Capacity and performance of MabSelect PrismA protein A chromatography resin

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Capacity and performance of MabSelect™ PrismA protein A chromatography resin

MabSelect™ PrismA is a protein A resin of which both the ligand and the base matrix have been optimized. The resin exhibits improved capacity over its predecessor MabSelect SuRe™ products, while maintaining purification performance. This application note demonstrates the binding capacity of MabSelect PrismA in comparison with its predecessor products for both polyclonal and monoclonal antibodies.

Introduction

As the area of therapeutic monoclonal antibodies (mAbs) is growing rapidly, much effort is put on intensifying processes to increase productivity and cost-efficiency. Ever-increasing titers in upstream production demand increased efficiency in downstream purification. To prevent the initial downstream protein A capture step from becoming rate-limiting for the manufacturing process, high efficiency of this step is crucial.

MabSelect PrismA is built on the heritage of alkaline-stabilized MabSelect products. However, with enhanced properties of both the agarose base matrix and the protein A ligand, MabSelect PrismA provides a significantly increased capacity compared with its predecessor resins. In this work, the binding capacity and the purification performance of MabSelect PrismA in comparison with its predecessors are evaluated.

Materials and methods

Determination of static binding capacity (SBC)

For determination of SBC, 96-well filter plates were each filled with 2 µL resin/well of either MabSelect PrismA, MabSelect SuRe LX, or MabSelect SuRe resin. Purified monoclonal antibody (mAb1) diluted in PBS to 3.92, 3, 1.96, 0.98, 0.49, and 0.245 mg/mL was used as sample. A sample volume of 200 µL was added to each well and the plates were incubated on a shaker for 2.5 h. Eluates were collected in a UV plate and measured at 280 and 254 nm. Sample pool of mAb1 purified on MabSelect SuRe LX was used as standard. SBC (Q) of the resins was calculated according to the following equation:

$$Q = V_{sample} (C_0 - C_{eq})/V_{resin}.$$

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C_0 is the applied sample concentration.

C_{eq} is the equilibrium concentration.

V_{sample} is the sample volume.

V_{resin} is the resin volume.

Q was plotted against the equilibrium concentration, and the curves were fitted to the Langmuir isotherm and maximum capacity and dissociation constant were calculated according to the following equation:

$$Q = (Q_{max} \times C_{eq})/(K_d + C_{eq})$$

Were

Q is the total amount of protein adsorbed per mL resin.

Q_{max} is the maximum capacity.

C_{eq} is the protein concentration in the supernatant.

K_d the dissociation constant.

Determination of dynamic binding capacity (DBC)

For determination of DBC, human IgG (gammanorm™, Octapharma) as well as two monoclonal antibodies (mAb1 and mAb2) were used as samples. For the human IgG, MabSelect PrismA, MabSelect SuRe LX, and MabSelect SuRe resins were each packed in HiScreen™ columns to a bed height of 10 cm. For the monoclonal antibodies, the resins were each packed in Tricorn™ 5/100 columns. All columns were operated on an ÄKTA™ pure system controlled by UNICORN™ software. Dynamic binding capacity at 10% breakthrough (Q_{B10}) was determined by frontal analysis according to the methods outlined in Table 1.

Determination of purification performance

For determination of purification performance, MabSelect PrismA was packed in a Tricorn 5/100 column. The study was conducted with three different mAbs (mAb1, mAb2, and mAb3). The used method is outlined in Table 2.

Table 1. Methods for determination of DBC

Sample	Human IgG (~ 5 mg/mL)	mAb1/mAb2 (4.3/2.2 mg/mL)
Equilibration	5 CV PBS, pH 7.4 (2 mL/min)	5 CV PBS, pH 7.4 (1 mL/min)
Sample load	Until ~ 20% breakthrough (residence time 1–10 min; 4,65–0,465 mL/min)	Until > 80% breakthrough (residence time 2.4, 4, and 6 min, 0.83–0.33 mL/min).
Wash	5 CV PBS, pH 7.4 (1 mL/min)	10 CV PBS, pH 7.4 (1 mL/min)
Elution	5 CV 0.1 M acetic acid, pH 3.0 (1 mL/min)	5 CV 50 mM sodium acetate, pH 3 (0.4 mL/min)
Cleaning in place	0.1 M NaOH	0.5 M NaOH (contact time 15 min)
Re-equilibration	5 CV , PBS, pH 7.4 (0.77 mL/min)	5 CV PBS, pH 7.4 (1 mL/min)

CV = column volumes, PBS = 20 mM phosphate, pH 7.4 + 150 mM NaCl.

