Optimized purification of antibodies using a new high-capacity and alkaline stable protein A resin

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Introduction

A new protein A chromatography resin has been designed for purification of mAbs, Gabs and polyclonal antibodies. The new resin, WorkBeads™ affimAb, is designed for high dynamic binding capacity even at short residence times and has stabile binding capacity over extended alkaline treatment with 0.5 M NaOH.

These benefits are obtained by the design of the specific protein A ligand in combination with an optimized basematrix. The WorkBeads affimAb alkaline-stable resin is thus suitable for demanding purification of antibodies in biopharmaceutical production.

High dynamic binding capacity

WorkBeads affimAb has a DBC of more than 40 mg IgG/ml resin even at a short residence time (RT) such as 2.4 min. DBC at different flow rates (residence times) determined by frontal analysis at 10% breakthrough for WorkBeads affimAb compared to MabSelect™ SuRe from GE Healthcare is shown in Figure 1.

Resins/Columns:	WorkBeads affimAb (6.6 × 100 mm, 3.4 ml) MabSelect SuRe (6.6 × 100 mm, 3.4 ml)
Sample:	1 mg/ml IgG in binding buffer
Residence times:	2.4, 4.8, 6 and 10 min (250, 125, 100 and 60 cm/h)
Binding buffer:	PBS, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 2.7

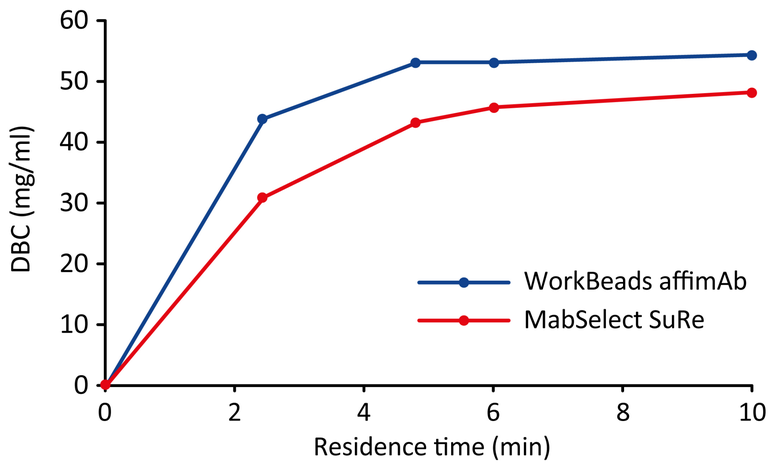

Figure 1. Dynamic binding capacity for human polyclonal IgG determined at different flow rates (residence times) by frontal analysis.

Particle size distribution

The combination of optimized basematrix rigidity and narrow particle size distribution gives columns with higher efficiency and low backpressure even at higher flow rates. Particle size distribution for WorkBeads affimAb compared to MabSelect SuRe is shown in Figure 2.

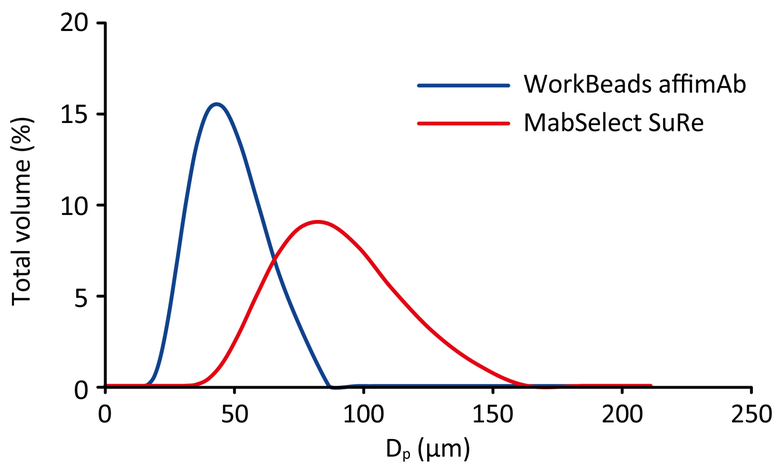

Figure 2. Particle size distribution for WorkBeads affimAb (blue) and MabSelect SuRe (red).

Preserved binding capacity over many cleaning-in-place (CIP) cycles

WorkBeads affimAb and MabSelect SuRe were investigated for stability towards a typical CIP by continuous incubation with 0.5 M NaOH at 1.0 ml/min for 15 minutes and regular DBC testing, see Figure 3.

