MabSelect

AFFINITY CHROMATOGRAPHY

MabSelect™ is a high-throughput Protein A chromatography resin that has been designed into several new and second-generation processes for the production of therapeutic monoclonal antibodies (mAbs). The hydrophilic, high-flow agarose particle, optimized for both capacity and throughput, and the oriented coupling of the rProtein A ligand, deliver a product pool that is high in purity and yield. The rProtein A ligand is expressed in *E. coli* and is free of components of mammalian origin.

Key performance characteristics of MabSelect include:

- High-flow agarose matrix results in high flow velocities at production scale allowing for the processing of more than 10 000 L of feed in one working day
- Enhanced binding capacity due to oriented coupling of the ligand and optimized matrix
- Well established in industry
- High dynamic binding capacity reduces resin volume requirements

Fig 1. MabSelect for capture of monoclonal antibodies.

Resin characteristics

MabSelect is a member of the MabSelect product line of rProtein A-based affinity resins for capturing monoclonal antibodies. Like its companion products, MabSelect is based on an innovative, high-flow agarose base matrix platform, optimized for maximum capacity. The rProtein A is coupled to the base matrix at the C-terminal cysteine via a stable thio-ether bond. This oriented coupling contributes to the increased capacity seen with this resin. The basic characteristics for MabSelect are summarized in Table 1.

MabSelect family

The MabSelect family of resins for process-scale purification of monoclonal antibodies comprises MabSelect, MabSelect Xtra™, MabSelect SuRe™, and MabSelect SuRe LX.

MabSelect Xtra is based on the same high-flow agarose base matrix platform but with slightly increased porosity and a decreased particle size. This results in higher dynamic binding capacity. In addition, MabSelect Xtra has a higher capacity for many Fc-fusion proteins.

MabSelect SuRe is composed of the same rigid, high flow agarose matrix, but is based on an alkali-stabilized rProtein A-based ligand. The ligand provides greater stability than conventional rProtein A-based resins, allowing extended use of 0.1–0.5 M NaOH for cleaning in place. MabSelect SuRe LX has been further developed from MabSelect SuRe to give even higher binding capacity at longer residence time.

For more information on MabSelect Xtra, MabSelect SuRe and MabSelect SuRe LX refer to data files 11001157, 11001165, and 28987062, respectively.

Table 1. Characteristics of MabSelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50V}[1]	~ 85 µm
Ligand	Recombinant protein A (*E. Coli*)
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) 6M guanidine hydrochloride 8M urea 20% ethanol 2% benzyl alcohol
pH stability, operational[3] pH stability, CIP[5]	3 to 10[4] 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] Median 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 a XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M NaH_2PO_4, 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 may 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 e.g. 100 mM 1-Thioglycerol followed by 15 mM NaOH is among the most efficient CIP for MabSelect
[7] In a BPG 300 column with 30 cm diameter and a 20 cm bed height using buffers with the same viscosity as water at 25°C

Enhanced binding capacity due to oriented coupling

The recombinant protein A has been engineered to include a C-terminal cysteine. The coupling conditions are controlled to favor a thioether coupling providing single point attachment of the protein A as seen in Figure 2.

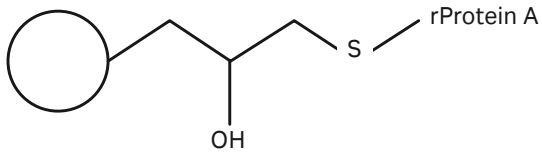


Fig 2. C-terminal cysteine favors oriented thioether coupling.

The oriented coupling also enhances the binding of IgG. At flow velocities of 500 cm/h and with an optimally packed bed height of 20 cm, the dynamic binding capacity of MabSelect is typically 30 mg polyclonal antibody/mL resin (residence time 2.4 min; see Fig 3).