Table 2. Method for determination of purification performance

Sample	mAb1 (4.3 mg/mL)	mAb2 (2.2 mg/mL)	mAb3 (1.6 mg/mL)
Equilibration	5 CV 20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	5 CV 20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	5 CV 20 mM Tris-acetic acid, pH 7.4 + 150 mM NaCl
Sample load	Until 80% of Q_{B10} (residence time 6 min)	Until 80% of Q_{B10} (residence time 6 min)	Until 80% of Q_{B10} (residence time 5 min)
Wash 1	5 CV 20 mM sodium phosphate, pH 7 + 500 mM NaCl	5 CV 20 mM sodium phosphate, pH 7 + 500 mM NaCl	3 CV 20 mM Tris-acetic acid, pH 7.4 + 150 mM NaCl
Wash 2	1 CV 50 mM sodium acetate, pH 6	1 CV 50 mM sodium acetate, pH 6	3 CV 50 mM sodium acetate, pH 5.5 + 1 M NaCl
Wash 2	N/A	N/A	3 CV 50 mM sodium acetate, pH 5.5
Elution	3 CV 50 mM sodium acetate, pH 3.5	3 CV 50 mM sodium acetate, pH 3.5	3 CV 50 mM sodium acetate, pH 3.6
Strip	2 CV 100 mM acetic acid, pH 2.9	2 CV 100 mM acetic acid, pH 2.9	3 CV 1 M acetic acid
Rinse	N/A	N/A	3 CV 20 mM Tris-acetic acid, pH 7.4 + 150 mM NaCl
Cleaning in place	0.1 M NaOH, 3 CV (15 min contact time)	0.1 M NaOH, 3 CV (15 min contact time)	0.1 M NaOH, 3 CV
Re-equilibration	3 CV 20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	3 CV 20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	5 CV 20 mM Tris-acetic acid, 150 mM NaCl pH 7.4

CV = column volumes. N/A = not applicable.

Analyses

Concentration of mAb in the cell culture supernatant was measured by surface plasmon resonance (SPR) using the Biacore™ T100 system.

Aggregate clearance was determined by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column, using PBS as mobile phase. Peaks were integrated and the percentage of aggregates was determined.

Host cell protein (HCP) content was analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies Inc.) and Gyrolab™ workstation (Gyros AB).

Protein A content was determined using a commercially available ELISA kit (Repligen Corp.). The MabSelect SuRe ligand was used as reference for MabSelect SuRe and MabSelect SuRe LX, and the MabSelect PrismA ligand was used as a reference for MabSelect PrismA.

Host cell DNA (hcDNA) was determined by an in-house qPCR method, using primers and probes as described previously (1). Samples were automatically prepared using a MagMax™ Express 96-deepwell magnetic particle processor and PrepSEQ™ Residual DNA Sample Prep kit. Real-time PCR was performed using the StepOnePlus™ system (Thermo Fisher Scientific), and by using the StepOne™ software for evaluation.

Results

Static binding capacity

Adsorption isotherms were determined in 96-well filter plates, and the static binding capacity (Q) was calculated and plotted versus the equilibrium concentration (C_{eq}) (Fig 1). The isotherm data was fitted to the Langmuir isotherm (not shown), and maximum capacity (Q_{max}) and dissociation constant (K_d) were calculated. The results are summarized in Table 3.

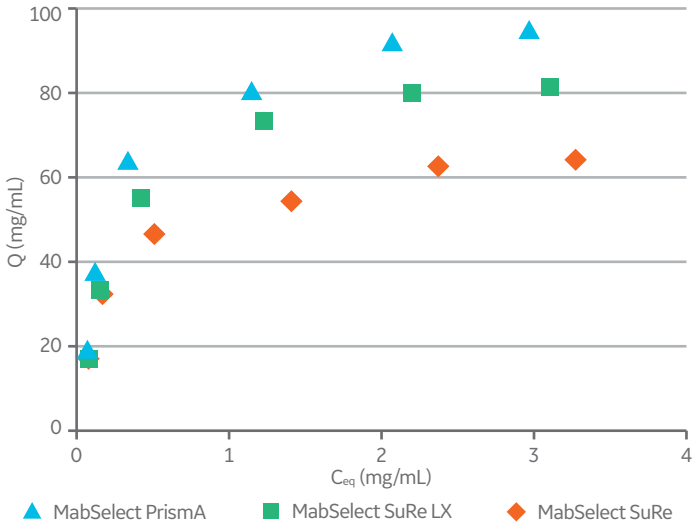

Fig 1. Adsorption isotherms of MabSelect PrismA, MabSelect SuRe LX, and MabSelect SuRe resins.

