GE Healthcare
Life Sciences

MabSelect SuRe™ LX

MabSelect SuRe LX is an alkali-stabilized, protein A-derived affinity medium with a binding capacity for monoclonal antibodies (MAbs) exceeding that of MabSelect SuRe at longer residence times. As an example, at 6 min residence time, the dynamic binding capacity of MabSelect SuRe LX for human IgG is approximately 60 g/l. This special combination of high binding capacity plus alkaline stability gives manufacturers of MAbs many opportunities to improve process economics and product quality.

High dynamic binding capacity (DBC) helps users process feed from high-expression cell cultures with increased antibody titers in shorter time or with smaller chromatography unit operations. Enhanced alkaline stability means that regular cleaning-in-place (CIP) can be performed with cost-effective agents such as sodium hydroxide (NaOH) to prolong the medium's working lifetime.

Key performance benefits of MabSelect SuRe LX include:

- Outstanding binding capacity, for example, approx. 60 g/l medium for human IgG at 6 min residence time
- Quick, efficient processing of large volumes of high-titer bioreactor feeds
- Higher density eluates increase operating flexibility and allow smaller unit operations
- Effective CIP with 0.1 M NaOH over hundreds of purification cycles improves process economy
- Enhanced protease resistance of the protein A ligand reduces leakage
- Generic elution conditions for different MAbs enable platform purifications

Fig 1. The significantly higher binding capacity of MabSelect SuRe LX protein A-based affinity media allows MAb manufacturers to process high-titer feeds in relatively small production columns.

Medium characteristics

Well-established in bioprocessing

The MabSelect™ media family for the process-scale capture and purification of MAbs comprises MabSelect, MabSelect Xtra™, MabSelect SuRe, and MabSelect SuRe LX.

MabSelect SuRe LX has been further developed from MabSelect SuRe to give even higher binding capacity at longer residence time. Table 1 lists the main characteristics of MabSelect SuRe LX.

Table 1. Main characteristics of MabSelect SuRe LX

Matrix	Rigid, highly cross-linked agarose
Ligand	Alkali-stabilized, protein A-derived (*E. coli*)
Ligand coupling	Single-point attachment
Coupling chemistry	Epoxy
Average particle size (d_{50v})*	85 µm
Dynamic binding capacity†	Approx 60 mg human IgG/ml medium at 6 min residence time
Maximum mobile phase velocity‡	500 cm/h
pH working range	3–12
Chemical stability	Stable in all aqueous buffers commonly used in protein A chromatography
Cleaning-in-place stability	0.1–0.5 M NaOH
Delivery conditions	20% ethanol

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

† Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h in a column with a bed height of 10 cm, residence time is 6 min. Residence time is equal to bed height (cm) divided by nominal fluid velocity (cm/h) during sample loading. Nominal fluid velocity is equal to volumetric flow rate (ml/h) divided by column cross-sectional area (cm^2).

‡ Determined in an AxiChrom™ 300 column, bed height 20 cm, operating pressure less than 2 bar.

High binding capacity meets modern processing demands

Increasing demand for MAbs as biopharmaceuticals has promoted the development of cell cultures with increased expression levels. Over recent years, the antibody titers of mammalian cell cultures have risen dramatically. Titers of 5 to 10 g/l are not unusual today. If MAb purification processes are to handle high-titer bioreactor feeds quickly and cost-effectively, they must offer significant increases in dynamic binding capacity (DBC). Several studies show that this can be achieved by increasing residence time with MabSelect SuRe LX (Fig 2).

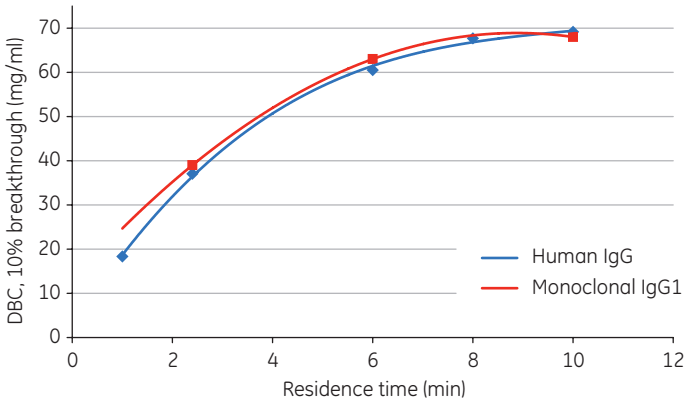

Fig 2. Dynamic binding capacity increases as a function of residence time.

