MabSelect PrismA

AFFINITY CHROMATOGRAPHY

MabSelect™ PrismA is a next-generation Protein A chromatography resin that offers significantly enhanced alkaline stability and binding capacity for improved process economy in monoclonal antibody (mAb) processing. The resin builds on the proven track record of MabSelect and MabSelect SuRe™ resins in commercial mAb production. In comparison with its predecessors, however, MabSelect PrismA has been improved with an optimized high-flow agarose base matrix and a genetically engineered Protein A-derived ligand, allowing future demands in mAb processing to be met (Fig 1).

Key features of MabSelect PrismA include:

- Enhanced dynamic binding capacity (DBC) allows high mass throughput of processed mAb per resin volume unit.
- Excellent alkaline stability enables efficient cleaning and sanitization using 0.5–1.0 M NaOH for improved process economy and robustness.
- Covered by a comprehensive security of supply program, including dual sources of the agarose base matrix and Protein A ligand.

Since the first commercially approved mAb in 1980s, this class of therapeutic molecules has grown to represent a large part of biopharmaceutical sales. Today, mAbs represent the largest and fastest growing segment of biopharmaceuticals. Over the past 30 years, Protein A chromatography resins and mAbs have followed a highly synergistic evolutionary path, with annual productivity gains in the Protein A step of above 4.5% and increases in Protein A binding capacity of more than 5.5% (1).

With their high affinity for the antibody Fc region, Protein A resins provide an efficient mAb purification platform. The homology of the Fc region allows most of all mAbs to be purified using essentially the same standard approach, thereby significantly reducing process development time. This is an important factor, explaining why nearly all commercially approved mAb manufacturing processes utilize Protein A capture as the initial step in downstream purification. The main characteristics of MabSelect PrismA resin are summarized in Table 1.

Fig 1. MabSelect PrismA is developed to meet future demands in large-scale mAb processing.

Designed for high productivity in mAb capture

Recent advances in upstream procedures are driving up the mass of mAb being sent to downstream purification, thus putting pressure on the Protein A step. With the increasing mAb titers, cell culture feed also contains increased levels of impurities. Concurrently, the high nutrient load present in the cell culture harvest, combined with a low alkaline resistance of the Protein A resin, results in an elevated risk of resin fouling and bioburden issues. For efficient purification of the upstream batch, the resin capacity needs to match the mass of produced mAb. Historically, binding capacity of Protein A resins has lagged behind ion exchange chromatography resins, resulting in the need for larger resin volumes and chromatography column sizes.

Table 1. Main characteristics of MabSelect PrismA

Matrix	Highly cross-linked agarose, spherical
Ligand	Alkaline stabilized Protein A-derived (*E. coli*)
Ligand coupling	Single point attachment
Coupling chemistry	Epoxy
Particle size, d_{50V}*	~ 60 µm
Dynamic binding capacity, Q_{B10}†	~ 80 mg human IgG/mL resin at 6 min residence time ~ 65 mg human IgG /mL resin at 4 min residence time
Recommended maximum operating flow velocity	300 cm/h‡
pH stability, operational§	3–12
pH stability, CIP¶	2–14
Chemical stability	Stable to commonly used aqueous buffers in Protein A chromatography.
Delivery conditions	20% ethanol On request 2% benzyl alcohol (BnOH)

* Median particle size of the cumulative volume distribution.

† DBC at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h (6 min residence time) and 150 cm/h (4 min residence time)in a lab column column at 10 cm bed height for human IgG in PBS buffer, pH 7.4.

‡ Packed in an AxiChrom™ 300 column with 30 cm i.d. at 20 cm bed height, using buffers with the same viscosity as water at 20°C.

§ pH range where resin can be operated without significant change in function.

¶ pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

Due to the enhanced properties of both the Protein A ligand and the base matrix design, MabSelect PrismA offers significantly increased binding capacity compared with its predecessor, MabSelect SuRe LX resin (2). Figure 2 shows DBC of MabSelect PrismA as compared with MabSelect SuRe and MabSelect SuRe LX resins at different residence times. Compared with MabSelect SuRe LX, the optimized base matrix of MabSelect PrismA offers an up to 40% increase in DBC at a residence time of 2.4 min or up to 30% increased binding capacity at 4 min residence time. At 6 min residence time, a binding capacity of ~ 80 mg human IgG /mL resin was observed for MabSelect PrismA, which is 25% higher than for MabSelect SuRe LX.

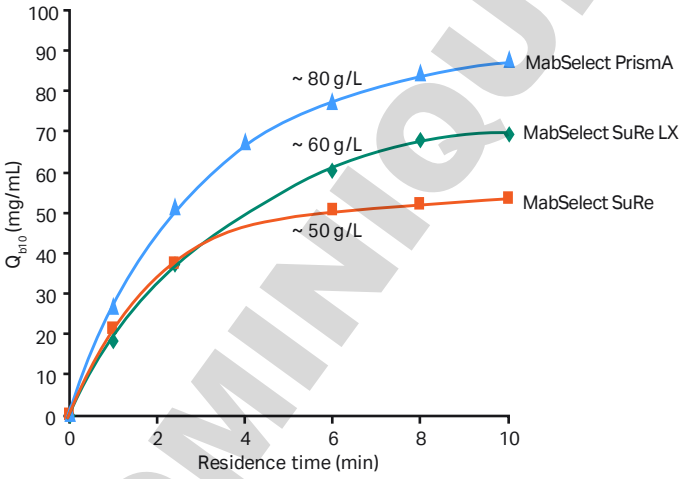

Fig 2. MabSelect PrismA is developed to meet future demands in large-scale mAb processing.