Table 3. Maximum capacity (Q_{max}) and dissociation constant (K_d) calculated out from the Langmuir

Resin	Q_{max} (mg/mL)	K_d
MabSelect PrismA	99.9	0.2
MabSelect SuRe LX	89.6	0.3
MabSelect SuRe	65.7	0.2

Dynamic binding capacity

As shown in Figure 2A for a human IgG antibody, MabSelect PrismA exhibits a significantly increased DBC compared with its predecessor products. The optimized base matrix of MabSelect PrismA offers an up to a 40% increase in DBC at a residence time of 2.4 min, or up to a 30% increase in binding capacity at 4 min residence time compared with MabSelect SuRe LX. At 6 min residence time, a binding capacity of 77 mg human IgG/mL resin was observed for MabSelect PrismA, which is 27% higher than for MabSelect SuRe LX (60.5 mg human IgG/mL) and 53% higher than for MabSelect SuRe resin (50.3 mg human IgG/mL resin). Figures 2B and 2C show DBC of MabSelect PrismA versus MabSelect SuRe and MabSelect SuRe LX for two monoclonal antibodies (mAb1 and mAb2). As shown, the DBC of MabSelect PrismA for the included mAbs is higher at all three residence times (2.4, 4, and 6 min) compared with MabSelect SuRe and MabSelect SuRe LX resins. Although the exact value in mg/mL resin is specific for the target molecule, the percent increase in DBC with increasing residence time is similar for human IgG.

Purification performance

Purification performance of MabSelect PrismA was investigated for three mAbs (mAb1, mAb2, and mAb3) and found to be similar to its predecessor MabSelect SuRe LX and MabSelect SuRe resins. Performance was evaluated with regards to mAb recovery; removal of HCP, hcDNA, and mAb aggregates; as well as protein A ligand leaching (Fig 2–7). MabSelect PrismA exhibits a similar elution pH as MabSelect SuRe LX (not shown). Table 4 shows the load (g mAb/L resin) to each resin for the results presented in Figures 3–8.