Moreover, direct comparisons between MabSelect SuRe LX and MabSelect SuRe, which already exhibits a high DBC, show that the former offers more than 20% higher DBC at extended residence times. At 6 min, for example, the DBC of MabSelect SuRe LX at 10% breakthrough for seven MAbs is clearly superior to that of MabSelect SuRe (Fig 3). These data confirm that the increased capacities range from approximately 20% to 50%.

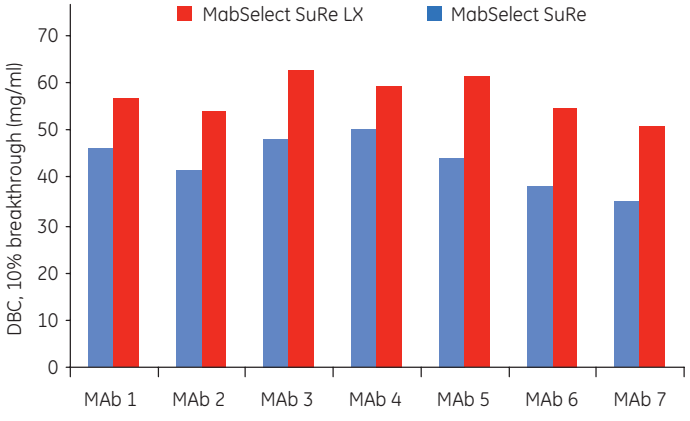

Fig 3. Significantly increased DBC of MabSelect SuRe LX compared to MabSelect SuRe at a residence time of 6 min.

This high capacity also translates to smaller elution pool volumes, that is, higher antibody concentrations (large pool volumes are a common bottlekneck in many manufacturing facilities today). Note that the high rigidity of the medium (see below) permits the use of higher bed heights, which increases the flexibility of process design and large-scale operations.

High alkaline stability extends working lifetime

CIP with NaOH is a key step in the cost-effective production of pure MAb products at industrial scale. However, affinity media based on native or recombinant protein A (rProtein A) ligands are particularly sensitive to NaOH and this is generally regarded as a drawback of the method. MabSelect SuRe LX overcomes this limitation by using the same alkali-stablilized ligand as MabSelect SuRe. The ligand was developed by protein engineering one of the IgG-binding domains of protein A. Amino acids particularly sensitive to alkali were identified and substituted with more stable ones. The final construct is a tetramer of the engineered domain with a C-terminal cysteine, which enables single-point attachment to the matrix. The resulting highly pure ligand is immobilized to the agarose matrix via a chemically stable thio-ether linkage.

The increased alkaline stability allows very effective CIP with 0.1 to 0.5 M NaOH over many purification cycles (Figs 8 and 9). In addition, the ligand shows improved stability to proteases compared with rProtein A. This attribute also extends working lifetime and minimizes ligand leakage. Furthermore, as the engineered protein A ligand of MabSelect SuRe LX does not exhibit affinity for the Fab region of antibodies, more generic elution conditions can be employed compared with rProtein A. Antibodies can thus be eluted at a more homogeneous pH range (1). See Figure 7 for more details.

Rigid, highly cross-linked matrix allows high flow rates

Like other media in the MabSelect family, MabSelect SuRe LX features a rigid, highly cross-linked agarose base matrix that allows much higher flow rates in process-scale purification than conventional cross-linked agaroses of similar porosity. This permits high-throughput purification of MAbs from large volumes of feed. Figure 4 shows pressure/flow curves for MabSelect SuRe LX packed in a range of column sizes.

High rigidity allows column capacity to be increased for high-titer feeds by increasing the column bed height. Running at higher bed heights and flow rates gives greater flexibility when designing processes, and can reduce the need to purchase new hardware. Higher bed heights also mean small diameter columns and thus a reduced equipment footprint.