For similar process setups and column sizes, the improved binding capacity of MabSelect PrismA enables significantly increased mass throughput per purification cycle compared with MabSelect SuRe LX (Fig 3). With the increased binding capacity, the productivity of current chromatography columns and systems can be improved without costly capital expenditures, making more efficient use of existing manufacturing footprint. Alternatively, the increased binding capacity can be used to decrease the resin volume (and concomitantly the buffer consumption) required to achieve a given mass throughput (Fig 4).

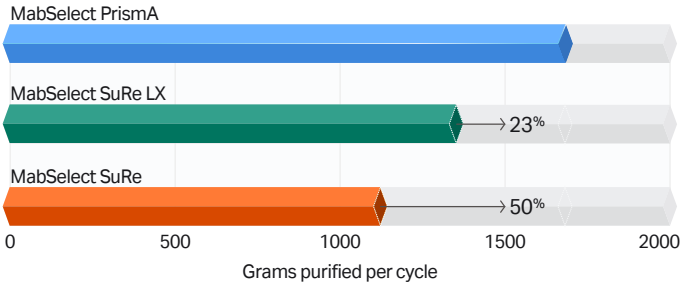

Fig 3. Example of the mass throughput increase enabled by using MabSelect PrismA in comparison with MabSelect SuRe and MabSelect SuRe LX. Here, a fixed column size of 450/200 mm, a mAb titer of 4 g/L, and a 20% safety factor in loading were used.

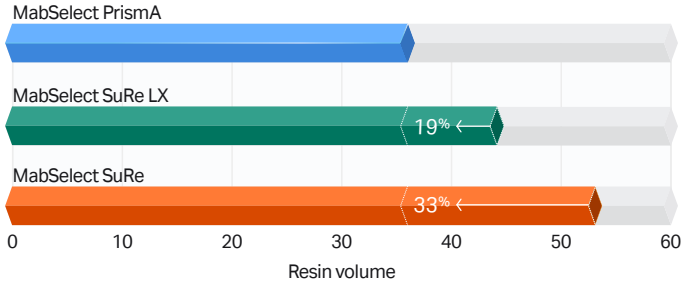

Fig 4. Example of the resin volume savings enabled by using MabSelect PrismA in comparison with MabSelect SuRe and MabSelect SuRe LX. Here, a safety factor of 20% was used in loading of a 2000 L bioreactor harvest with a mAb titer of 4 g/L.

Consistent purification performance

Purification performance of MabSelect PrismA was investigated and found to be similar to its predecessors MabSelect SuRe LX and MabSelect SuRe resins (2). Performance was evaluated with regards to mAb recovery; removal of host cell protein (HCP), host cell DNA (hcDNA), and mAb aggregates; as well as Protein A ligand leakage (Fig 5–10). In addition, MabSelect PrismA exhibits a similar elution pH profile as MabSelect SuRe LX (not shown). Table 2 shows the load to each resin for the results presented in Figures 5–10.

Quantitation of protein A ligand leakage can be performed using the same commercial kits available for MabSelect SuRe and MabSelect SuRe LX.

Table 2. Load on columns

Product	Load mAb1 (g/L resin)	Load mAb2 (g/L resin)	Load mAb3 (g/L resin)
MabSelect PrismA	58	63	51
MabSelect SuRe LX	46	43	40
MabSelect SuRe	39	36	37

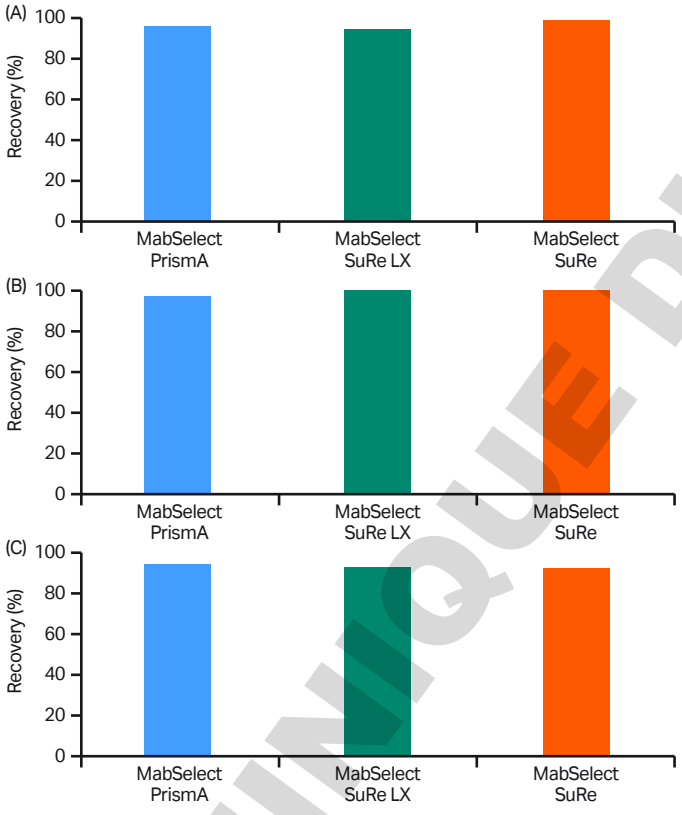

Fig 5. Recovery (%) of (A) mAb1 , (B) mAb2, and (C) mAb3.

