MabSelect SuRe

AFFINITY CHROMATOGRAPHY

MabSelect SuRe™ (Superior Resistance) is a member of the MabSelect™ family of affinity chromatography resins 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 resins under 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
- Novel ligand design gives enhanced protease resistance resulting in lower ligand leakage
- Generic elution conditions for different monoclonal antibodies enables platform approach to purification
- High dynamic binding capacity (DBC) reduces process time and amount of resin 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 designed 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.

The toxicology of the MabSelect SuRe ligand has been investigated in a toxicity study and is reported in Application note 11001164. Although a high dose of the ligand was administered, no toxic effects of the ligand were observed.

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 an excellent choice for purification of mAbs at process scale.

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Rigid, highly cross-linked matrix allows high flow rates

The MabSelect family of resins for process-scale purification of monoclonal antibodies comprises MabSelect, MabSelect Xtra™, MabSelect SuRe, and MabSelect SuRe LX. MabSelect is designed for high-throughput purification of monoclonal antibodies from large volumes of feed. MabSelect Xtra demonstrates even higher DBC and is also optimized for Fc-fusion proteins. For more information on MabSelect and MabSelect Xtra, refer to Data files 18114994 and 11001157 respectively.

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. 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 SuRe LX, refer to Data file 28987062.

The characteristics of MabSelect SuRe are summarized in Table 1.

Table 1. Characteristics of MabSelect SuRe.

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50V}[1]	~ 85 µm
Ligand	Alkali-stabilized, protein A-derived (*E. coli*)
Coupling chemistry	Epoxy activation
Dynamic binding capacity, Q_{B10}[2]	~ 35 mg *h*IgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers
pH stability, operational[3]	3 to 12
pH stability, CIP[4]	3 to 13.7
Recommended operating flow velocity	100 to 500 cm/h[5]
Recommended maximum operating flow velocity	500 cm/h[6]
Delivery conditions	20% ethanol
Storage	2-8°C, 20% ethanol

[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 range where resin can be subjected to cleaning-or sanitation-in-place without significant change in function.
[5] 30 cm diameter, 20 cm bed height, at 20°C using buffers with the same viscosity as water.
[6] In a BPG 300 column with 30 cm diameter and 20 cm bed height using buffers with the same viscosity as water at 25°C.

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 resins 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.

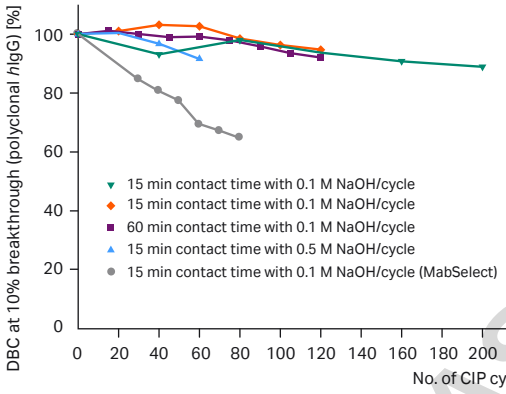


Fig 2. Dynamic binding capacity of MabSelect SuRe and MabSelect for polyclonal human IgG after CIP with 0.1–0.5 M NaOH for up to 200 cycles.

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 83% and 82% of the initial binding capacity respectively. 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)	DBC (dynamic binding capacity [% of initial DBC])
IgG_1	150 × 15 min	≥ 83%
IgG_4	100 × 15 min	≥ 82%

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 resin. 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. At longer residence times or if even higher binding capacity is required, MabSelect SuRe LX is the recommended resin as it is optimized for long residence times.

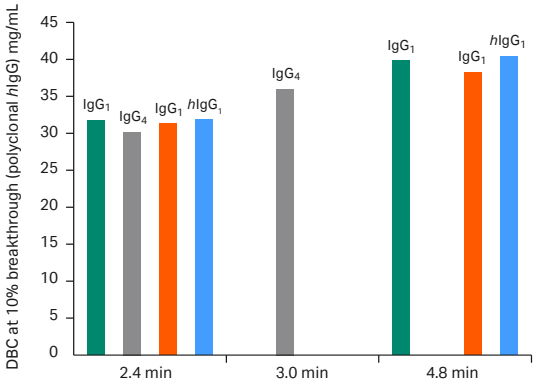


Fig 3. Dynamic binding capacity of MabSelect SuRe as a function of residence time of the protein on the resin.

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Protease stability and low ligand leakage

The ligand construction of MabSelect SuRe has an increased stability towards proteases. All feeds contain proteases and with all protein ligands there is always a risk for proteolytic degradation. The degree of proteolytic activity can differ significantly between feedstocks. This is one of the most important contributions to ligand leakage from Protein A resins.