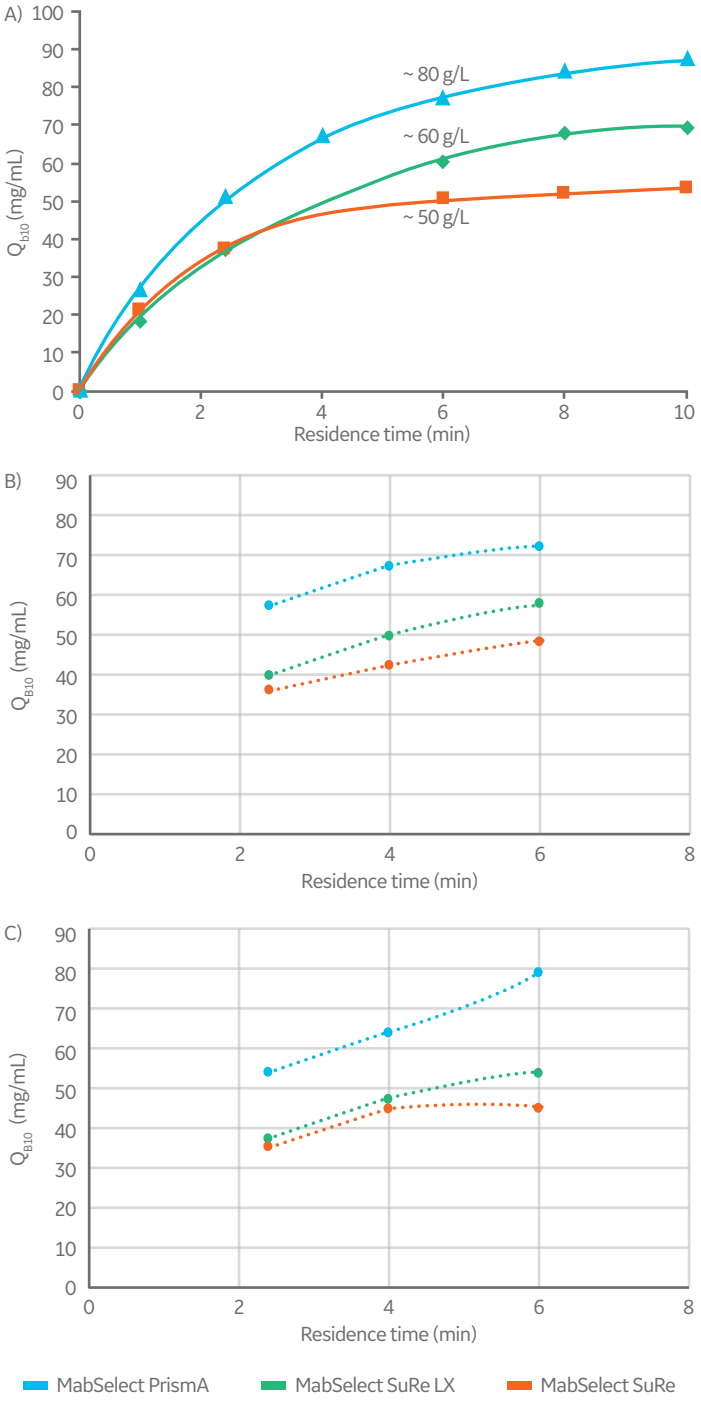

Fig 2. DBC of MabSelect PrismA as compared with MabSelect SuRe and MabSelect SuRe LX resins at different residence times for (A) human IgG, (B) mAb1, and (C) mAb2.

Table 4. Load on columns

Resin	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

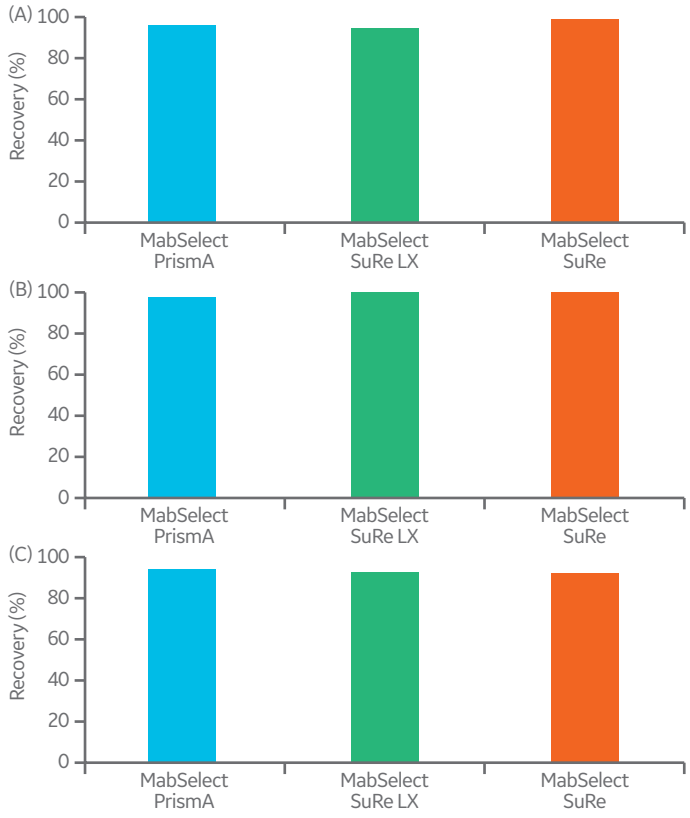

Fig 3. Recovery (%) of (A) mAb1, (B) mAb2, and (C) mAb3.

