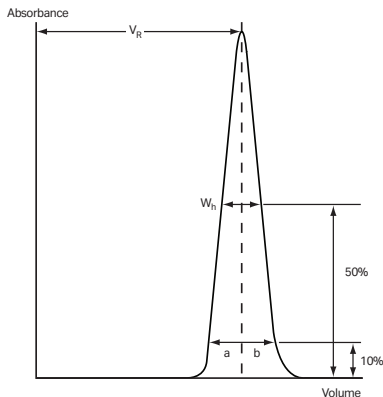


Fig 8. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

6 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 may affect the chromatographic properties of the resin. If the fouling is severe, it may block the resin, increase back pressure and reduce flow rate.

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

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 may have to be combined. If fouling is severe, the protocols may 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 7–8. Reversed flow direction.

One-step protocol

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

This solution might reduce the lifetime of the media. 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 (10–30 mM) are not efficient for cleaning.

As an alternative to sodium hydroxide, 6 M guanidine hydrochloride at contact times of 30–60 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 1–5% or isopropanol 5–30%.

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).

7 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum. Equilibrate the column with a solution 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 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.

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

8 Storage

Store unused medium in its container at a temperature of 2°C to 8°C. Ensure that the screw top is fully tightened. Equilibrate packed columns in binding buffer containing 20% ethanol or 2% benzyl alcohol to prevent microbial growth.

After storage we recommend performing a blank run, preferably including CIP, before use.

9 Scaling up

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

- Keep the residence time constant.
- Select bed volume according to required binding capacity.
- Select column diameter. Next, determine the bed height to give the desired residence time. Note that the back pressure is increasing proportionally with increasing bed height at a constant nominal flow velocity. Bed heights of 10–25 cm are generally considered appropriate.
- Keep sample concentration and elution conditions constant.

The larger equipment needed when scaling up may cause some deviations from the method optimized 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 Troubleshooting

Fault	Possible cause/corrective action
Back pressure increases during sample application	• Change the in-line filter.
Unstable pressure curve during sample application	• Remove air bubbles that might have been trapped in the sample pump. • Degas the sample using a vacuum degasser or an air trap. • Might be due to insufficient elution or CIP caused by contaminants accumulating in the column. Optimize elution conditions and/or perform CIP more frequently and/or optimize the CIP protocol.
Gradual increase in elution volume	• Perform wash with thioglycerol followed by CIP with NaOH, according to Section 6
Gradual decrease in yield	• Too high sample load. Decrease the sample load. • Might be due to insufficient elution and CIP. Perform CIP more frequently and/or optimize the CIP protocol.
Gradual increase in CIP peaks	• Insufficient CIP. Optimize the CIP protocol and/or perform CIP more frequently. • Perform wash with thioglycerol followed by CIP with NaOH, according to Section 6
High protein A leakage during the first purification cycle	• Make a blank run, including CIP, before the first purification cycle on a new column.

11 Further information

Please read these instructions carefully before using MabSelect Xtra media.

For further information visit cytiva.com or contact your local Cytiva representative.

For technical support, visit: cytiva.com/contact

12 Ordering information

Product	Pack size	Product code
MabSelect Xtra	25 ml	17-5269-07
	200 ml	17-5269-02
	1 l	17-5269-03
	5 l	17-5269-04
	10 l	17-5269-05
	60 l	17-5269-06

Related product	Quantity	Product code
HiTrap™ MabSelect Xtra	5 × 1 ml	28-4082-58
	1 × 5 ml	28-4082-60
	5 × 5 ml	28-4082-61
HiScreen MabSelect Xtra	1 × 4.7 ml	28-9269-76
PreDictor MabSelect Xtra, 6 µl	4 × 96-well filter plates	28-9432-75
PreDictor MabSelect Xtra, 20 µl	4 × 96-well filter plates	28-9432-76
PreDictor MabSelect Xtra, 50 µl	4 × 96-well filter plates	28-9432-77
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

Related literature

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	AxiChrom columns	28-9290-41
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