WorkBeads Protein A

Novel media for antibody purification

Johanna Tawe[1], Carina Engstrand[1], Laura Mesas[1], Anton Linde[2] and Lars Haneskog[1]

1) Bio-Works Technologies, Uppsala, Sweden

2) Medicago AB, Uppsala, Sweden

Introduction

The expression levels of monoclonal antibodies (MAbs) in biopharma are frequently up to 10 g/L. Efficient production is therefore often considered to require a capture step using chromatography media with high binding capacities. WorkBeads™ Protein A is an agarose-based media with high binding capacity and a competitive price. Purity of >99% is generally obtained using standard conditions without optimization. WorkBeads Protein A thus does not require optimization for most research-scale applications. Demanding large-scale purification usually require optimization of the washing and elution steps followed by one or more polishing purification steps using, *e.g.*, ion-exchange chromatography.

Results

WorkBeads Protein A was developed to have high dynamic binding capacity (DBC). Human polyclonal IgG was used to investigate the effect of residence time on DBC at 10% breakthrough ($Q_{b,10\%}$). A residence time of 3 minutes or higher gave a DBC of 45 mg/ml (Fig. 1).

Column: 6.6 x 100 mm *(Omnifit® glass column)*
Medium: WorkBeads Protein A
Sample: 1 mg/mL human normal serum IgG in *PBS (20 mM Na-phospate, 150 mM NaCl, pH 7.4) (PBS SmartBuffer from Medicago; Art. No. 09-9500-100)*

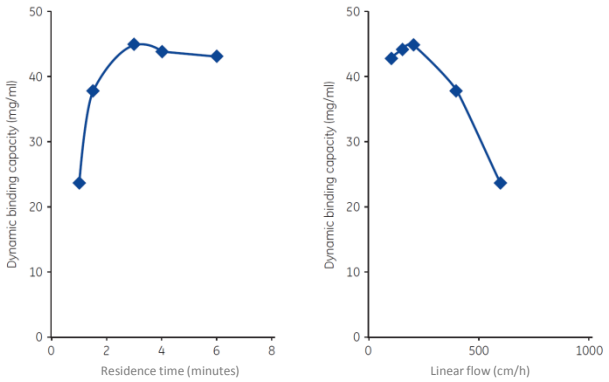

Figure 1. Effect of residence time and linear flow on dynamic binding capacity.

Elution of adsorbed IgG is perfromed by decreasing the pH. The pH needed for elution vary between Mabs, and can be determined running a decreasing pH-gradient. As high pH as possible should be used for elution to avoid denaturation and aggregation. Polyclonal IgG was applied to a column packed with WorkBeads Protein A and eluted using a linear pH gradient to investigate the typical pH range needed for efficient (Fig. 2). To test the efficiency of the binding and elution conditions human polyclonal IgG was applied in an amount corresponding to 65% of the DBC and eluted by a step gradient at pH 3.0. The yield was 95%. It should be noted that some IgG eluted at significantly higher pH indicating that the elution pH should be ivestigated for each individual antibody.

Column: 6.6 × 100 mm bed
Medium: WorkBeads Protein A
Flow: 1 ml/min
Cleaning-in-place (CIP): 10 column volumes (CV) 10 mM NaOH, contact time 30 minutes.

Run A.
Sample: 1 mg/mL human polyclonal IgG (Octagam from Octapharm) in PBS, pH 7.4
Binding buffer: PBS
Elution buffer A: 50 mM Na-citrate, pH 5.6
Elution buffer B: 50 mM Na-citrate, pH 2.1
Gradient 0-100% Elution buffer B over 15 column volumes

Run B.
Sample: 1 mg/mL human polyclonal IgG (Octagam from Octapharm) in PBS, pH 7.4
Binding buffer: PBS
Elution buffer : 100 mM Na-citrate, pH 3.0
Elution: Step gradient

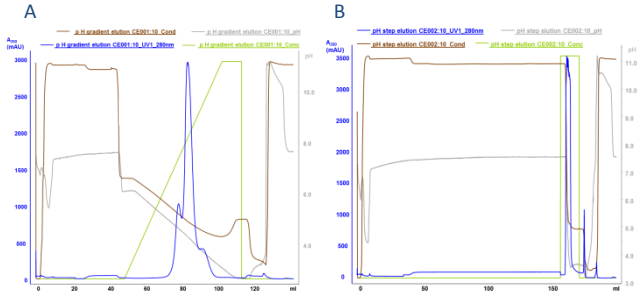

Figure 2. Binding and elution of human polyclonal IgG on WorkBeads Protein A. A) pH gradient elution pH 5.6-2.1 , B) Step elution at pH 3.2

The stability of the dynamic binding capacity during cleaning-in-place (CIP) was investigated using BabyBio™ A pre-packed with WorkBeads Protein A by frontal analysis after every 5th cleaning cycle (Fig. 3) . CIP using 15 mM NaOH give acceptable loss in DBC, and is recommended.

Column: BabyBio A 5 ml (pre-packed column)
Medium: WorkBeads Protein A
Flow: 2.5 ml/min
Sample: 1 mg/mL human polyclonal IgG in PBS, pH 7.4
Binding buffer: PBS, pH 7.4
Elution buffer: 100 mM glycine-HCl, pH 2.7
Elution: Step gradient, 10 CV
Cleaning-in-place: Step 1; 15 ml 15 minutes 100 mM 1-thioglycerol, pH 8.5. Step 2; A) 15 mM NaOH and B) 100 mM NaOH (not shown)

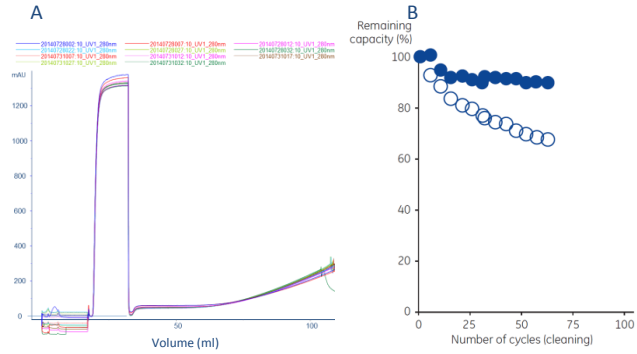

Figure 3. Alkaline stability of WorkBeads Protein A. A) Frontal analysis. B) Remaining DBC after CIP cycles using 15 mM NaOH (closed circles) or 100 mM NaOH (open circles).

PS40605010 AA