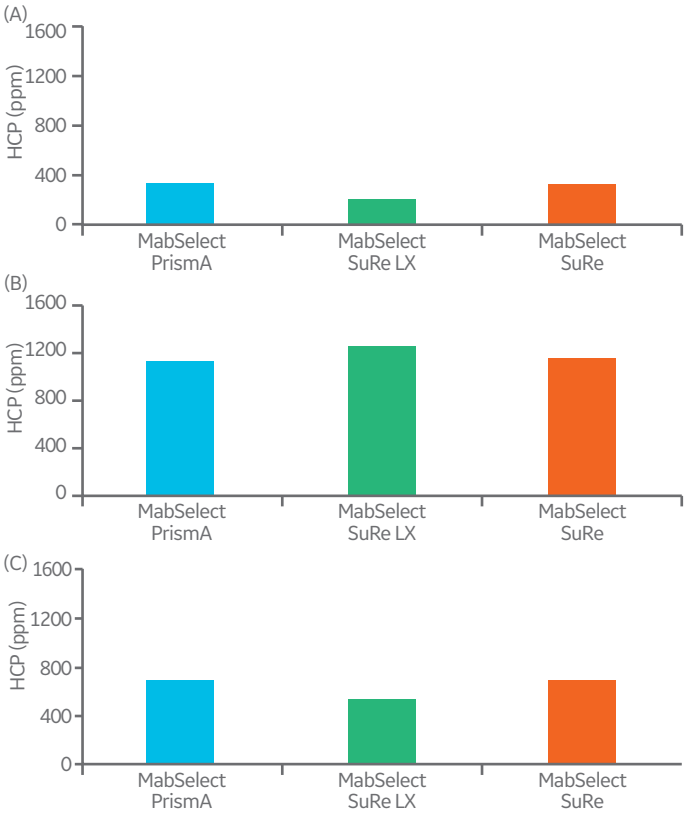

Fig 4. Removal of HCP from (A) mAb1, (B) mAb2, and (C) mAb3. HCP concentration in loaded was 1.4×10^5 for mAb1, 5.7×10^5 ppm for mAb2, and 11.7×10^5 for mAb3. Bar graphs show remaining HCP in elution pool.

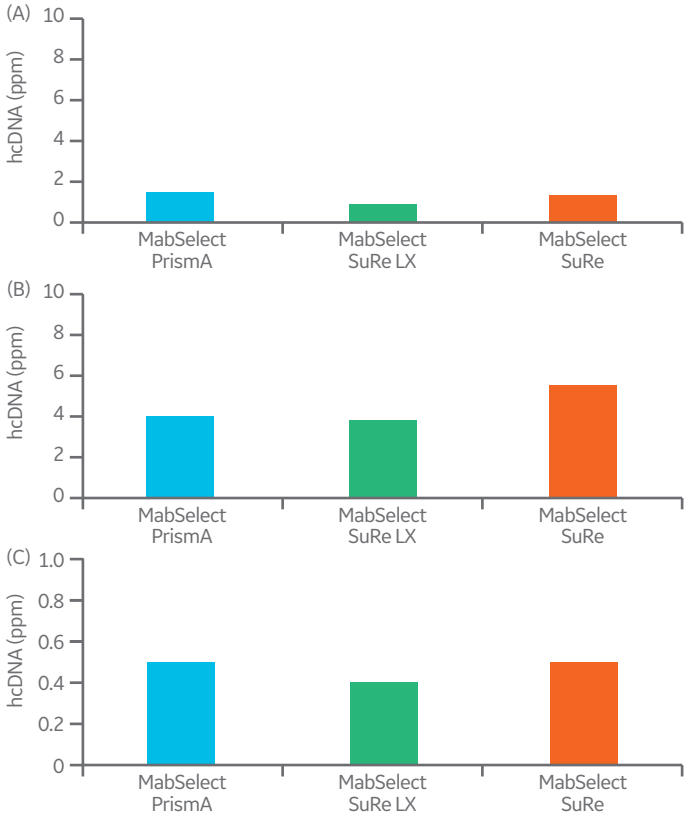

Fig 5. Removal of hcDNA from (A) mAb1, (B) mAb2, and (C) mAb3. DNA concentration in loaded was 8037 ppm for mAb1, 6785 ppm for mAb2, and 38200 ppm for mAb3. Bar graphs show remaining hcDNA in elution pool.