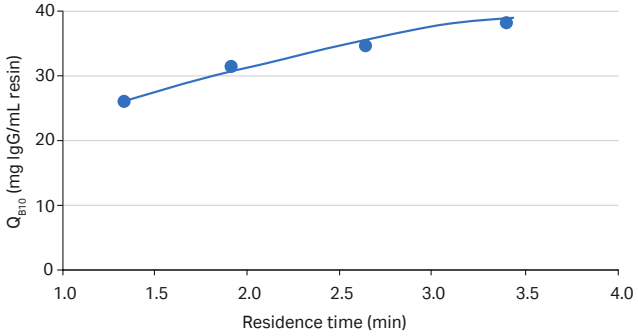


Fig 3. At flow velocities of 500 cm/h and with an optimal packed bed height of 20 cm, the dynamic binding capacity of MabSelect is typically 30 mg polyclonal antibody/mL resin (residence time: 2.4 min).

Highly purified rProtein A

The rProtein A (*E. coli*) is produced in validated fermentation and downstream processes. The purification process contains several chromatographic steps, but no material of animal origin is used in the manufacturing process. Each batch of protein is tested using validated Quality Control (QC) methods for IgG binding activity (> 95%), electrophoretic purity and purity by size exclusion chromatography (SEC, > 93%), as well as for endotoxin content (< 1 EU/mg). Results from QC analysis of five production batches are shown in Table 2.

Table 2. QC analysis of five production batches of recombinant protein A

Production batch	IgG binding activity (%)	Purity by SEC (%)	Endotoxin (EU/mg)
1	97	96.2	< 0.1
2	98	94.9	< 0.1
3	96	95.2	0.2
4	98	95.9	< 0.1
5	96	96.0	0.6

The recombinant protein A has also been tested and found to have no mitogenic activity in human lymphocytes *in vitro*.

Low ligand leakage

Leakage of recombinant protein A from MabSelect is low. The leakage during purification of human polyclonal IgG and a monoclonal IgG has been analyzed using a non-competitive ELISA[1]. The ELISA was developed to analyze native protein A in the presence of IgG, and has been adapted and evaluated for measurement of this specific recombinant protein A. Typical values found in the IgG-containing eluents after purification on MabSelect are shown in Figure 4. Leakage data for rProtein A Sepharose™ Fast Flow are included for comparison. Note that there is no significant difference in the leakage levels between rProtein A Sepharose Fast Flow and MabSelect.

In pharmaceutical production processes protein A must be removed from the final product. Leached rProtein A (is in most cases) efficiently removed by subsequent polishing steps. Commonly used technologies include cation exchange, anion exchange, and hydrophobic interaction chromatography. Novel multimodal resins such as Capto™ adhere are particularly efficient in removing leached protein A.

Methods to remove leached rProtein A are further described in the Instructions enclosed with each pack of MabSelect.

[1] The ELISA was developed and adapted for recombinant protein A by Franz Steindl, Institute of Applied Microbiology, University of Agricultural Sciences, Vienna, Austria.

