MabSelect

MabSelect™ is a BioProcess™ affinity medium for capturing monoclonal antibodies from large volumes of feed by packed bed chromatography. MabSelect offers the following advantages:

- Designed to process more than 10 000 l feed from high expression fermentations in a working day, with high recovery and high purity
- High flow velocities under process conditions
- Enhanced binding capacity for immunoglobulins due to oriented coupling of the ligand and optimized matrix
- Straightforward scale-up in BPG™, INdEX™, FineLINE™ and Chromaflow™ columns
- Withstands effective and rigorous CIP procedures

Fig 1. MabSelect for capture of monoclonal antibodies.

Characteristics

The matrix of MabSelect is a highly cross-linked agarose, produced using a new manufacturing process that gives a very rigid matrix.

This development is part of our continuous programme to extend the usefulness of our products. Although other polymers were evaluated during this programme, agarose was selected because of its macroporous hydrophilic structure and its high chemical stability. The use of evaluation tools, such as multivariate analysis applied to a systematic study of agarose stabilization, resulted in a new cross-linking technique and a matrix highly optimized for high throughput adsorption chromatography of IgG.

The matrix of MabSelect allows at least five times higher flow velocities to be used in process scale compared with conventional cross-linked agarose of similar porosity.

The recombinant protein A ligand 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 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. The epoxy-based coupling chemistry ensures low ligand leakage. The high capacity, low ligand leakage and specially developed base matrix make MabSelect ideal for purification of monoclonal antibodies at process scale. The basic characteristics are summarized in Table 1.

Table 1. Characteristics of MabSelect

Composition	Highly cross-linked agarose
Particle size[1]	40–130 µm (d_{50v} ≈ 85 µm)
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Epoxy
Binding capacity, dynamic[2]	30 mg human IgG/ml medium
Chemical stability	No significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M gua-HCl, 2% benzyl alcohol or 20% ethanol
Cleaning-In-Place stability	No significant change in chromatographic performance after 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 hours using 50 mM NaOH + 1 M NaCl or 50 mM NaOH + 1 M Na_2SO_4 or 6 M Gua-HCl
Recommended pH working range	3 to 10
cleaning-in-place	2 to 12
Recommended mobile phase velocity[3]	500 cm/h
Temperature stability[4]	4 to 40°C
Delivery conditions	20% ethanol

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

[2] Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a column with a bed height of 20 cm

[3] In BPG 300 column, bed height 20 cm, operating pressure < 2 bar, 20°C, H_2O as test solution

[4] Recommended long term storage conditions: +4 to +8°C, 20% ethanol

Table 2. 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 medium)	20.0
Bead size (µm)	85.0
Selected Out-parameters	**Optimized values**
Column diameter (cm)	80.0
Number of cycles	5.0
Bed height (cm)	20.0
Process time (h)	7.8
Column volume (l)	100.0
Max volumetric flow (l/h)	2512
Productivity (g protein/(l MabSelect × h))	0.042

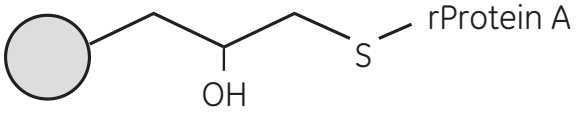

Fig 2. C-terminal cysteine favors oriented thioether coupling.

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

In designing MabSelect 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 media.

The software is used as an in-house process optimization tool for the design of chromatographic processes and development of new chromatographic media. 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 the give highest possible productivity within a defined time, without exceeding relevant technical limitations (e.g., column dimension, dynamic capacity).

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.

The oriented coupling also enhances the binding of IgG. This is illustrated in Figure 3 where breakthrough curves of human IgG for MabSelect are compared with those for other commercial media.

The binding capacity at 10% break-through (20 cm bed height, 500 cm/h mobile phase velocity) was 32 mg hIgG/ml bed volume for MabSelect, compared to 25 mg/ml for Product A, and ca 22 mg/ml for Product B (Fig 4).