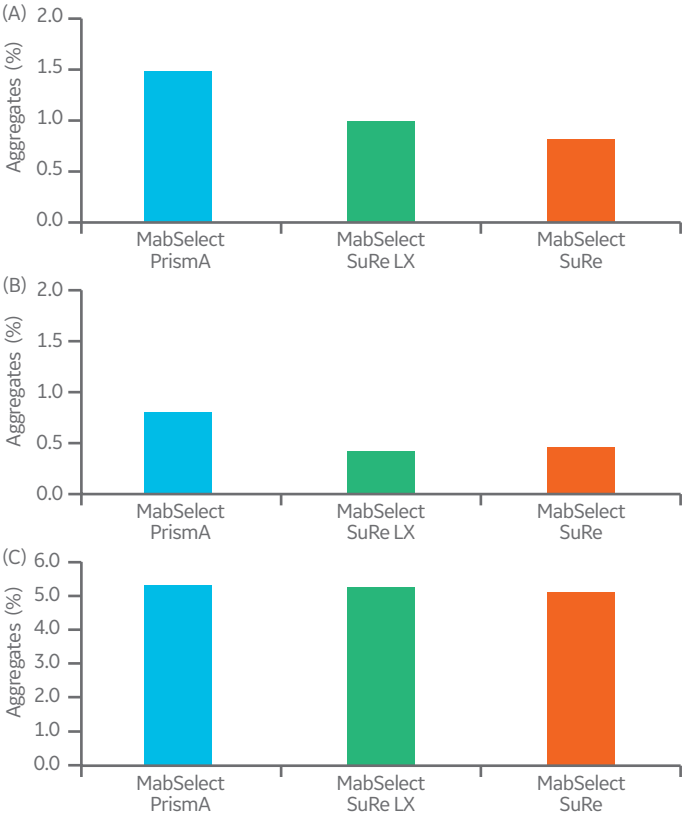

Fig 6. Aggregate removal from (A) mAb1, (B) mAb2, and (C) mAb3. Bar graphs show remaining mAb aggregates in elution pool. The increased aggregate level observed with MabSelect PrismA can be associated with the higher load.

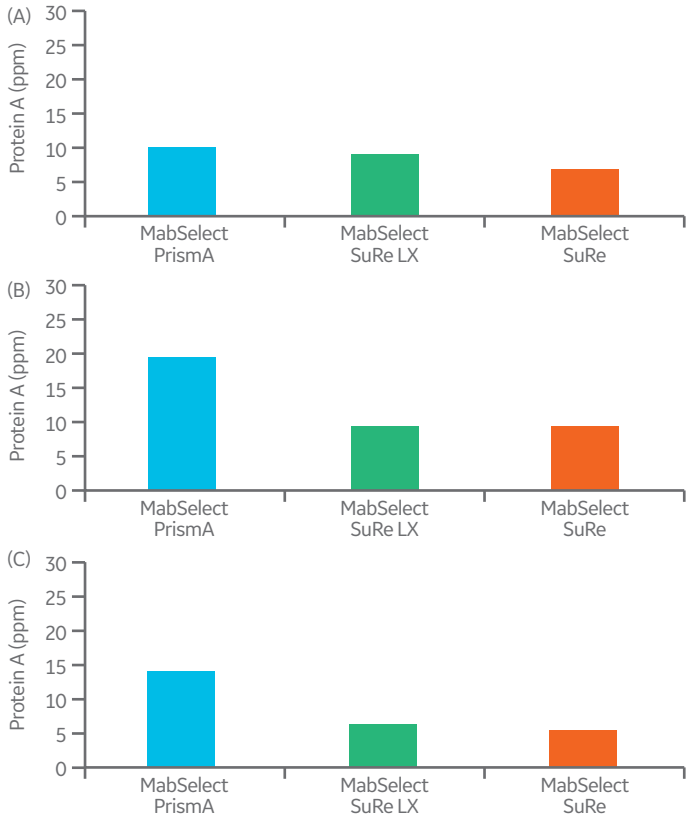

Fig 7. Leached protein A in elution pool of (A) mAb1, (B) mAb2, and (C) mAb3. The slightly increased ligand levels observed with MabSelect PrismA is a result of the higher ligand density and higher ligand molecular weight for this resin compared with the predecessor MabSelect SuRe resins. Remaining leached protein A ligand will be removed in subsequent purification steps (results not shown).