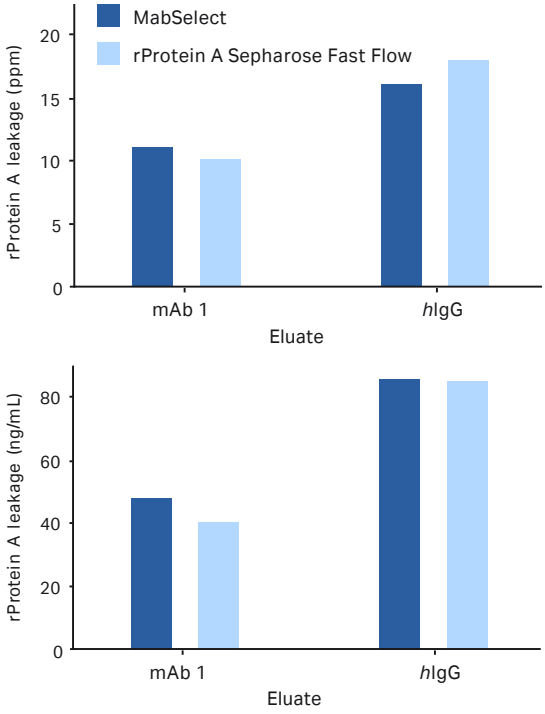


Fig 4. Leakage (ng/mL = ng protein A/mL elutate; ppm = ng protein A/mg purified IgG) of rProtein A in the antibody eluate during purification of a monoclonal antibody (mAb 1) and human polyclonal IgG (hIgG). MabSelect and rProtein A Sepharose Fast Flow were loaded to 24 mg of antibody per mL packed bed. mAb 1 was loaded in Chinese Hamster Ovary (CHO) cell culture supernatant (feed concentration 1 mg/mL) and *h*IgG was loaded in equilibration buffer. The eluate was collected in five column volumes of low pH buffer (pH 3.6 for mAb, pH 3.0 for *h*IgG) and neutralized with Tris-buffer. Aliquots of the buffers, pure antibody samples and eluates were then analyzed by ELISA for their rProtein A content. The only fractions that contained rProtein A were the eluates.

Operation

Method development

As for most affinity chromatography resins, MabSelect offers high selectivity that 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 will bind the highest amount of target molecule, in the shortest time and with the highest product recovery. The degree to which IgG binds to protein A varies with respect to both origin and antibody subclass. This is an important consideration when developing the purification protocol.

Typical binding conditions are low salt concentration buffers at neutral pH. To achieve efficient capture of weakly bound antibodies, it is often necessary to increase the pH and/or salt concentration in the binding buffer. Elution is normally achieved at reduced pH, down to pH 3.5 depending on species and subclass.

PreDictor™ 96-well filter plates or miniaturized columns, prefilled with MabSelect resin, are suitable for efficient high-throughput screening of chromatographic conditions during process development. Defined conditions can then be verified and optimized with HiScreen™ or HiTrap™ prepacked columns.

Cleaning and sanitization

A high-throughput screening approach of cleaning-in-place conditions for MabSelect using PreDictor plates has been performed [1]. The most favorable results were obtained using a two-step sequence with reducing agent (100 mM 1-Thioglycerol) followed by 15mM NaOH. Stronger NaOH can be used, but might reduce the lifetime of the resin. The two-step CIP protocol was verified in column experiments using CHO cell supernatant with humanized IgG_1. CIP was performed in every cycle using a contact time of 15 min and a blank cycle was run every 10th cycle for measuring of carryover. 110 CIP cycles were performed (Fig 5) with consistent results and no carry-over was observed.

Of the one step CIP protocols evaluated in the screening, 50 mM NaOH gave best results but this solution might reduce the lifetime of the resin. Addition of salt (e.g., NaCl and Na_2SO_4) to the caustic CIP solution can increase the rProtein A stability but might decrease the cleaning efficiency. Lower NaOH concentrations (10–30 mM) were not efficient for cleaning of MabSelect in this specific mAb process.

As an alternative cleaning protocol, 6 M guanidine hydrochloride can be used, also in combination with reducing agent.

To remove hydrophobically bound substances a solution of non-ionic detergent or ethanol is recommended. To sanitize MabSelect we recommend treatment with 0.1 M acetic acid/ 20% ethanol, or 2% hibitane digluconate/20% ethanol.

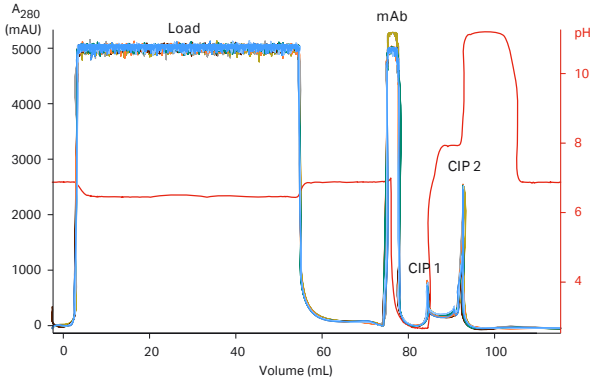


Fig 5. The recommended CIP protocol for MabSelect is reducing agent (e.g., 100 mM 1-Thioglycerol) followed by 15mM NaOH. Overlay of chromatograms from the column lifetime study (110 cycles).

