# MabSelect SuRe

## Alkali-stabilized protein A-derived medium for capture of monoclonal antibodies

MabSelect SuRe™ (Superior Resistance) is a member of the MabSelect™ family* of affinity chromatography media for the capture of monoclonal antibodies (MAbs) at process scale. MabSelect SuRe is composed of a rigid, high-flow agarose matrix and alkali-stabilized protein A-derived ligand. This ligand provides greater stability than conventional protein A-based media in the alkaline conditions used in cleaning-in-place (CIP) protocols. The enhanced alkali stability of MabSelect SuRe improves process economy; cleaning can be performed with cost-effective reagents such as sodium hydroxide, which improves process economy and product quality.

Key performance characteristics of MabSelect SuRe are:

- Novel, alkali-stabilized protein A ligand allows the use of 0.1–0.5 M sodium hydroxide for CIP.
- Improves product quality and reduces overall costs.
- Eliminates the need for expensive and corrosive CIP reagents.
- High dynamic binding capacity (DBC) reduces process time and amount of medium used.
- High-flow agarose matrix allows processing of large volumes of feed.

### High stability in alkaline conditions

The MabSelect SuRe ligand was developed by protein engineering of 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.

**Fig 1.** MabSelect SuRe is ideal for purification of large volumes of monoclonal antibodies and is resistant to cleaning- and sanitization-in-place using alkali.

The ligand is produced by validated fermentation and downstream processes and the entire production process is free of components of mammalian origin. The resulting highly purified ligand is immobilized to the agarose matrix through a chemically stable thio-ether linkage.

MabSelect SuRe is stable under alkali conditions and has been tested for up to 200 cycles of CIP using 0.1 M NaOH. The combination of low ligand leakage and high DBC together with the high-flow matrix makes MabSelect SuRe ideal for the purification of MAbs at process scale.

* The MabSelect family of media for process-scale purification of monoclonal antibodies comprises MabSelect, MabSelect Xtra™, and MabSelect SuRe. MabSelect is designed for high-throughput purification of monoclonal antibodies from large volumes of feed. MabSelect Xtra is designed for maximum DBC, which allows capture of high expression levels of MAbs from feedstock. For more information on MabSelect and MabSelect Xtra, refer to data files 18-1149-94 and 11-0011-57 respectively.

## Rigid, highly cross-linked matrix allows high flow rates

MabSelect SuRe has been developed from the same rigid, highly cross-linked agarose matrix used for MabSelect. The matrix of MabSelect SuRe allows the use of higher flow rates in process-scale purifications of Mabs compared with conventional cross-linked agarose of similar porosity.

The characteristics of MabSelect SuRe are summarized in Table 1.

## High dynamic binding capacity after numerous CIP cycles

Cleaning-in-place is an essential step in the production of pure Mabs in industrial applications. The main drawback with using sodium hydroxide for CIP of conventional protein A-based media is the sensitivity of native and recombinant Protein A (rProtein A) to alkaline conditions. MabSelect SuRe, however, retains dynamic binding capacity after repeated CIP cycles with 0.1–0.5 M NaOH.

Figure 2 shows DBC (10% breakthrough) of polyclonal human IgG (*h*IgG) as a function of exposure to alkaline conditions. MabSelect, with its conventional rProtein A ligand, was used for comparison. Approximately 85–90% of the initial dynamic binding capacity of MabSelect SuRe is retained after numerous CIP cycles with sodium hydroxide.

The dynamic binding capacity of MabSelect SuRe remains high after CIP in conjunction with purification of humanized $IgG_1$ and $IgG_4$ from clarified cell culture (Table 2). DBC was retained to approximately 85% of the initial binding capacity. The recovery of both MAbs was consistently over 95%.

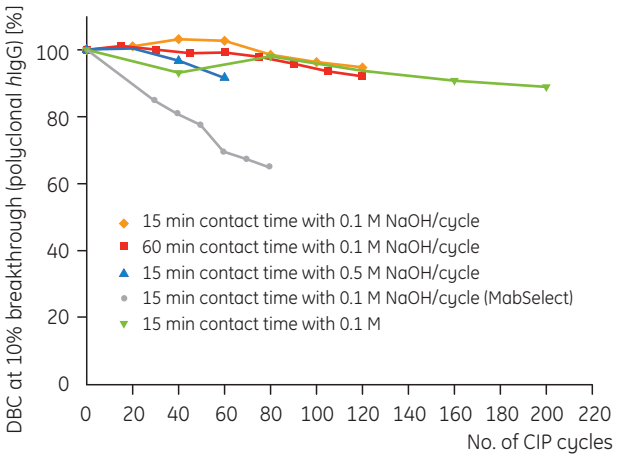



**Fig 2.** Dynamic binding capacity of MabSelect SuRe and MabSelect for polyclonal human IgG after CIP with 0.1–0.5 M NaOH for up to 200 cycles.

