A next-generation Protein A resin for improved productivity and bioburden control

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A next-generation Protein A resin for improved productivity and bioburden control

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Abstract

This poster presents a newly designed, high-capacity Protein A resin, MabSelect™ PrismA, which offers alkali stability on par with ion exchange and hydrophobic interaction chromatography resins. MabSelect PrismA offers new possibilities in cleaning and sanitization efficiency to address bioburden risks and process economy.

The more alkali resistant ligand was developed by replacing sensitive amino acids and screening and evaluating more than 400 constructs on a Biacore™ surface plasmon resonance (SPR) system. A new high-flow agarose base matrix was developed, optimized for high binding capacity to enable increased mass throughput and productivity.

Performance benchmarking shows that MabSelect PrismA has significantly improved process performance compared to its predecessors MabSelect SuRe™ and MabSelect SuRe LX with similar product quality results.

Introduction

mAb production using a Protein A capture step has followed a highly successful synergistic path the last 30 yr. The high purity and yield provided by Protein A resins is a large part of the success. There are, however, remaining challenges.

The increased upstream titers in mAb production seen in recent years may make the Protein A capture step a bottleneck. Protein A columns columns are also more prone to bioburden contamination due to heavy impurity load and weak tolerance for common concentrations of NaOH cleaning-in-place solutions. Regulators are therefore increasingly asking manufacturers if they understand the sources of bioburden and have them under control.

Ligand development

Sensitive amino acids and/or exposed protein surfaces were mutated to increase alkaline stability (Fig 1). More than 400 constructs were screened using the Biacore SPR system. The MabSelect SuRe ligand was used as a reference. The most alkali-stable constructs were selected and coupled to base matrix and further evaluated in packed columns. A new base matrix was also developed and optimized towards the new ligand. To increase binding capacity, base matrix properties such as particle size, matrix volume, and pore size were optimized in combination with ligand length and ligand density.

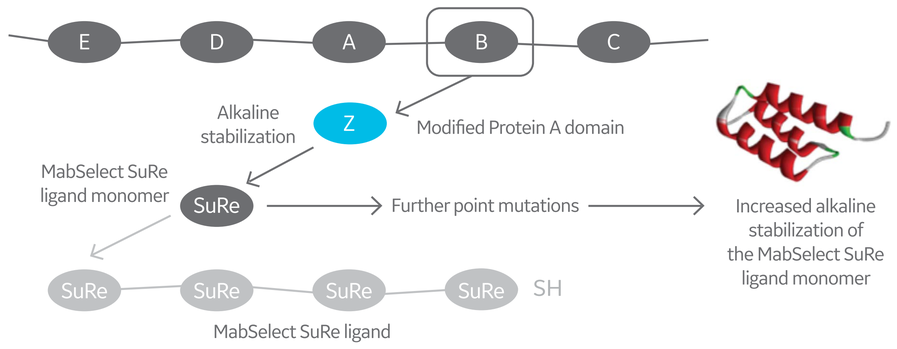

Fig 1. Further development of MabSelect SuRe Protein A ligand using point mutation of sensitive amino acids.

Results

Dynamic binding capacity (DBC)

The DBC for polyclonal IgG was measured at different residence times (RT). MabSelect SuRe and MabSelect SuRe LX were used as references.

The results show that MabSelect PrismA offers:

- Up to 40% increased DBC compared with MabSelect SuRe LX at 2.4 min RT
- Up to 30% increased DBC compared with MabSelect SuRe LX at 4 min RT
- Up to 25% increased DBC compared with MabSelect SuRe LX at 6 min RT

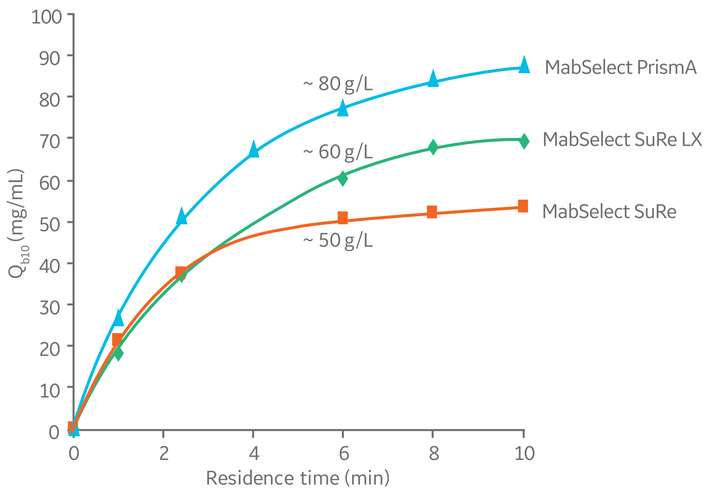

Fig 2. Dynamic binding capacity for polyclonal IgG.