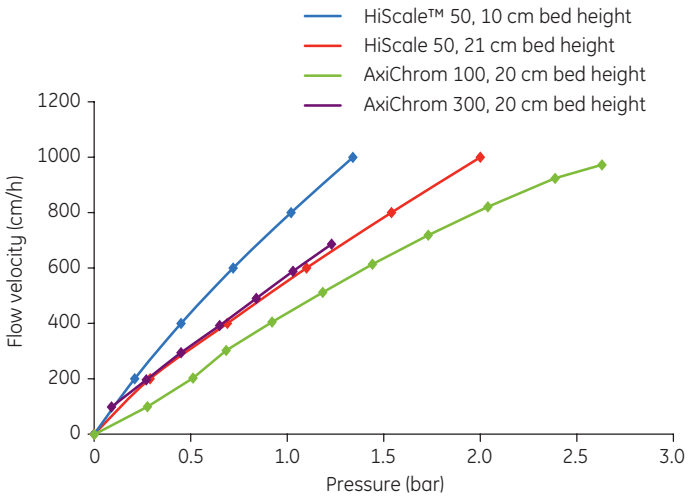

Fig 4. Pressure/flow curves for MabSelect SuRe LX packed at different bed heights in HiScale 50 and AxiChrom 100 and 300 columns. The medium's rigid base matrix allows high flow rates in process-scale purifications.

BioProcess™ medium with full support

MabSelect SuRe LX also belongs to the BioProcess media family that is developed and supported for the large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term media supply, safe and easy handling, and Regulatory Support Files (RSF) to assist process validation and submissions to regulatory authorities. In addition, Fast Trak Training & Education provide high-level, hands-on training for all key aspects of bioprocess development and manufacturing.

Operation

Equipment

MabSelect SuRe LX can be used with most modern chromatography equipment from laboratory to production scale. Table 2 lists suitable empty columns from GE Healthcare. To ensure best performance at process scale, pack MabSelect SuRe LX at bed heights of 10 to 30 cm.

Table 2. Recommended GE Healthcare column families for packing MabSelect SuRe LX

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

* Intelligent Packing method for MabSelect SuRe can be used.

† The pressure rating of BPG 450 is too low for use with MabSelect media.

‡ Packing instructions for MabSelect media in Chromaflow columns are described in Application note 11-0007-52.

§ Larger pack stations can be required for larger diameters.

Method development and scale-up

The primary aim of method development is to establish and optimize 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. In general, however, human or humanized antibodies, except for subclass 3, have high affinity for protein A.

MabSelect SuRe LX is supplied prefilled in 96-well Predictor™ plates, which support high-throughput process development (HTPD) by allowing parallel screening of chromatographic conditions such as pH and conductivity.

Defined conditions for loading, washing, elution, CIP, etc., can then be verified and optimized with small prepacked HiScreen™ (4.7 ml) columns. Together with a chromatography system such as ÄKTA™ avant, the HiScreen format helps develop an efficient and robust separation method.

Further development and optimization using HiScale columns then permits straightforward scale-up for GMP clinical trials or full-scale manufacturing on AxiChrom columns.

Loading and elution

The high selectivity of MabSelect SuRe LX 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, therefore, to establish the conditions that will bind the highest amount of target molecule and give the highest product recovery.

Typically, clarified feedstock is loaded directly onto the column. The amount of antibody that can be loaded is determined by the dynamic binding capacity (DBC). Since DBC increases as a function of residence time and varies between different MAbs and starting materials, we recommend determining DBC at different residence times (Fig 2). To obtain high and reproducible yields over hundreds of cycles, the load should normally not exceed 90% of DBC.

After intermediate washing, the target MAb is normally eluted at pH 3 to 4. Elution conditions for a specific MAb can be determined by performing elution in a linear pH gradient. Figure 5 shows an example run for MabSelect SuRe. Elution pH was 3.67 at peak maximum and 3.56 at 10% peak maximum (i.e. descending). Based on this result, an elution pH of 3.5 would result in high yields and narrow elution peaks.

Running conditions

Column: HiScreen MabSelect SuRe, 4.7 ml
Sample: 1 ml of clarified CHO feed containing 1.72 mg MAb/ml
Binding buffer: 20 mM citrate, pH 6.0
Elution buffer: 20 mM citrate, pH 3.0
Gradient: Linear, 20 mM citrate buffer pH 6.0 to 3.0 in 10 CV
Flow rate: 0.5 ml/min
System: ÄKTA avant 25