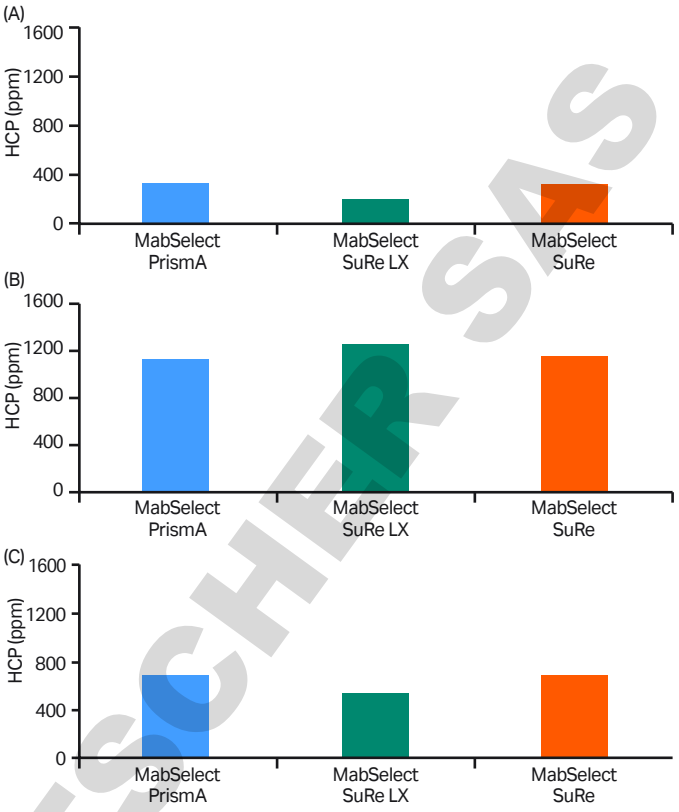

Fig 6. Removal of HCP from (A) mAb1, (B) mAb2, and (C) mAb3. HCP concentration in loaded feed: 1.4 × 105 for mAb1 and 5.7 × 105 ppm for mAb2. Bar graphs show remaining HCP (ppm) in elution pool.

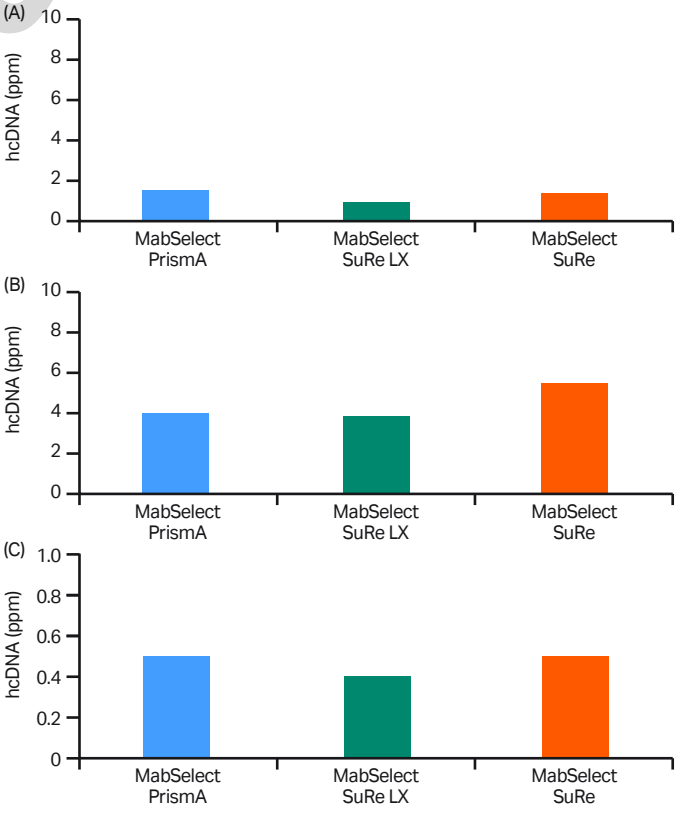

Fig 7. Removal of hcDNA from (A) mAb1, (B) mAb2, and (C) mAb3. DNA concentration in loaded feed: 8037 ppm mAb1 and 6785 ppm mAb2. Bar graphs show remaining hcDNA (ppm) in elution pool.

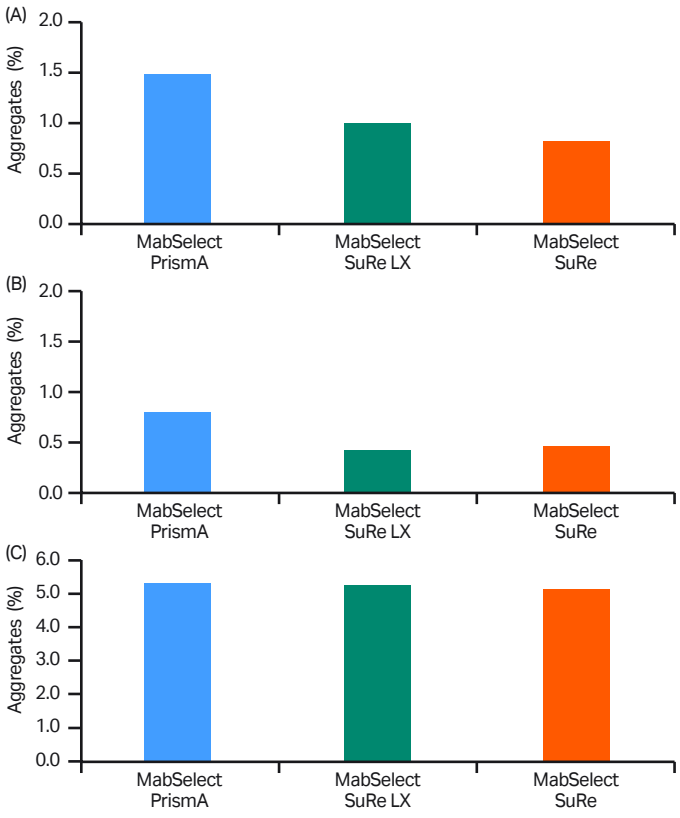

Fig 8. Aggregate removal from (A) mAb1, (B) mAb2, and (C) mAb3. Bar graphs show remaining mAb aggregates (%) in elution pool. The observed increased aggregate level is associated with the higher load.