Highly purified recombinant protein A

The recombinant protein A (*E. coli*) is produced in validated fermentation and downstream processes. The purification process contains several chromatographic steps, but no affinity or other steps in which the protein A comes into contact with substances of mammalian origin. Each batch of protein is tested using validated Quality Control (QC)

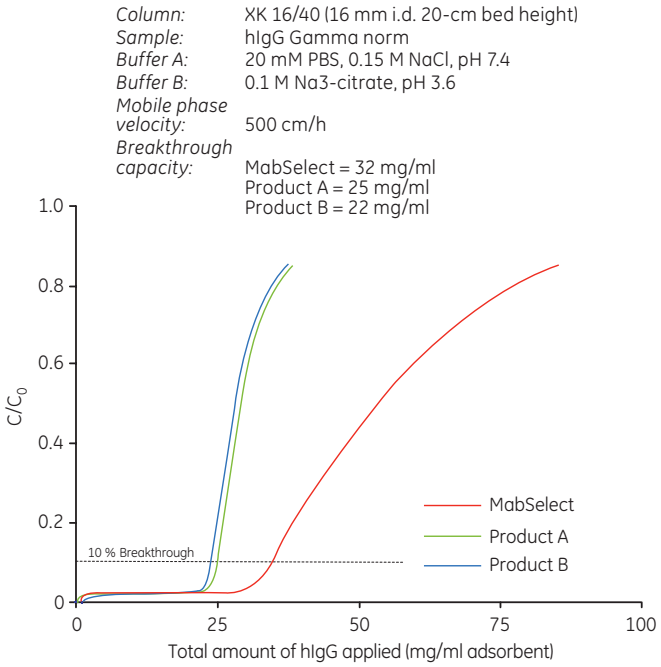

Fig 3. Comparison of three different protein A media with respect to breakthrough capacity. Breakthrough capacity is defined as mg hIgG bound per ml medium at the point where the concentration of hIgG in the column effluent reaches a value of 10% of the concentration in the sample.

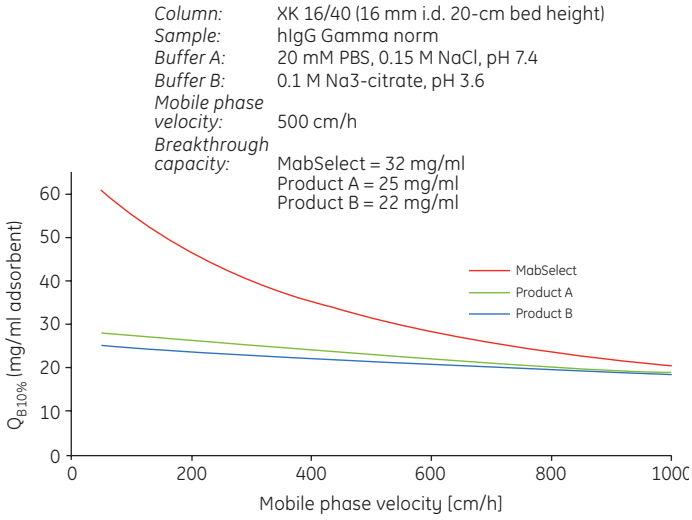

Fig 4. Comparison of the dynamic binding capacities at 10% breakthrough (QB10%) at different flow velocities for protein A affinity media based on high rigidity agarose (MabSelect, GE Healthcare), synthetic polymer (Product A) and porous glass (Product B). Running conditions – see Figure 3.

methods for IgG binding activity (>95%), electrophoretic purity and reversed phase- (RP-) HPLC purity (>98%), as well as for endotoxin content (<1 EU/mg). Results from QC analysis of five production batches are shown in Table 3.

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

Table 3. QC analysis of five production batches of recombinant protein A

Production batch	IgG binding activity (%)	Purity by RP-HPLC (%)	Endotoxin (EU/mg)
1	97	99.5	<0.1
2	98	99.3	<0.1
3	96	98.9	0.2
4	98	99.6	<0.1
5	96	99.0	0.6

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 5. 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 recombinant protein A can be removed efficiently from the IgG containing fraction using gel filtration or ion exchange chromatography.