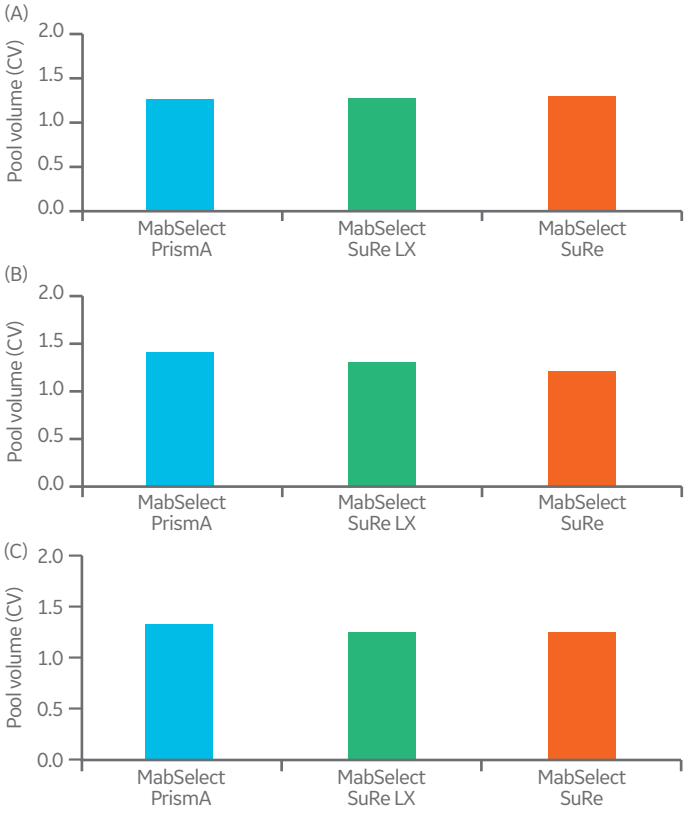

Fig 8. Elution pool sizes from capture of (A) mAb1, (B) mAb2, and (C) mAb3.

Binding capacity and purification performance of four antibody constructs

The performance of MabSelect PrismA was evaluated in an independent end-user evaluation. Binding capacity, product quality, and recovery of four Fc-based antibody constructs (Molecules 1, 2, 3, and 4) were compared between the use of MabSelect PrismA and MabSelect SuRe resins in the capture step. The experiments were performed in 2 mL columns (10 cm bed height) at a residence time of 6 min.

Optimal elution pH was determined for Molecule 4 and was found to be 4.1 for MabSelect PrismA and 3.9 for the MabSelect SuRe resin (Fig 9). For all included antibody constructs, the DBC of MabSelect PrismA was determined to be 73% to 101% higher compared with MabSelect SuRe resin (Fig 10). Purification results for MabSelect PrismA show that antibody recovery and quality in terms of amount of monomer were comparable with the predecessor resin (Fig 11).

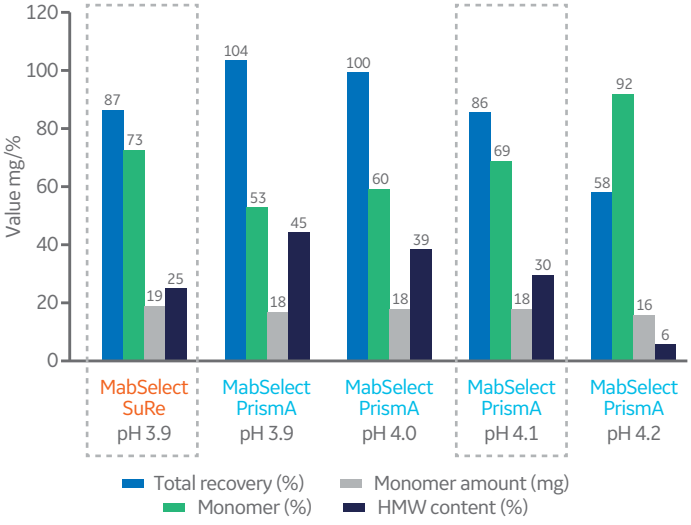

Fig 9. Screening of pH for elution of Molecule 4 for MabSelect PrismA and MabSelect SuRe resins.

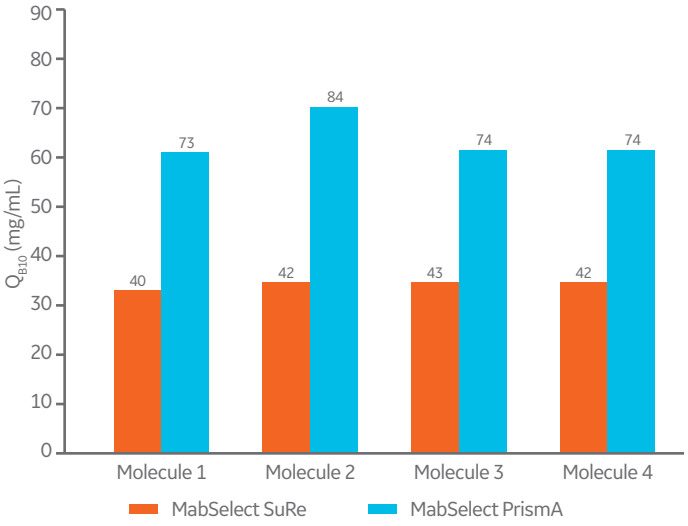

Fig 10. DBC of MabSelect PrismA and MabSelect SuRe resins determined for four different Fc-based antibody constructs.