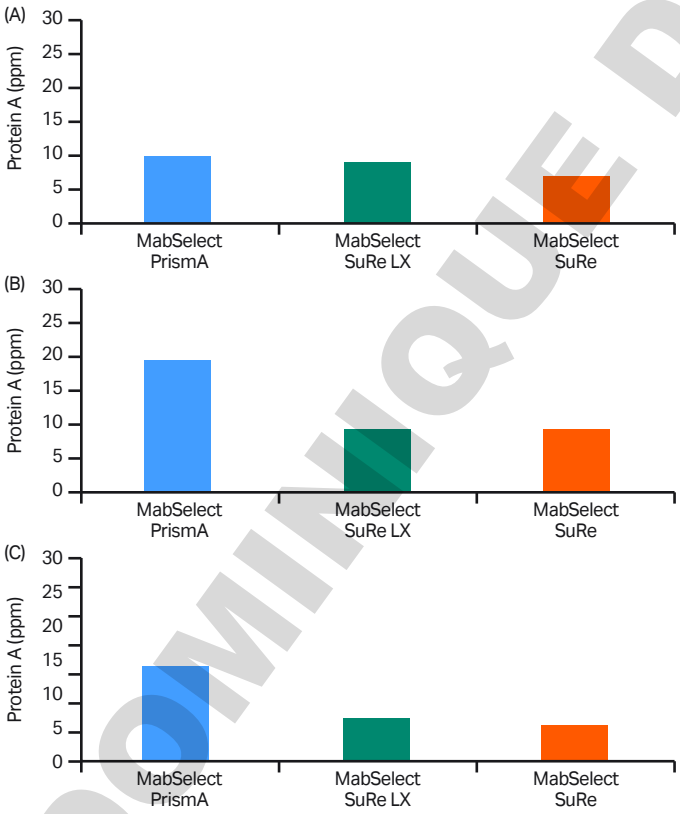

Fig 9. Leached Protein A (ppm) in (A) mAb1, (B) mAb2, and (C) mAb3 elution pools. The slightly increased ligand levels observed with MabSelect PrismA is a result of the higher ligand density and higher ligand molecular weight for this resin compared with the predecessor MabSelect SuRe resins.

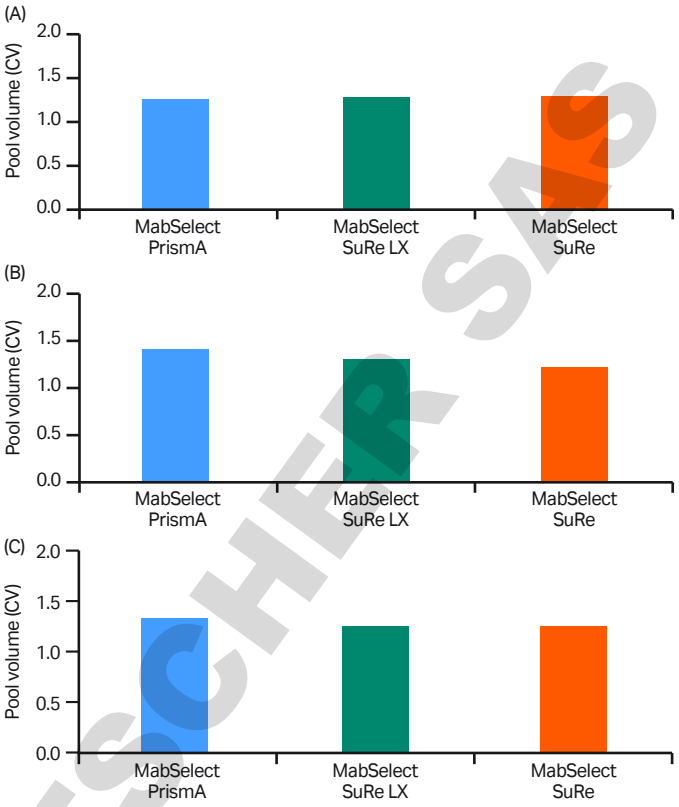

Fig 10. Elution pool sizes from capture of (A) mAb1, (B) mAb2, and (C) mAb3.

MabSelect PrismA and binding to non-Fc regions

The binding of protein A mainly takes place between constant heavy chain domains, C_H2 and C_H3, in the Fc region of the mAb (Fig 11). The protein A ligand in MabSelect PrismA has enhanced binding affinity for the V_H3 sequence located on the variable heavy chain of the Fab region compared to its predecessors, MabSelect SuRe and MabSelect SuRe LX.