Resins/Columns:	WorkBeads affimAb (6.6 × 100 mm, 3.4 ml) MabSelect SuRe (6.6 × 100 mm, 3.4 ml)
Sample:	1 mg/ml human polyclonal IgG in binding buffer
Flow rate:	1.4 ml/min (2.4 min RT)
Binding buffer:	PBS, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 2.7

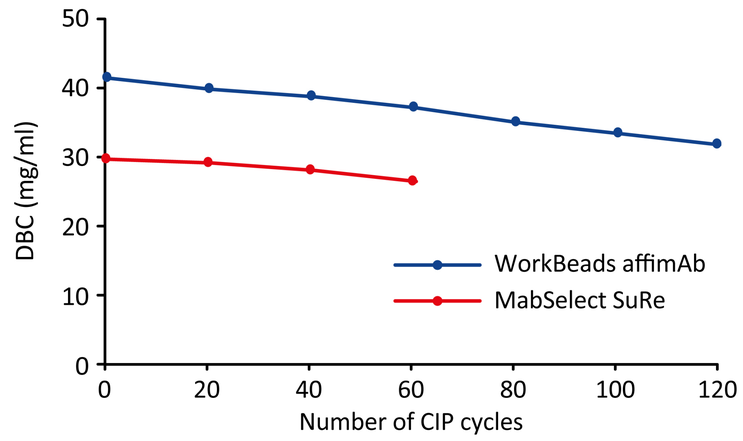

Figure 3. Comparison of dynamic binding capacity of WorkBeads affimAb and MabSelect SuRe after 120 resp. 60 CIP cycles with 0.5 M NaOH with 15 minutes contact time. The DBC was determined at every 20th CIP cycle, at 10% breakthrough by frontal analysis at 2.4 minutes residence time using 1 mg/ml human polyclonal IgG in PBS, pH 7.4.

Purification of a monoclonal antibody

An example of using WorkBeads affimAb for a purification of a monoclonal antibody expressed in CHO cells is shown in Figure 4A. Purity was analyzed by SDS-PAGE, see Figure 4B. A comparison with MabSelect SuRe is included.

Resin/Column:	WorkBeads affimAb (6.6 × 100 mm, 3.4 ml)
Sample:	18 ml clarified cell supernatant from CHO cells
Flow rates:	100 cm/h (sample application) 300 cm/h (washing) 150 cm/h (elution)
Binding buffer:	PBS, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 2.7

Lanes
1. Marker
2. Sample
3. WorkBeads affimAb, flow through
4. WorkBeads affimAb, eluted pool
5. MabSelect SuRe, flow through
6. MabSelect SuRe, eluted pool

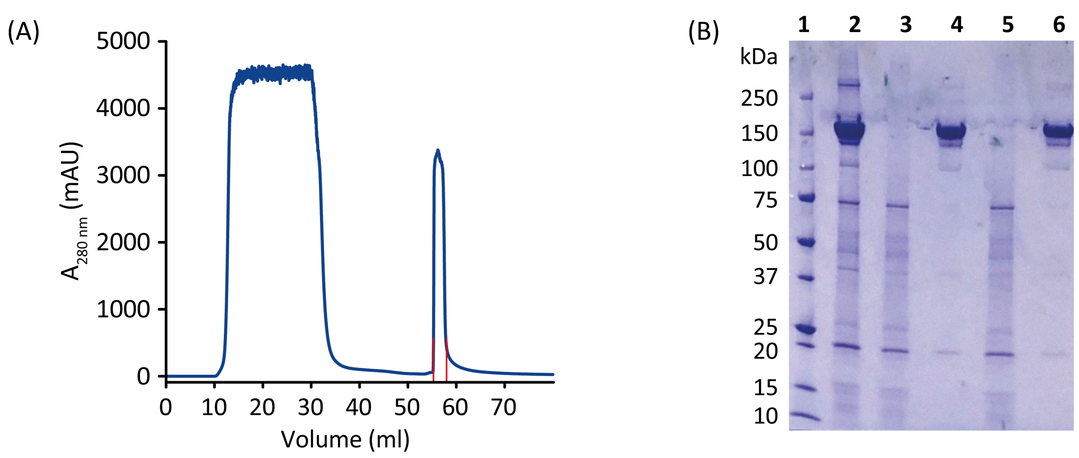

Figure 4. Purification profile of a monoclonal antibody from clarified CHO cell supernatant. Eluted pool is shown in red (A). Purity check on SDS-PAGE (B).

Conclusions

WorkBeads affimAb shows high binding capacity, > 40 mg IgG/ml resin at as short residence time as 2.4 minutes. This new resin has high alkaline stability with > 90% of DBC remaining after 60 purification cycles including CIP with 0.5 M NaOH for 15 minutes contact time. WorkBeads affimAb thus presented superior performance when compared with another market leading protein A resin.

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