Scale-up

After optimizing the antibody purification at laboratory scale, the process can be scaled up by increasing the column diameter, and keeping the mobile phase velocity and sample to bed volume ratio constant. A typical bed height could be around 20 cm so that the high capacity of MabSelect can be used at high flow rates. Pressure/flow curves for different columns are shown in Figure 6.

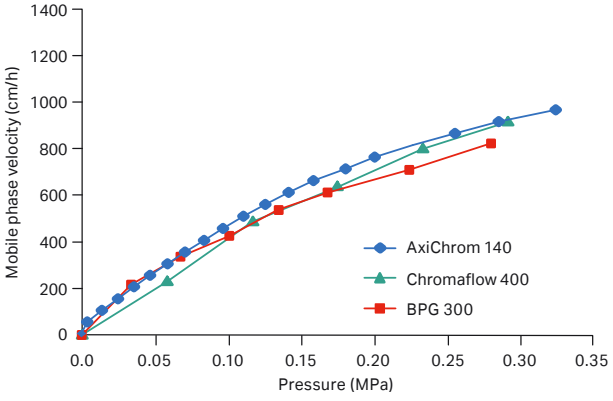


Fig 6. Pressure-flow curves for MabSelect packed to a bed height of 20 cm in AxiChrom, BPG, and Chromaflow columns

Table 3. Recommended columns for MabSelect SuRe. For maximum productivity and robust performance, bed heights of 10–30 cm are normally used

Column family range	Inner diameter (mm)
Lab scale:	
Tricorn™	5, 10
HiScale™	16, 26, 50
Pilot and production scale:	
AxiChrom™	50–1000
BPG™	100–300†
Chromaflow™	400–800‡

† The pressure rating of BPG 450 is too low to use it with MabSelect resins
‡ Larger pack stations might be required at larger diameters

Storage

Recommended storage solutions for MabSelect are 20% ethanol or solutions containing 2% benzyl alcohol. The recommended storage temperature is 2°C to 8°C.

Recommendations for column packing, cleaning and sanitization, method design, and optimization can be found in the instructions delivered with each pack of resin.

Application

An example of a purification of monoclonal antibody is shown in Figure 7. Clarified supernatant from a large-scale culture of CHO cells was purified on MabSelect. The sample load was 24 mg IgG/mL bed volume and the recovery was 99% of highly purified antibody. Non-reducing SDS PAGE analysis results are shown in Figure 8.

Column:	XK 16/40 (16 mm i.d., 20 cm bed height)
Sample:	Clarified feed conc., 1mg mAb/mL
Sample load:	24 mg IgG/mL resin
Buffer A:	20 mM NaH_2PO_4, 0.15 M NaCl, pH 7.2
Buffer B:	0.1 M Na_3-citrate, pH 3.6
Mobile phase velocity:	500 cm/h

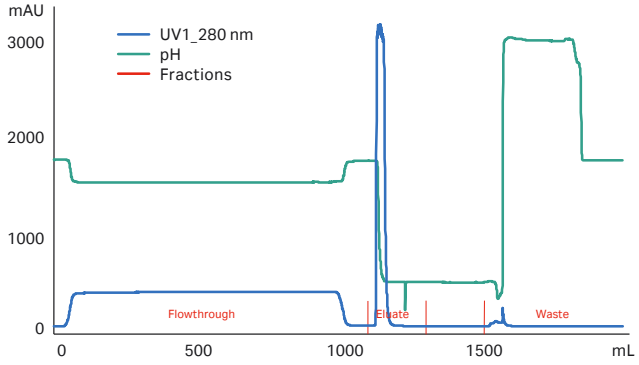
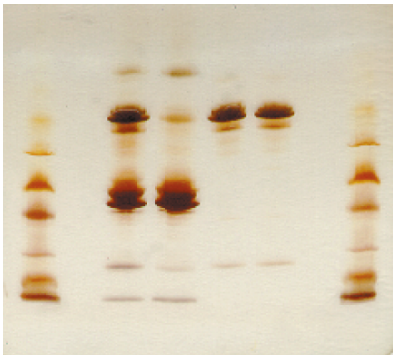