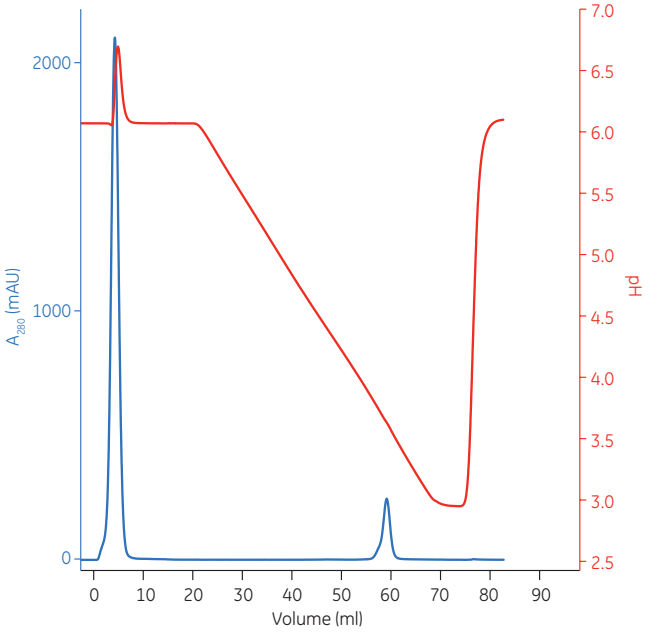

Fig 5. Determining optimum elution pH helps ensure a high yield of the MAb product.

Design of Experiments (DoE) can also be used to optimize elution conditions. In the DoE example shown in Figure 6, elution pH was varied between 3.25 to 4.0 and flow rate between 0.3 and 1 ml/min (100 to 300 cm/h). Highest yields and lowest pool volumes were obtained at low elution pH, while no major effect of flow rate could be observed. The optimal elution pH for the investigated MAb was 3.6 or below.

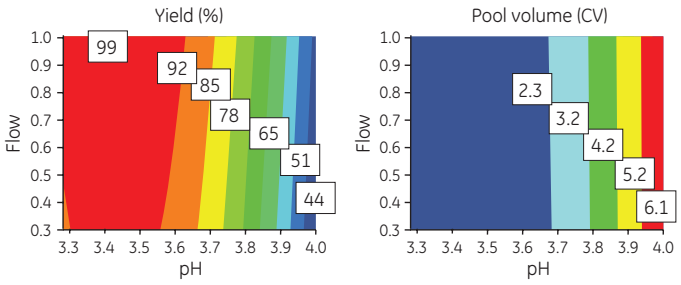

Fig 6. DoE optimization of elution conditions on MabSelect SuRe LX. CV=column volume.

Generic elution promotes platform purification

MabSelect SuRe LX allows the use of generic elution conditions for different monoclonal antibodies, which is advantageous when designing generic purification platform processes. Figure 7 shows the elution pH for different MAbs with both MabSelect (with a traditional rProtein A ligand) and with MabSelect SuRe. With MabSelect, the MAbs elute at different pHs, but for MabSelect SuRe, almost all elute at a more homogeneous pH. A similar behavior is expected for MabSelect SuRe LX.

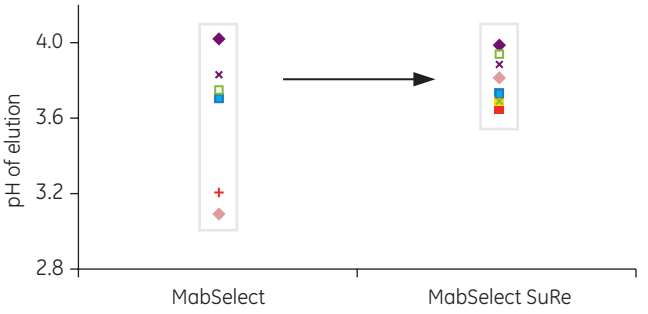

Fig 7. Scatter plot showing the distribution of elution, pH of various human antibodies and Fc fusion proteins on MabSelect and MabSelect SuRe. Reprint from *Biotechnol. Bioeng.* (1), with courtesy of Amgen.

Effective CIP with retained dynamic binding capacity

Use of 0.1 to 0.5 M NaOH is recommended for CIP and sanitization. As well as being an effective cleaning agent, NaOH is also inexpensive and easy to handle in bulk quantities. It is thus an attractive choice for large-scale commercial manufacturers of monoclonal therapeutic antibodies.

A lifetime study with MAb-containing feedstock has shown that the DBC and yield of MabSelect SuRe LX are stable over 100 purification cycles with 0.1 M NaOH, and that the levels of leached protein A and host cell proteins remain constant. Figure 8 shows DBC data and Figure 9 overlay chromatograms from this study.

Effective sanitization of MabSelect SuRe LX is also possible using a combination of 0.1 M NaOH and 40% isopropyl alcohol.