Figure 4 shows the increased stability of MabSelect SuRe against proteases. Using fluroscent dyes, conventional rProtein A ligand (green) and SuRe ligand (red) were labelled. After incubation in CHO cell lysate, the conventional rProtein A ligand clearly showed degradation, whereas the SuRe ligand remained intact. The results illustrate why the use of MabSelect SuRe leads to lower ligand leaching levels. 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.

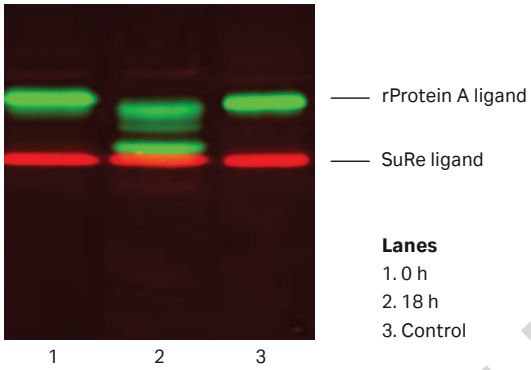


Fig 4. Electrophoresis of rProtein A ligand (marked with green fluorescent dye) and SuRe ligand (marked with red fluorescent dye). Ligands were incubated for 18 h at 37°C at pH 5 with or without 50% cell culture supernatant from non-mAb expressing CHO cells.

Figure 5 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 5 confirms the low leakage of the MabSelect SuRe ligand over numerous purification/CIP cycles.

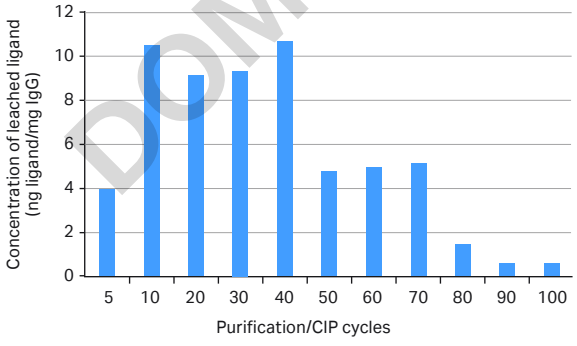
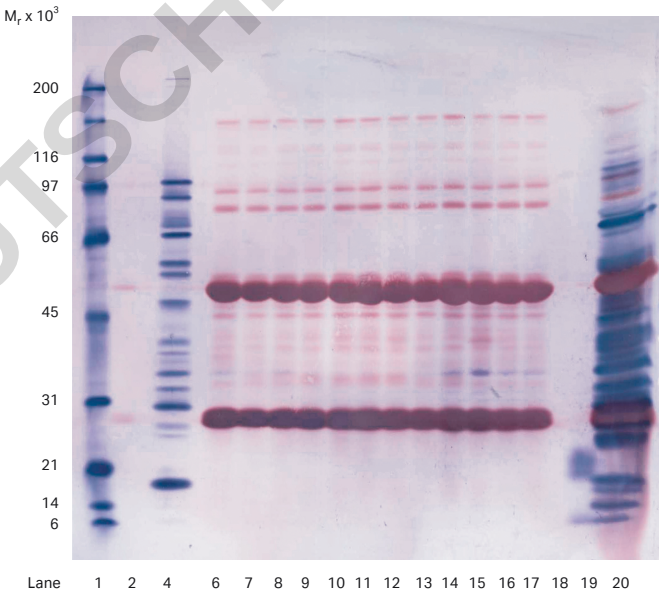


Fig 5. Ligand leakage (ppm) of MabSelect SuRe over 100 cycles of CIP in conjunction with the purification of humanized IgG_4. Cleaning-in-place was performed with 0.1 M NaOH and the contact time was 15 min/cycle.

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 packed column, as well as carryover in the product pools.

Figure 6 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 (lanes 18 and 19). Comparison of the 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 eluates. The absence of protein bands in lanes 18 (blank cycle after 50 cycles) and 19 (blank cycle 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.



Lanes

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

Fig 6. 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.

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Comparison of MabSelect SuRe with rProtein A-based resins

Figures 7 and 8 show the performance of MabSelect SuRe and MabSelect in the purification of humanized IgG_1 and IgG_4 from HCCF (harvested cell culture fluid). Selectivity and specificity of the two resins 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 resins was similar and contamination of host cell proteins was minimal as seen on the Western blot in Figure 9. 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