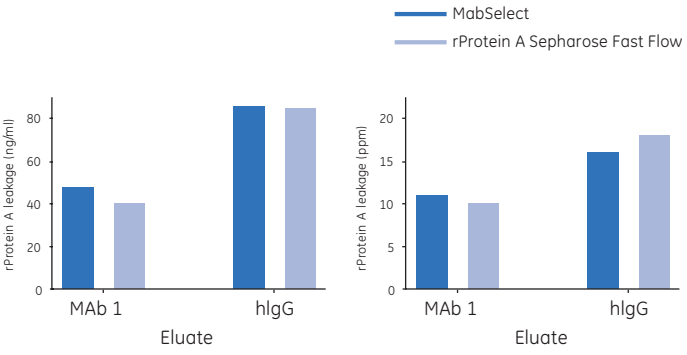

Fig 5. Leakage (ng/ml = ng protein A/ml elutate; ppm = ng protein A/mg purified IgG) of rProtein A in the antibody eluate during purification of a monoclonal antibody (Mab 1) and human polyclonal IgG (hIgG). MabSelect and rProtein A Sepharose Fast Flow were loaded to 24 mg of antibody per ml packed bed. MAb 1 was loaded in Chinese Hamster Ovary (CHO) cell culture supernatant (feed concentration 1 mg/ml) and hIgG 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 hIgG) and neutralized with Tris-buffer. Aliquots of the buffers, pure antibody samples and eluates were then analysed by ELISA for their rProtein A content. The only fractions that contained rProtein A were the eluates.

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

Column: HiTrap™ SP (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

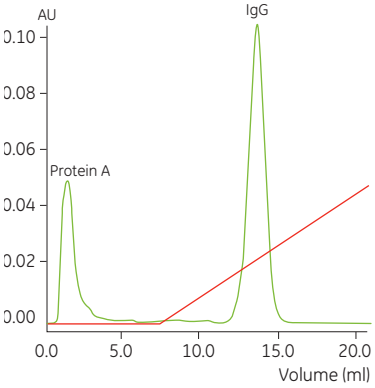

Fig 6. Removal of protein A from mouse IgG_{2b} by cation exchange chromatography on HiTrapTM SP. Recombinant protein A was spiked into mouse IgG_{2b}.

Figure 6 shows purification of mouse IgG_{2b} on a cation exchanger after spiking with a large amount of recombinant protein A. Similar results can be achieved with gel filtration and anion exchange chromatography.

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

Operation

Method development

As for most affinity chromatography media, MabSelect offers high selectivity that renders efficiency related parameters such as sample load, flow rate, bead 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.

Cleaning and sanitization

The general recommendation for cleaning MabSelect is to use a mixture of 50 mM NaOH and 1 M NaCl. As an alternative cleaning protocol 6 M guanidine hydrochloride can be used. Phosphoric acid (100 mM) has also been used for cleaning. To remove hydrophobically bound substances a solution of non-ionic detergent or ethanol is recommended. To sanitize MabSelect we recommend treatment with solutions containing benzyl alcohol (2%), 0.1 M acetic acid/20% ethanol, or 2% hibitane digluconate/20% ethanol. These solutions can also be used as storage solutions.

Detailed recommendations for column packing, cleaning and sanitization, method design and optimization can be found in the instructions that are enclosed with each pack of medium.

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. We recommend a bed height of about 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 Figures 7 and 8.

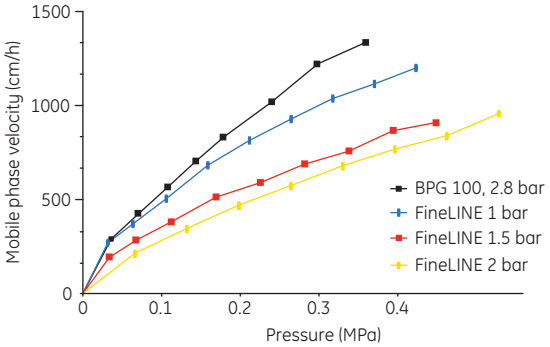

Fig 7. Pressure-flow curves for MabSelect packed to 20-cm bed height in FineLINE 200 at different packing pressures. Results from BPG 100, flow-packed at 2.8 bar, are included for comparison.