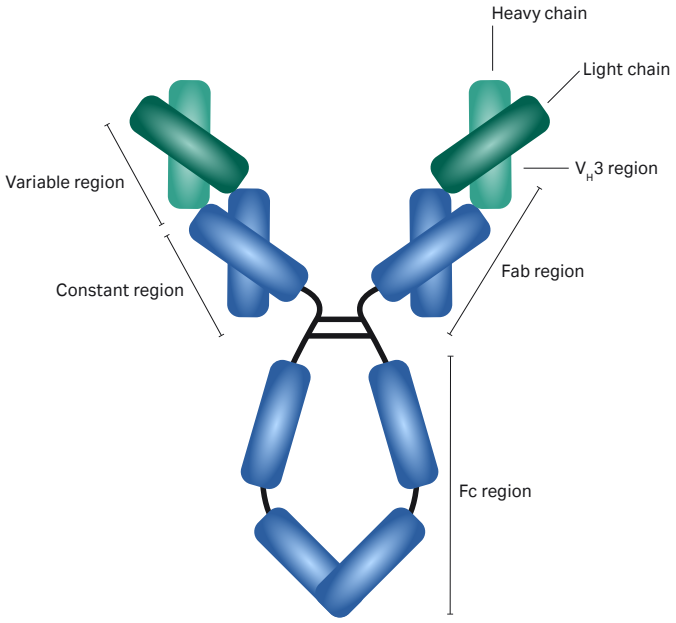

Fig 11. Different interaction points on mAb for alternative purification possibilities.

Table 3. Protein A binding amino acids in V_H3 region of IgG antibodies. Table shows amino acids tolerated and amino acids not tolerated for binding according to literature

V_H3 position	Tolerated	Not tolerated
15 (G)	G, D[a]	
17 (S)	S, A[a]	
19 (R)	R	K[b], S[b], A[a], Q[a]
57 (K/T)	K, R, T, P, S	I, E, A[b]
59 (Y)	Y	V[b]
64 (K)	K, R, T, E	
65 (G)	G	D, S[c]
66 (R)	R	
68 (T)	T, A[b]	S[b]
70 (S)	S, T	
81 (Q)	Q	E[c]
82a (N)	N	D[b], S[b,c], A[a]
82b (S)	S,G,N[b]	D

a = Data from Fridy *et al.* Engineered high-affinity nanobodies recognizing staphylococcal Protein A and suitable for native isolation of protein complexes. *Anal. Biochem.* **477**, 92–94 (2015). https://doi.org/10.1016/j.ab.2015.02.013

b = Data from Henry *et al.* A rational engineering strategy for designing Protein A-binding Camelid single-domainantibodies. *PLoS ONE* **11(9)**, 1–18 (2016). https://doi.org/10.1371/journal.pone.0163113

c = Data from Ollier *et al.* Single-step Protein A and Protein G avidity purification methods to support bispecific antibody discovery and development. *mAbs* **11(8)**, 1464–1478 (2019). https://doi.org/10.1080/19420862.2019.1660564

MabSelect, MabSelect SuRe, and MabSelect PrismA interact to different degrees with V_HH fragments. These V_HH fragments are unique to Camelids and are single chain antibody mimics of the V_H3 region of IgG, with mutations at different positions. The data in Table 4 shows different amino acid sequences of V_HH fragments where mutations have been introduced in selected positions known to have high variability. The table demonstrates that MabSelect SuRe can interact with the V_H3 region in some cases, but MabSelect and MabSelect PrismA are less sensitive to sequence variation. The results also show that MabSelect PrismA has a similar binding pattern for the tested V_HH sequences as the recombinant protein A ligand present in MabSelect and MabSelect Xtra™ resins, which provides new opportunities for purification of increasing molecular diversity of antibody fragments.

Purification performance of antibody fragments

The binding capacity for V_HH was determined by frontal analysis and elution pH using a pH gradient from pH 6 to pH 3 on MabSelect PrismA (Table 5).

Table 5. Binding capacity and elution pH of a V_HH fragment using MabSelect PrismA

Entity	Q_{B10} (g/L)	Q_{B10} (mM)	Elution pH
V_HH	42	2.7	3.8

A 150 mL volume of clarified *E. coli* harvest containing 0.2 g/L VHH (30 g of V_HH/L resin) was loaded at 6 min residence time to a MabSelect PrismA column (CV 1 mL). The V_HH fragment was eluted in 50 mM sodium acetate, pH 3.5. Results showed that a 4.3 $\log_{10}$ reduction of HCP was achieved. The leached protein A ligand concentration was 10 ppm (Table 6).

Table 6. HCP and leached protein A ligand concentration in the purification of a V_HH fragment from *E. coli* on MabSelect PrismA resin

Sample	Pool volume (CV)	eHCP (ppm*)	Leached ligand (ppm†)
Load	NA	4 252 500	NA
Elution pool	1	200	10

* Nanogram (ng) of eHCP/mg V_HH
† ng of leached ligand/mg V_HH

Table 4. Interaction of MabSelect, MabSelect SuRe, and MabSelect PrismA with V_HH fragments

V_HH sequence	MabSelect	MabSelect SuRe	MabSelect PrismA
T57	Yes	Yes	Yes
T57K	Yes	No	Yes
T57I	No	No	No
T57E	No	No	No
N82aT, S82bD	No	No	No
T57P	Yes	No	Yes
T57S	Yes	No	Yes
T57R	Yes	No	Yes
K64R	Yes	Yes	Yes
K64T	Yes	Yes	Yes
K64E	Yes	N/A	Yes
G65D	No	No	No
S70T	Yes	No	Yes
S82bD	No	No	No
S82bG	Yes	Yes	Yes

Rigid, highly cross-linked agarose base matrix allows for high flow velocities

MabSelect PrismA features a highly cross-linked agarose base matrix that allows for high flow velocities in process-scale operations. This permits high-throughput purification of mAbs and other antibody molecules from large feed volumes. Figure 12 shows pressure-flow curves for MabSelect PrismA, enabling flow velocities as high as 300 cm/h, as verified in large BioProcess™ columns. For more information on packing MabSelect PrismA in large-scale columns, *see* reference 3.