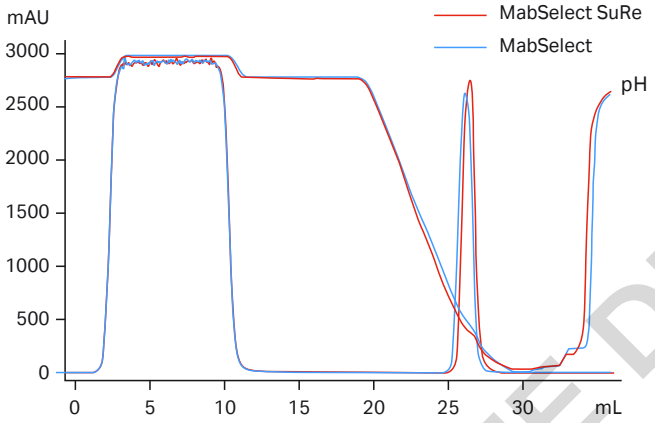


Fig 7. Similar desorption characteristics of MabSelect SuRe and MabSelect in the purification of humanized IgG_1 from HCCF.

Column:	Tricorn 5/100
Sample:	5 mg humanized IgG_4 in HCCF
Equilibration buffer:	20 mM phosphate, 150 mM NaCl, pH 7.4
Elution buffer:	100 mM citrate, pH 3.0
System:	ÄKTAexplorear 10

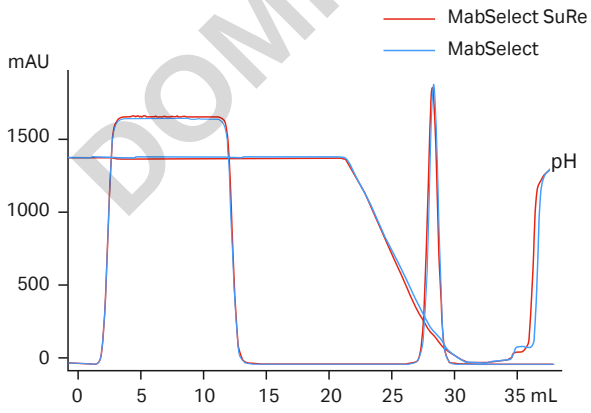
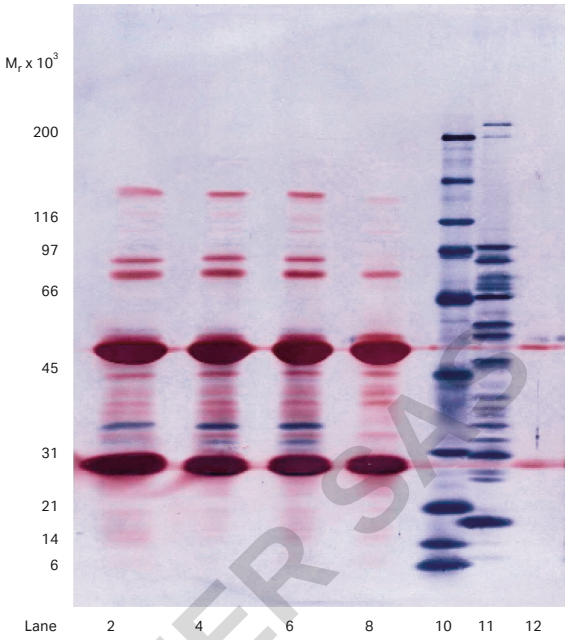


Fig 8. Similar desorption characteristics of MabSelect SuRe and MabSelect in the purification of humanized IgG_4 from HCCF.



Lanes

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

Fig 9. Western blot confirming the purity of humanized IgG_4 purified on MabSelect SuRe, MabSelect, and MabSelect Xtra.

Generic elution conditions

MabSelect SuRe has been shown to give generic elution conditions for different monoclonal antibodies, which is an advantage when designing generic purification platform processes. This feature is also correlated with the construction of the MabSelect SuRe ligand. In Figure 10, the elution pH for a range of different monoclonal antibodies is shown. With MabSelect (with a traditional rProtein A ligand) the different mAbs are eluted at different pH, but for MabSelect SuRe they are all eluted in a more narrow pH interval.

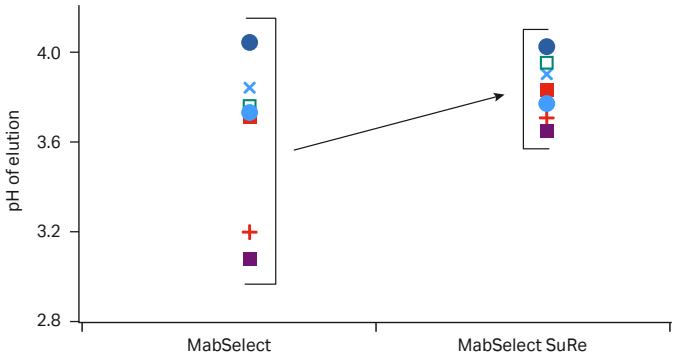


Fig 10. Scatter plot showing the distribution of elution, pH of various human antibodies, and Fc fusion proteins on MabSelect and MabSelect SuRe. Redrawn from (1), by courtesy of Amgen.