Alkali stability

Repetitive purification cycles using 1.0 M NaOH as cleaning agent for 15 min/cycle were performed. The results show that MabSelect PrismA had significantly more alkali stability than MabSelect SuRe LX. After 150 cycles at 1.0 M NaOH, MabSelect PrismA still had ~ 90% relative remaining binding capacity left while MabSelect SuRe LX was down to 50%.

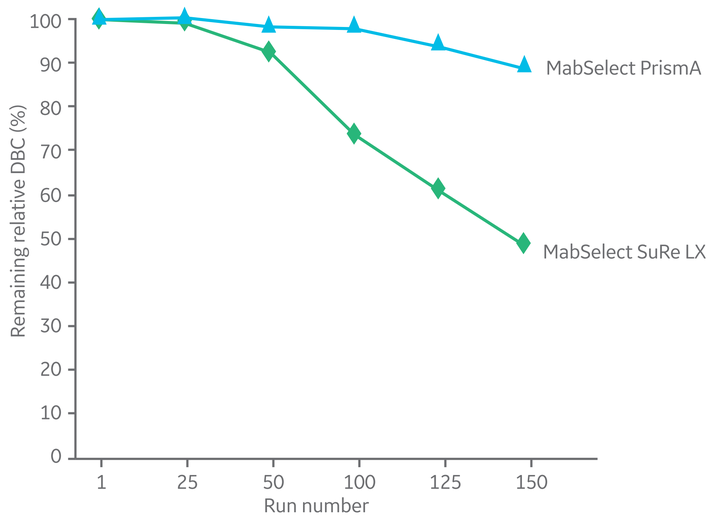

Fig 3. Relative remaining dynamic binding capacity using 1.0 M NaOH as cleaning agent with a contact time of 15 min in each purification cycle.

Pressure-flow properties

MabSelect PrismA is based on a new high-flow agarose base matrix designed for optimal performance of binding capacity and high flow velocities. Flow velocities of 300 cm/h are possible with MabSelect PrismA (Fig 4).

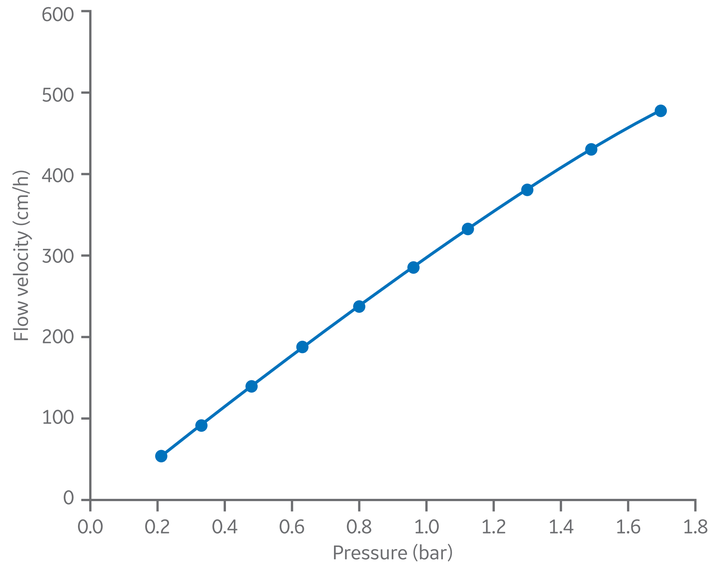

Fig 4. Pressure-flow properties determined in a AxiChrom™ 300, 20 cm bed height, temperature 20˚C.

mAb purification

A mAb purified using MabSelect PrismA was compared with the same mAb purified on MabSelect SuRe and MabSelect SuRe LX. The sample load was 80% of Qb10 (58 mg/mL for MabSelect PrismA; 46 mg/mL for MabSelect SuRe LX; and 39 mg/mL for MabSelect SuRe. Purification performance, in terms of yield and purity, was comparable for the different Protein A resins.

Table 1. Summary of purification data from capture step on different Protein A resins. Start HCP: 137 518 ppm Start DNA: 8037 ppm

	Yield (%)	HCP (ppm)	Aggregates (%)	DNA (ppm)	Pool volume (CV)	Leached Protein A (ppm)
MabSelect PrismA	96	336	1.5	1.4	1.3	10
MabSelect SuRe LX	95	204	1.0	0.9	1.3	9
MabSelect SuRe	99	322	0.8	1.3	1.3	7

Discussion and conclusions

A next-generation Protein A resin, MabSelect PrismA, with significantly increased alkali stability allowing for improved cleaning and sanitization efficiency is presented. The new resin offers the potential of improved process economy and reduces costly bioburden-related process deviations.

By combining high process flow velocities with exceptional DBC, MabSelect PrismA increases the mass throughput and productivity of chromatography columns for large-scale biomanufacturing.

The increased binding capacity enables improved productivity from existing equipment and will also increase the possibility to use prepacked bioprocess Protein A columns, even for high titer 2000 L bioreactor harvests. The key characteristics of the resin also enable new opportunities in continuous chromatography.

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