Fig 7. Purification of a monoclonal antibody from a large-scale culture of CHO cells using MabSelect



Lanes

1. LMW standards
2. CHO cell culture supernatant containing mAb
3. Flowthrough fraction
4. Eluate
5. Pure MAb standard
6. LMW standards

Fig 8. Analysis of purification of monoclonal antibody on MabSelect shown in Figure 9. PhastSystem™, PhastGel™ Gradient 10–15, silver staining

Designed to process more than 10 000 L feed from high expression levels in a working day

At the time MabSelect was designed, many different prototypes were evaluated using a process optimization simulation software. The software helps determine the prototype that can best meet the high throughput demands from large-scale manufacturers of monoclonal antibodies. The goal set for each prototype matrix was the capability to process 10 000 L fermentation broth in 8 h using reasonable column dimensions and reasonable amounts of chromatography resin.

The software is used as an in-house process optimization tool for the design of chromatographic processes and development of new chromatographic resins. It works with iterative calculations to find optima for selected parameters using known relationships and equations. The selected in-parameters can be set as fixed values or as an interval between two values. These parameters are optimized to give the highest possible productivity within a defined time, without exceeding relevant technical limitations (e.g., column dimension, dynamic capacity).

Table 4. Example of a computer-optimized process with MabSelect enabling 10 000 L to be processed in 7.8 h

Selected In-parameters	Set values
Total loading per day (g)	10 000
Loading concentration (g/L)	1
Mobile phase velocity for equilibration (cm/h)	500
Mobile phase velocity for sample load (cm/h)	500
Mobile phase velocity for elution (cm/h)	500
Mobile phase velocity for CIP (cm/h)	500
Capacity (mg/mL resin)	20
Particle size (µm)	85
Selected Out-parameters	**Optimized values**
Column diameter (cm)	80
Number of cycles	5
Bed height (cm)	20
Process time (h)	7.8
Column volume (L)	100
Max volumetric flow (L/h)	2512
Productivity (g protein/[L MabSelect × h])	0.042

Ordering information

Product	Pack size	Product code
MabSelect	25 mL	17519901
	200 mL	17519902
	1 L	17519903
	5 L	17519904
	10 L	17519906
MabSelect in BnOH	5 L	17519921
	10 L	17519922
HiScreen MabSelect	1 × 4.7 mL	28926973
HiTrap MabSelect	5 × 1 mL	28408253
	5 × 5 mL	28408256
PreDictor MabSelect	6 µL (4 × 96-well plates)	28925820
	20 µL (4 × 96-well plates)	28925821
	50 µL (4 × 96-well plates)	28925822
PreDictor RoboColumn™ MabSelect	50 µL (one row of 8 columns)	28986202
	200 µL (one row of 8 columns)	28986106

For additional information, please contact your local Cytiva representative.

Related Literature

Data files	
MabSelect Xtra	11001157
MabSelect SuRe	11001165
MabSelect SuRe LX	28987062
PreDictor 96-well filter plates and Assist software	28925839
PreDictor RoboColumn	28988634
HiScreen prepacked columns	28930581
AxiChrom columns	28929041
BPG columns	18111523
Chromaflow columns	18113892
Handbooks	
High-throughput process development with PreDictor plates, principles and methods	28940358
Antibody purification, principles and methods	18103746

Reference

1. 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).

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