**Table 1.** Characteristics of MabSelect SuRe

| | |
|---|---|
| Ligand | Alkali-stabilized protein A-derived (*E. coli*) |
| Ligand coupling method | Epoxy activation |
| Matrix | Rigid, highly cross-linked agarose |
| Average particle size ($d_{50v}$)* | 85 µm |
| Dynamic binding capacity† | At least 30 mg human IgG/ml medium at 2.4 min residence time |
| Recommended mobile phase velocity‡ | 100–500 cm/h |
| Chemical stability | Stable in all aqueous buffers commonly used in protein A chromatography. |
| pH working range | 3–12 |
| 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 250 cm/h in a column with a bed height of 10 cm.

‡ Determined in a BPG 300 column, bed height 20 cm, operating pressure less then 2 bar.

The dynamic binding capacity of MabSelect SuRe remains high after CIP in conjunction with purification of humanized $IgG_1$ and $IgG_4$ from clarified cell culture (Table 2). DBC was retained to approximately 85% of the initial binding capacity. The recovery of both MAbs was consistently over 95%.

**Table 2.** Effect of CIP cycles using 0.1 M NaOH on the DBC of MabSelect SuRe in the purification of $IgG_1$ and $IgG_4$.

| Antibody | CIP (no. of cycles × duration in minutes) | DBC (dynamic binding capacity [% of initial DBC]) |
|---|---|---|
| $IgG_1$ | 150 × 15 min | ≥ 85% |
| $IgG_4$ | 100 × 15 min | ≥ 85% |

## Increased residence time increases dynamic binding capacity

The already high dynamic binding capacity of MabSelect SuRe is further improved by increasing sample residence time on the medium. With a residence time of 2.4 min, the dynamic binding capacity at 10% breakthrough of humanized $IgG_1$, humanized $IgG_4$, and polyclonal *h*IgG is ≥ 30 mg/ml (Fig 3). Increasing the residence time to 4.8 min increases the dynamic binding capacity of the humanized immunoglobulins and *h*IgG to more than 38 mg/ml.

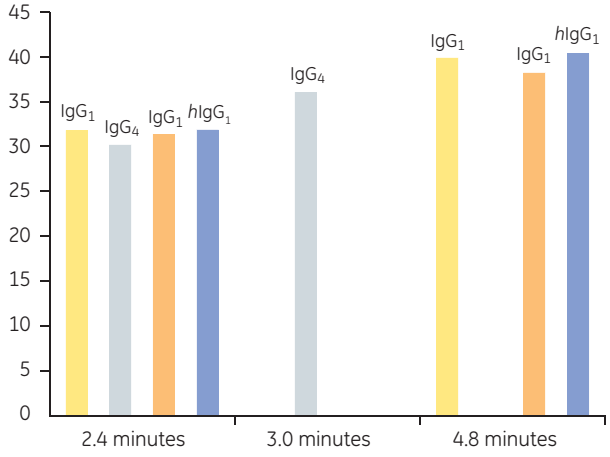



**Fig 3.** Dynamic binding capacity of MabSelect SuRe as a function of residence time of the protein on the medium.

## Low ligand leakage

The level of leakage of the MabSelect SuRe ligand during elution is low. A normal range of leakage is estimated to be 5–20 ppm (ng ligand/mg IgG). However, leakage is affected by chromatographic running conditions and the composition of the feedstock.

Figure 4 shows the ligand leakage from MabSelect SuRe over 100 cycles of purification of a monoclonal antibody from clarified cell culture. Fractions collected from the purification were analyzed by noncompetitive ELISA. Figure 4 confirms the low leakage of the MabSelect SuRe ligand over numerous purification/CIP cycles.