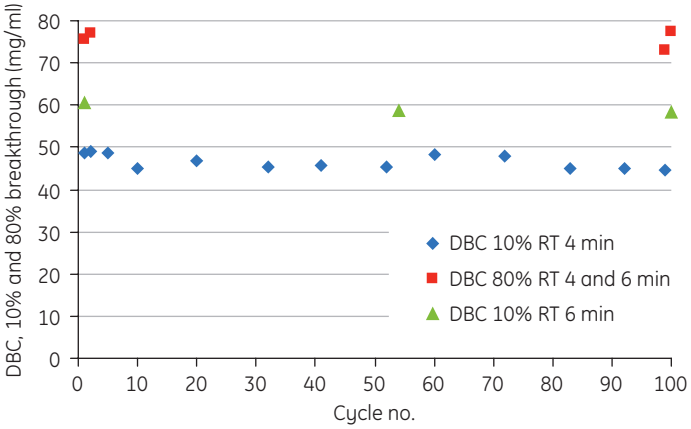

Fig 8. Dynamic binding capacity measurements for MabSelect SuRe LX over 100 purification cycles with 0.1 M NaOH as CIP agent. RT = residence time.

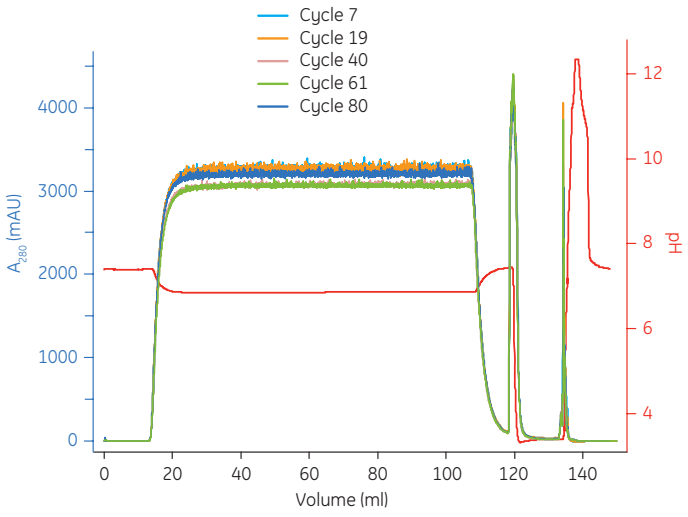

Fig 9. Overlay of chromatograms from runs with harvested cell culture feed (HCCF), (0.8 g MAb/l from cycle 3). A new batch of HCCF with a MAb concentration of 1.1 g/l was applied from cycle 87.

An extended lifetime study on human IgG with repeated buffer cycles also demonstrated that DBC is stable (> 95%) for more than 300 cycles with 0.1 M NaOH. For 0.5 M NaOH, DBC gradually decreases after cycle 25 but still remains above 90% for 100 cycles at this higher concentration.

Full details can be found in Application note: Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place (see Related literature)

Storage

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

After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

Reference

1. Ghose G, *et al.* Antibody variable region interactions with protein A: Implications for the development of generic purification processes. *Biotechnol. Bioeng.* **92(6)** 665-673 (2005).

Ordering information

Product*	Quantity	Code no.
MabSelect SuRe LX	25 ml	17-5474-01
	200 ml	17-5474-02
	1 l	17-5474-03
	5 l	17-5474-04
	10 l	17-5474-05
PreDictor MabSelect SuRe LX, 6 µl	4 × 96-well plates	17-5474-30
PreDictor MabSelect SuRe LX, 20 µl	4 × 96-well plates	17-5474-31
PreDictor MabSelect SuRe LX, 50 µl	4 × 96-well plates	17-5474-32
HiScreen MabSelect SuRe LX	1 × 4.7 ml	17-5474-15

* MabSelect Sure LX will be made available in prepacked, prequalified, and presanitized ReadyToProcess™ columns. Please ask for details.

Related products

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

Data files

MabSelect	18-1149-94
MabSelect Xtra	11-0011-57
MabSelect SuRe	11-0011-65

Application notes

Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place	28-9872-96
Dynamic binding capacity study on MabSelect SuRe LX for capturing high-titer monoclonal antibodies	28-9875-25
High-throughput process development for design of cleaning-in-place protocols	28-9845-67

Handbooks

Antibody Purification, Principles and methods	18-1037-46
High-throughput Process Development with PreDictor Plates, Principles and methods	28-9403-58

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