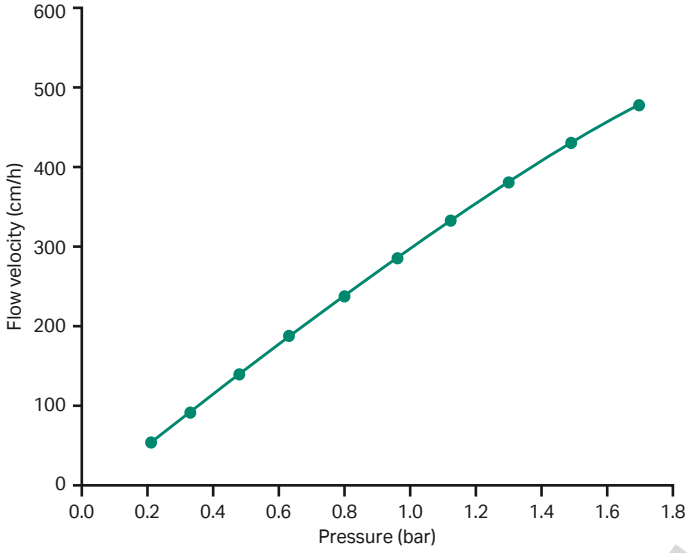

Fig 12. Pressure-flow curve for MabSelect PrismA packed in a AxiChrom 300 column to a 20 cm bed height, generated at a temperature of 20°C. Recommended maximum operating flow velocity is 300 cm/h.

Improved alkaline stability enables new standards for cleaning and sanitization

Due to its efficacy, low cost, as well as ease of detection, removal, and disposal, sodium hydroxide (NaOH) has gained popularity for cleaning and sanitization in the bioprocessing industry. Commonly, 1 M NaOH is used for cleaning and sanitization of chromatography columns and resins. Apart from its ability to inactivate endotoxins and many microorganisms, NaOH can remove bound proteins, nucleic acids, and lipids from the resins. Fouled resins can increase cross-contamination risk and also negatively impact DBC over time (4).

Historically, Protein A chromatography resins have been sensitive to NaOH due to the limited alkaline stability of the protein ligand. Later generations of Protein A resins exhibit improved alkaline stability and have recommended cleaning procedures using 0.1 M NaOH, with the option of occasional use of 0.5 M NaOH. Given that Protein A capture is the first purification step and therefore exposed to the crudest feed and highest impurity loads, the use of weak CIP agents has been highlighted as a challenge and risk with using this technique.

With enhanced alkaline stability of its Protein A ligand, MabSelect PrismA meets this challenge (5). The ligand was developed using high-throughput screening to identify amino acids sensitive to alkaline degradation and substitution of these amino acids with more stables ones. The final construct constitutes a hexamer of the engineered domain. Highly pure ligand is immobilized to the agarose base matrix via a chemically stable thioether linkage. The enhanced alkaline stability enables efficient cleaning of the resin using 0.5 to 1.0 M NaOH over many purification cycles.

Figure 13 shows relative remaining DBC of MabSelect PrismA as compared with MabSelect SuRe and MabSelect SuRe LX over repeated CIP cycles. As shown, MabSelect PrismA retains more than 90% of its initial DBC after more than 150 cycles with 1.0 M NaOH, while only 50% of the initial DBC of MabSelect SuRe LX remains after an equivalent number of CIP cycles. Using 0.5 M NaOH, MabSelect PrismA retains more than 93% of its initial DBC after 300 cycles (6).

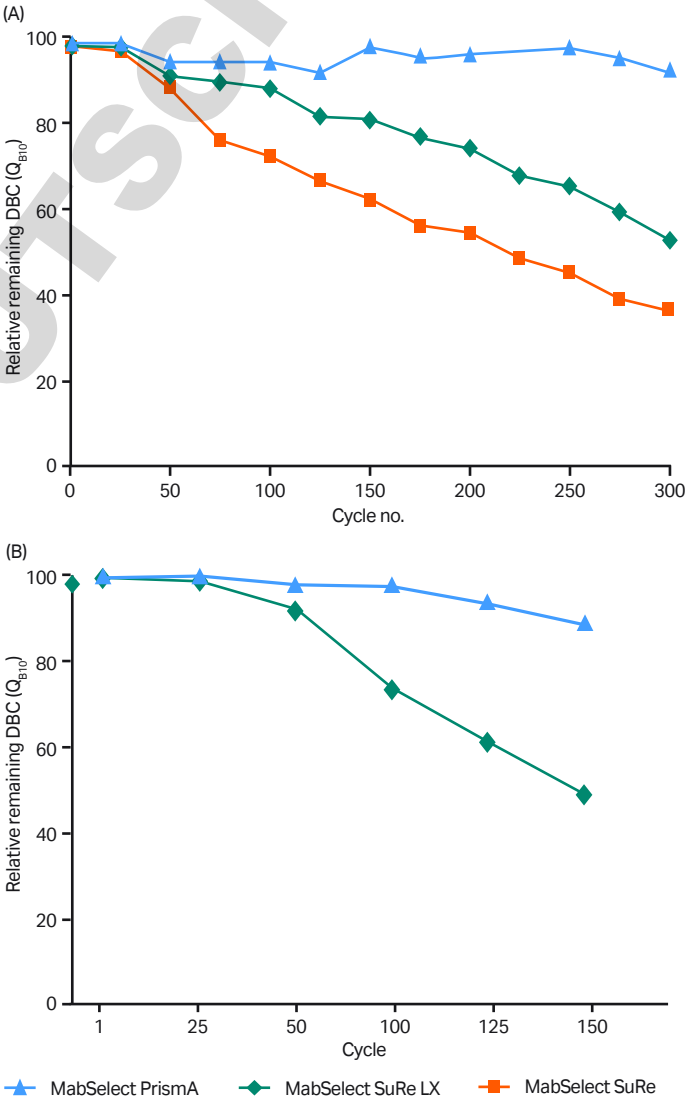

Fig 13. Relative remaining DBC using (A) 0.5 M NaOH and (B) 1.0 M NaOH as cleaning agent at 15 min contact time between cycles.