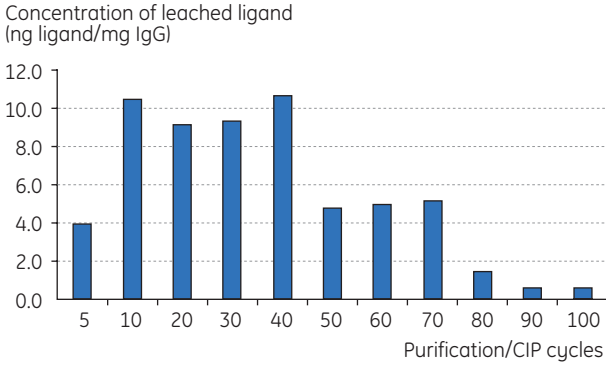
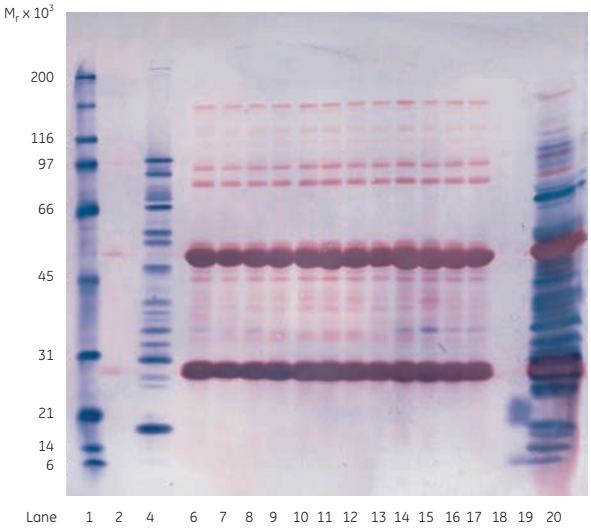



**Fig 4.** Ligand leakage (ppm) of MabSelect SuRe over 100 cycles of CIP in conjunction with the purification of humanized $IgG_4$. Cleaning-in-place was performed with 0.1 M NaOH and the contact time was 15 min/cycle.

## Low risk of host cell protein contamination or carryover

Rigorous CIP or sanitization-in-place with sodium hydroxide reduces the risk of both contamination from host cell proteins and microbial growth in the medium, as well as carryover in the product pools.

Figure 5 is a Western blot of humanized $IgG_4$ fractions eluted in the ligand-leakage study described above. The Western blot confirms that no contamination from host cell proteins or carryover is detected after 50–100 purification cycles on MabSelect SuRe. Comparison of the separation of host cell protein standard in lane 4 with the purified $IgG_4$ in lanes 6–17 obtained from 100 affinity purifications on MabSelect SuRe indicates no presence of the host cell proteins in the bound fraction eluates. The absence of protein bands in lanes 18 (carryover after 50 cycles) and 19 (carryover after 100 cycles) indicates no carryover or cross-contamination between two consecutive purification cycles with an intermittent CIP cycle. Sample, data, and image were supplied by kind courtesy of Lonza Biologics plc, Slough, U.K.




| | | | |
|---|---|---|---|
| Lane 1: | HMW marker | Lane 12: | Cycle 50 |
| Lane 2: | BSA IgG standard | Lane 13: | Cycle 60 |
| Lane 4: | Host cell protein | Lane 14: | Cycle 70 |
| Lane 6: | Purification/CIP cycle 1 | Lane 15: | Cycle 80 |
| Lane 7: | Cycle 5 | Lane 16: | Cycle 90 |
| Lane 8: | Cycle 10 | Lane 17: | Cycle 100 |
| Lane 9: | Cycle 20 | Lane 18: | Carryover after 50 cycles |
| Lane 10: | Cycle 30 | Lane 19: | Carryover after 100 cycles |
| Lane 11: | Cycle 40 | Lane 20: | Start material |

**Fig 5.** Western blot of humanized $IgG_4$ from 1–100 purification/CIP cycles on MabSelect SuRe. The Western blot was developed with two sets of polyclonal antibodies; one raised against the host cell proteins and another raised against the MAb. The presence of bovine serum albumin (BSA) and IgG in lane 2, as the negative and positive controls respectively, marks the specificity of the two sets of antibodies.

## Comparison of MabSelect SuRe with rProtein A-based media

Figures 6 and 7 show the performance of MabSelect SuRe and MabSelect in the purification of humanized $IgG_1$ and $IgG_4$ from HCCF (host cell culture fluid). Selectivity and specificity of the two media were comparable.