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Operation

Method development

MabSelect SuRe offers high selectivity, which 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. 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 to 4.

PreDictor™ 96-well filter plates or miniaturized columns, prefilled with MabSelect SuRe, are suitable for efficient high-throughput screening of chromatographic conditions during process development. Defined conditions can then be verified and optimized with HiTrap™ or HiScreen™ prepacked columns. An example of a high-throughput screening and optimization of MabSelect SuRe step is described in Application note 28946858.

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. For challenging feed-stocks, CIP protocols with reducing agent (e.g., thioglycerol or DTT) followed by 0.1–0.5 M NaOH is an option. A high throughput screening methodology, where numerous cleaning agents and sequences of cleaning steps can be evaluated in parallel using PreDictor 96-well filter plates, is described in Application Note 28984564.

Effective sanitization of MabSelect SuRe is possible using a solution containing 0.1 M NaOH and 40% isopropyl alcohol.

Storage

Recommended storage solutions for MabSelect SuRe 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.

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. At process scale, the preferred packing technique is axial compression. Using AxiChrom™ columns with Intelligent Packing and preprogrammed packing methods for all MabSelect products, is the most optimal and fastest approach. For more details on packing in process-scale columns, see Application note 11000752.

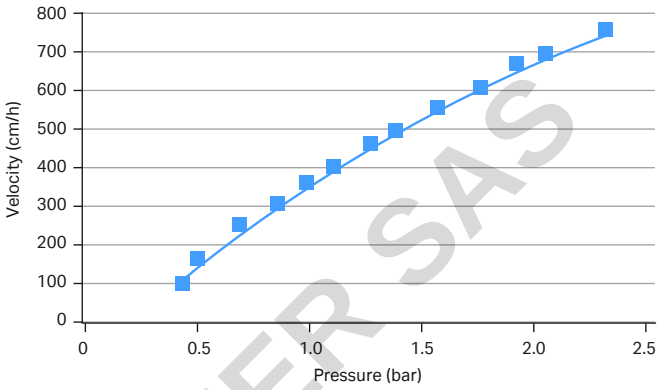


Fig 11. Typical pressure/flow profile for MabSelect SuRe in a packed bed (bed height 20 cm) in an AxiChrom 300 column (i.d. 300 mm).

Equipment

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.

MabSelect SuRe is also available within the ReadyToProcess™ platform, with prepacked, prequalified and presanitized ReadyToProcess columns ranging in size from 1 to 20 L, designed for purification of biopharmaceuticals for clinical phase I and II studies.

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 SuRe resins
‡ Larger pack stations might be required at larger diameters

Reference

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

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Ordering information

Product	Pack size	Product code
MabSelect SuRe	25 mL	17543801
	200 mL	17543802
	1 L	17543803
	5 L	17543804
	10 L	17543805
RTP MabSelect SuRe	1 L	28951110
	2.5 L	28901717
	10 L	28901718
	20 L	28901719
HiScreen MabSelect SuRe	1 × 4.7 mL	28926977
HiTrap MabSelect SuRe	1 × 1 mL	29049104
	1 × 5 mL	11003494
	5 × 1 mL	11003493
	5 × 5 mL	11003495
PreDictor MabSelect SuRe	6 µL (4 × 96-well plates)	28925823
	20 µL (4 × 96-well plates)	28925824
	50 µL (4 × 96-well plates)	28925825
PreDictor RoboColumn™ MabSelect SuRe	50 µL (one row of 8 columns)	28986203
	200 µl (one row of 8 columns)	28986107

Related literature	
Data files	
MabSelect SuRe LX	28987062
MabSelect	18114994
MabSelect Xtra	11001157
AxiChrom columns	28929041
BPG columns	18111523
Chromaflow columns	18113892
HiScale columns	28975523
PreDictor 96-well filter plates and Assist software	28925839
PreDictor RoboColumn	28988634
HiTrap MabSelect, MabSelect Xtra, MabSelect SuRe	11003490
HiScreen prepacked columns	28930581
ReadyToProcess Columns	28915987
Application notes	
MabSelect SuRe: studies on ligand toxicology, leakage, removal of leached ligand, and sanitization	11001164
High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates	28927792
High-throughput screening and optimization of a protein A capture step in a monoclonal antibody process	28946858
A flexible antibody purification process based on ReadyToProcess products	28940348
Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom columns	28940349
Purification of a monoclonal antibody using ReadyToProcess columns	28919856
High-throughput process development for design of cleaning-in-place protocols	28984564
Handbooks	
High-throughput process development with PreDictor plates, principles and methods	28940358
Antibody purification, principles and methods	18103746

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

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