Addressing spore-forming bacteria for improved bioburden control

The increased alkaline stability helps maintain bioburden growth control as well as decrease process-deviation risks and associated costs. Advancements in the design of sanitization methods have resulted in efficient removal also of spore-forming bacteria. Studies have shown that the oxidizing agent peracetic acid (PAA) is highly efficient in reducing bacterial spores (5). A more than 6.0 $\log_{10}$ reduction of *Bacillus subtilis* spores was obtained already after a 15 min treatment with 30 mM PAA or after a 30 min treatment with 20 mM PAA (Fig 14).

PAA is a strong oxidizing agent that might be expected to have a negative impact on the performance of chromatography resins, in particular those containing protein ligands. However, studies indicate that PAA at limited contact (2 to 3 times during the lifetime of the MabSelect SuRe resin) can provide a good safeguard against spore-forming bacteria, without negatively impacting resin performance or lifetime (7). Studies are on-going to develop a similar recommendation for MabSelect PrismA.

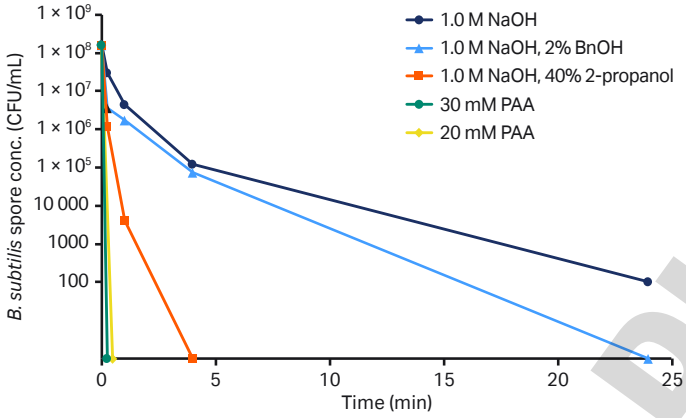

Fig 14. *B. subtilis* spore challenge test in a 50% MabSelect SuRe resin slurry, showing sporicidal effects of 30 and 20 mM PAA already at 15 to 30 min contact time.

Proven technology for seamless scale-up

Protein A affinity chromatography is an established method for fast and low-risk capture of mAb molecules. The method is scalable from microliter levels to tens of thousands of liters of cell culture feed. Defined conditions for loading, wash, elution, and CIP can be verified and optimized using small prepacked HiScreen™ columns (4.7 mL). Together with a chromatography system, such as ÄKTA™ avant or ÄKTA pure, the HiScreen column format helps develop an efficient and robust separation method. Further development and optimization for process scale-up can be conducted using the well-established user-packed AxiChrom or prepacked ReadyToProcess™ columns.

Adds flexibility to production

MabSelect PrismA prepacked in ReadyToProcess columns provides sufficient capacity for purification of mAbs even from large quantities of cell culture feed (Fig 15). With a DBC reaching 80 mg/mL, MabSelect PrismA prepacked in the ReadyToProcess 57 L (600/200 mm) column enables processing of high-titer bioreactor harvests of up to 2000 L in a single working day. The disposable ReadyToProcess columns offer the benefits of single-use technology with regard to fast setup, reduced cross-contamination risk, and high flexibility for quick adaption of production scales to market needs.

For reuse columns, the high productivity of MabSelect PrismA offers a reduced facility footprint and processing time. The enhanced alkaline stability of MabSelect PrismA also provides opportunities for mAb processing using continuous chromatography, where the extended exposure to high impurity loads causes increased risks of bioburden growth. The high alkaline stability of the resin allows for significantly improved control of both cleaning and sanitization under the more challenging conditions of continuous chromatography setups.