In a separate study, the purification of humanized $IgG_4$ expressed in cell culture fluid using MabSelect SuRe was compared to the purification performance of MabSelect and MabSelect Xtra. Purification performance of the three media was similar and contamination of host cell proteins was minimal as seen on the Western blot in Figure 8. Sample, data, and image were supplied by kind courtesy of Lonza Biologics plc, Slough, U.K.

| | |
|---|---|
| *Column:* | Tricorn™ 5/100 |
| *Sample:* | 8 mg humanized $IgG_1$ in HCCF |
| *Equilibration buffer:* | 20 mM phosphate, 150 mM NaCl, pH 7.4 |
| *Elution buffer:* | 100 mM citrate, pH 3.0 |
| *System:* | ÄKTAexplorer™ 10 |

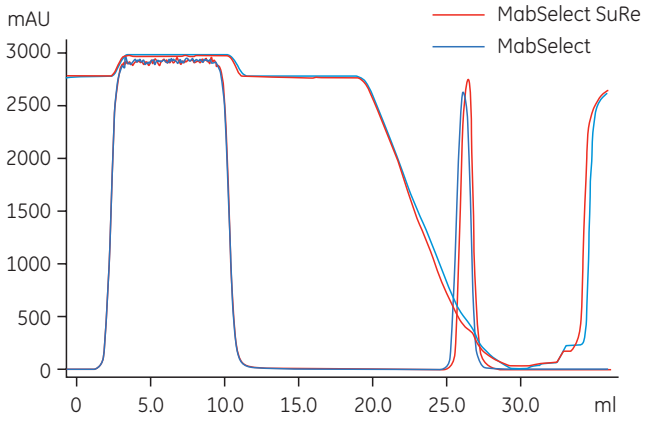



**Fig 6.** Similar desorption characteristics of MabSelect SuRe and MabSelect in the purification of humanized $IgG_1$ from HCCF.

| | |
|---|---|
| Column: | Tricorn 5/100 |
| Sample: | 5 mg humanized IgG4 in HCCF |
| Equilibration buffer: | 20 mM phosphate, 150 mM NaCl, pH 7.4 |
| Elution buffer: | 100 mM citrate, pH 3.0 |
| System: | ÄKTAexplorer 10 |

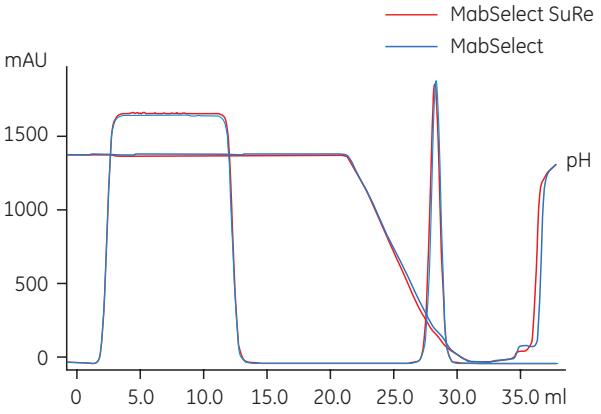
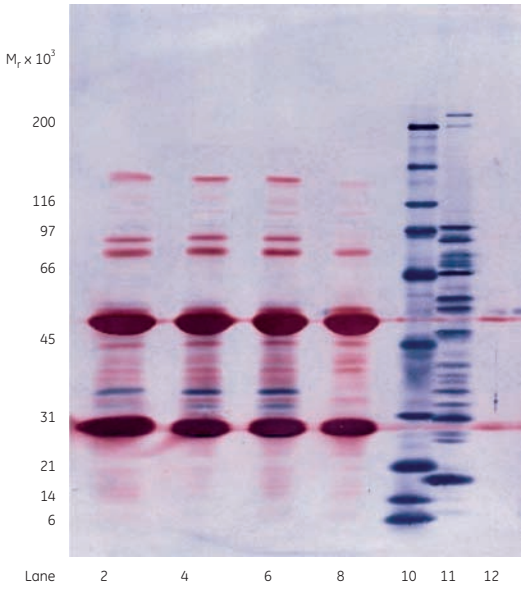



**Fig 7.** Similar desorption characteristics of MabSelect SuRe and MabSelect in the purification of humanized $IgG_4$ from HCCF.




Lane 2: MabSelect eluate
Lane 4: MabSelect SuRe eluate
Lane 6: MabSelect Xtra eluate
Lane 8: Antibody reference marker
Lane 10: HMW markers
Lane 11: Host cell protein standard
Lane 12: BSA/IgG standards

**Fig 8.** Western blot confirming the purity of humanized $IgG_4$ purified on MabSelect SuRe, MabSelect, and MabSelect Xtra.