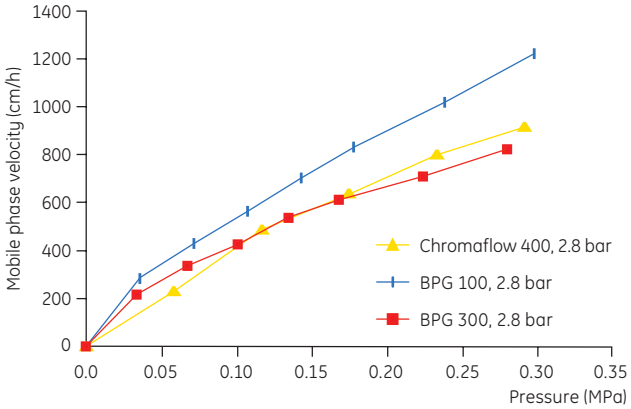

Fig 8. Pressure-flow curves for MabSelect packed to a bed height of 20 cm in BPG and Chromaflow columns.

Equipment

MabSelect can be used together with most equipment available for chromatography, from lab-scale to production-scale. To ensure best performance, it is recommended to use MabSelect at 20 cm bed height. Recommended columns from GE Healthcare are listed in Table 4.

Table 4. Recommended columns for MabSelect

Columns	Inner diameter (mm)	Bed volume	Bed height (cm)
Lab scale			
XK 16/40	16	8 to 74 ml	max. 35
XK 26/40	26	32 to 196 ml	max. 35
Production scale			
BPG variable bed, glass column	100 to 450	2.4 to 131 l	max. 83
BioProcess Stainless Steel (BPSS) fixed bed columns	400 to 1400	12 to 1500 l	10 to 100
INdEXvariable bedcolumns	70 to 200	Up to 24.8 l	max. 79
Chromaflow variable bed columns	280 to 2000	Up to 942 l	100 to 300

Application

An example of a purification of monoclonal antibody is shown in Figure 9. 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 10.

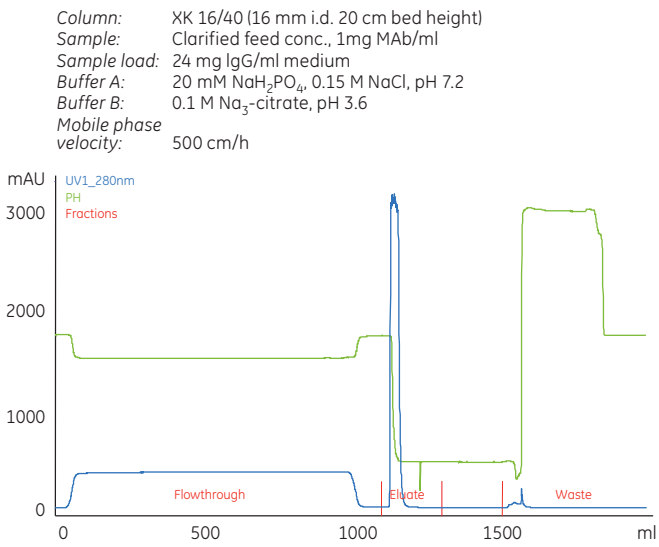

Fig 9. Purification of a monoclonal antibody from a large-scale culture of CHO cells using MabSelect.

Lanes

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

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

Ordering information

Product	Pack size	Code No
MabSelect	25 ml	17-5199-01
	200 ml	17-5199-02
	1 l	17-5199-03
	5 l	17-5199-04
Lab scale columns	XK 16/40 (16 mm i.d.)	18-8774-01
	XK 26/40 (26 mm i.d.)	18-8768-01
Process columns	FineLINE 350 columns	*

Related Literature

Data Files

Process scale columns	BPG columns	18-1115-23
	BioProcess Stainless Steel (BPSS) columns	18-1121-08
	INdEX columns	18-1115-61
	BPG 450 column	18-1060-59
	FineLINE 35 Pilot column	18-1104-95
	FineLINE 70/70l, 100P/100LP, 200P/200LP	18-1130-00
	Chromaflow columns	18-1138-92

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

* Custom designed columns, contact your local GE Healthcare representative for further information.

www.gehealthcare.com/protein-purification-bioprocess

www.gehealthcare.com

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imagination at work

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