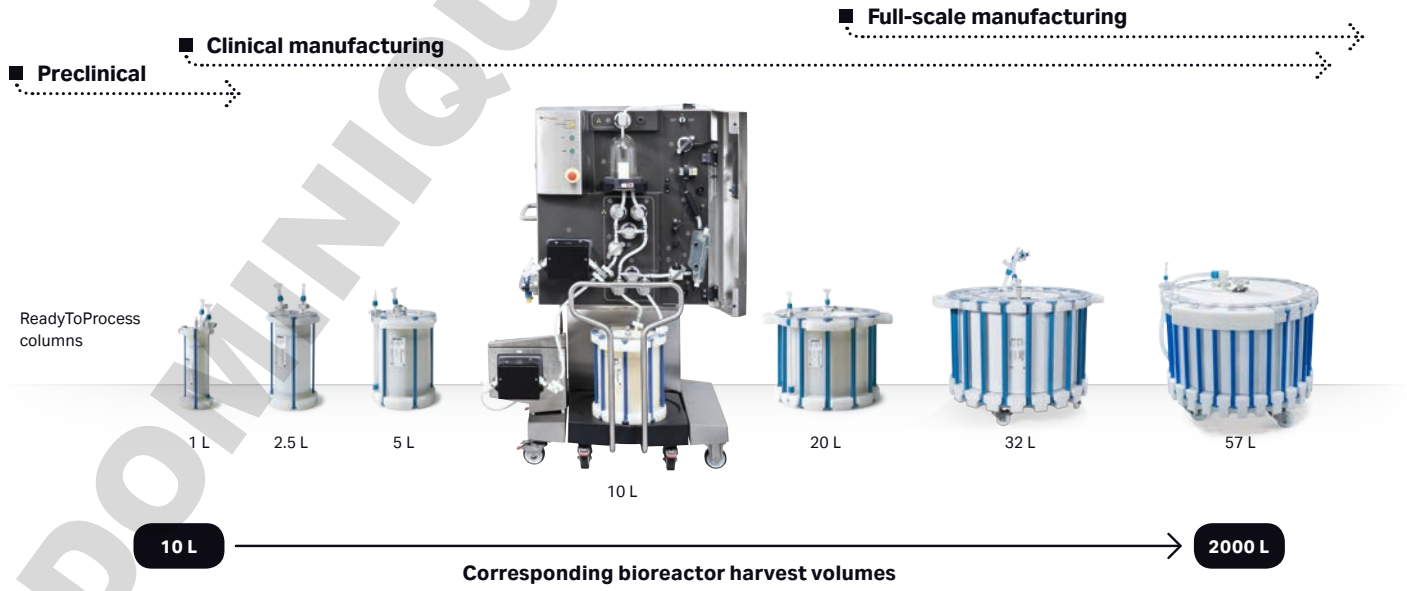

Fig 15. The enhanced binding capacity of MabSelect PrismA enables use of smaller columns. Prepacked ReadyToProcess columns support mAb purification from high-titer bioreactor harvests of up to 2000 L.

Supply chain stability

The complex nature of biopharmaceuticals makes manufacturing a challenge, in which a consistent, high-quality end product is depending on the use of equally consistent, high-quality manufacturing components. Cytiva has made significant investments in supply stability to ensure security of supply of MabSelect PrismA. For critical raw materials, such as the Protein A ligand and the agarose base matrix, dual sourcing has been validated and implemented. Protein A ligand production has been partially in-sourced to ensure supply security (the remaining ligand is produced by a contract manufacturer using our developed process).

For emergency preparedness, large investments have been made and efforts implemented to minimize risk and impact of supply interruptions in our manufacturing. Cytiva's chromatography product manufacturing has been certified to ISO22301 business continuity management. As an extra precaution in the event of a disruption of our supply chain, a strategic reserve of chromatography resins used in approved manufacturing processes has been created to ensure coverage during the recovery phase (8).

BioProcess resin support

MabSelect PrismA belongs to the BioProcess family of products that is developed and supported for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and regulatory support files (RSF) to assist process validation and submission to regulatory authorities. In addition, Fast Trak training and education provide high-level, hands-on training in all key aspects of process development and manufacturing.

Resin storage

Store unused MabSelect PrismA 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% BnOH.

After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use. In case of bioburden incidents, treatment with PAA can be performed to reduce spore-forming bacteria.

References

1. Bolton G. R. and Mehta, K. K. The role of more than 40 years of improvement in Protein A chromatography in the growth of the therapeutic antibody industry. *Biotechnol. Prog.* **32**, 1193–1202 (2016). https://doi.org/10.1002/btpr.2324
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3. Article: Verified chromatography column packing methods for MabSelect PrismA resin.
4. Zhang J. *et al.* Maximizing the functional lifetime of Protein A resins. *Biotechnol. Prog.* **33**, 708–715 (2017). https://doi.org/10.1002/btpr.2448
5. Whitepaper: Efficient cleaning-in-place methods for protein-based antibody affinity chromatography resins. Cytiva. KA1619220218WP (2018).
6. Application note: Lifetime performance study of MabSelect PrismA during repeated cleaning-in-place cycles. GE Healthcare, KA1061080618AN (2018).
7. Application note: Impact of sporicidal agent on MabSelect SuRe Protein A resin lifetime. GE Healthcare, 29262168, Edition AA (2017).
8. Whitepaper: Security of supply for chromatography media. GE Healthcare, 29085383, Edition AA (2014).

Ordering information

Product	Size	Product code
MabSelect PrismA	25 mL	17549801
MabSelect PrismA	200 mL	17549802
MabSelect PrismA	1 L	17549803
MabSelect PrismA	5 L	17549804
MabSelect PrismA	10 L (made to order)	17549805
MabSelect PrismA in BnOH	1 L (made to order)	17549823
MabSelect PrismA in BnOH	5 L (made to order)	17549824
MabSelect PrismA in BnOH	10 L (made to order)	17549825
HiTrap™ MabSelect PrismA	1 × 1 mL	17549851
HiTrap MabSelect PrismA	5 × 1 mL	17549852
HiTrap MabSelect PrismA	1 × 5 mL	17549853
HiTrap MabSelect PrismA	5 × 5 mL	17549854
HiScreen MabSelect PrismA	1 × 4.7 mL	17549815
PreDictor™ MabSelect PrismA	6 µL	17549830
PreDictor MabSelect PrismA	20 µL	17549831
PreDictor MabSelect PrismA	50 µL	17549832
PreDictor RoboColumn™ MabSelect PrismA	200 µL	17549833
PreDictor RoboColumn MabSelect PrismA	600 µL	17549834
ReadyToProcess MabSelect PrismA	column 1.0 L (80/200)	17549861
ReadyToProcess MabSelect PrismA	column 2.5 L (126/200)	17549862

Related literature

Data files	Product code
MabSelect	18114994
MabSelect Xtra	11001157
MabSelect SuRe	11001165
MabSelect SuRe LX	28987062
MabSelect SuRe pcc	28987062

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