# Operation

## *Purification*

MabSelect SuRe offers high selectivity, which 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. In general, however, all human or humanized antibodies, except for subclass 3, have a high affinity for protein A.

Typically, the clarified feedstock is loaded onto the column directly. After sufficient washing, the Mab is normally eluted at pH 3–4.

## *Cleaning and sanitization*

Use of 0.1–0.5 M NaOH is recommended for cleaning and sanitization. Optimization of contact time, concentration, and frequency of CIP cycles is required to achieve the best possible results.

## *Storage*

Recommended storage solutions for MabSelect SuRe are 20% ethanol or solutions containing 2% benzyl alcohol. The recommended storage temperature is 4–8 °C.

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

## *Scale-up*

After optimizing the antibody purification at laboratory scale, the process can be scaled up by keeping the residence time constant in order to maintain capacity. This can be achieved by increasing the column diameter, and keeping the mobile phase velocity and sample-to-bed volume ratio constant.

MabSelect SuRe is based on the same matrix as MabSelect and has similar pressure and flow characteristics. The pressure/flow curve shown in Figure 9 is for MabSelect SuRe packed in a large-format BPG™ column. For more details on packing in Chromaflow™ columns, see Application Note 11-0007-52.

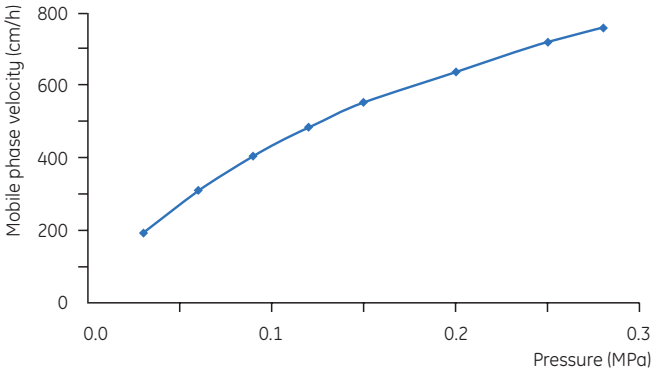



**Fig 9.** Pressure/flow profile for MabSelect SuRe in a packed bed (bed height 20 cm) in a BPG 300 column (i.d. 300 mm).

## Recommended columns

MabSelect SuRe can be used together with most equipment available for chromatography from laboratory scale to process scale. To ensure best performance at process scale, pack MabSelect SuRe to a bed height of 10–30 cm.

Recommended columns are listed in Table 3.

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

| Column* | Inner diameter (mm) | Bed volume | Bed height (cm) |
|---|---|---|---|
| **Lab scale** | | | |
| XK 16/40 | 16 | 16–60 ml | 8–30 |
| **Production scale** | | | |
| BPG | 100–300 | 0.8–21 l | 10–30 |
| BioProcess | 100–1200 | 0.8–339 l | 10–30 |
| Chromaflow | 400–2000 | 13–942 l | 10–30 |

All large-scale columns can be supplied as variable bed height columns. Note that all bed height and diameter combinations above are possible but not necessarily suitable. For example, do not choose large-diameter columns if the bed height is low. Another general recommendation is not to choose a bed height above 30 cm.

## Ordering Information

| Product | Pack size | Code No |
|---|---|---|
| MabSelect SuRe | 25 ml | 17-5438-01 |
| | 25 ml | 17-5438-01 |
| | 200 ml | 17-5438-02 |
| | 1 l | 17-5438-03 |
| | 5 l | 17-5438-04 |
| | 10 l | 17-5438-05 |
| Lab-scale columns | XK 16/20 column | 18-8773-01 |
| | XK 16/40 column | 18-8774-01 |
| | Packing connector XK 16 | 18-1153-44 |
| | XK 16/20 tube | 19-0315-01 |
| | AK 16 adapter | 18-8778-01 |
| | RK 16/26 reservoir | 18-8793-01 |
| Prepacked columns | MabSelect SuRe, Tricorn 10/100 GL | Inquire |
| **Related literature** | | |
| Data Files | BPG columns | 18-1115-23 |
| | BioProcess columns | 18-1167-76 |
| | Chromaflow columns | 18-1138-92 |
| Application notes | MabSelect SuRe - Leakage and Toxicity | 11-0011-64 |
| | MabSelect - Column packing | 11-0007-52 |

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

For contact information for your local office, please visit, www.gelifesciences.com/contact

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imagination at work



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