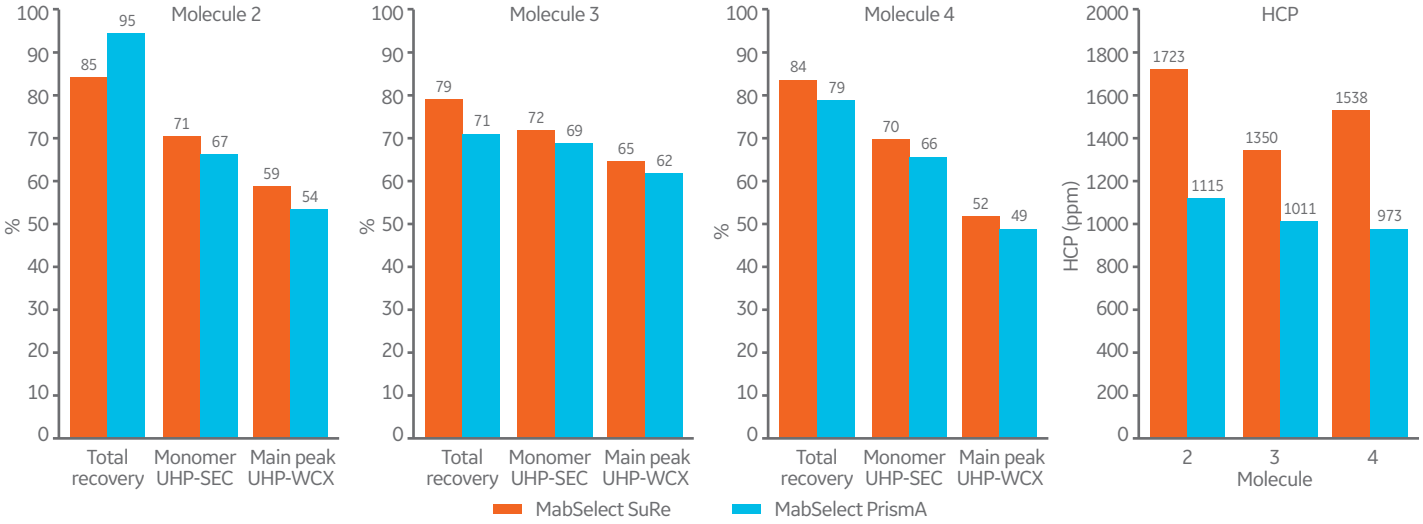

Fig 11. Quality and recovery of three different Fc-based antibody constructs after the capture step using either MabSelect PrismA or MabSelect SuRe resin. UHP-SEC = ultra high performance size-exclusion chromatography, UHP-WCX = ultra high performance weak cation exchange chromatography.

Conclusion

This work demonstrates the improved binding capacity of MabSelect PrismA, at maintained purification performance in terms of mAb purity and recovery. The enhanced performance of MabSelect PrismA over other commercially available protein A resins is confirmed in a third-party case study. The high capacity of the resin allows for handling of increasing upstream titers to resolve bottlenecks in downstream mAb processing. The improved capacity enables an increased mass throughput per purification cycle, enhancing productivity of current chromatography columns and systems without costly capital expenditures. MabSelect PrismA allows for up to 30% more product to be purified using current equipment. Alternatively, the increased binding capacity can be used to decrease the resin volume required to achieve a given mass throughput.

References

1. Hu, B., Sellers, J., Kupec, J., Ngo, W., Fenton, S., Ynag, T.Y., Grebanier, A. Optimization and validation of DNA extraction and real-time PCR assay for the quantitative measurement of residual host cell DNA in biopharmaceutical products. *J Pharm Biomed Anal* **88**, 92–95 (2014).

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