BabyBio A
Novel columns for antibody purification

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Introduction

BabyBio™ A 1ml and BabyBio A 5 ml are pre-packed columns with WorkBeads™ Protein A for antibody purification. BabyBio A columns can be used for determination of expression levels and purification in research scale, or used for method development for bioprocess. The high binding capacity allows for efficient purification of high-titer feeds. We have investigated the properties of the columns.

Results

The dynamic binding capacity (DBC) at 10% breakthrough ($Q_{b,10\%}$) on BabyBio A 1 ml was determined by frontal analysis uner standard conditions for IgG purification at different flow rates (Fig. 1). A typical residence time of 3 minutes (obtained at 0.33 ml/min. gave a binding capacity of 40 mg&ml. Increase in flow rate reduces the binding capacity. Full-height chromatography beds gave even higher binding capacities. Data for competitor columns were included for comparison. Figure 2 shows comparative DBC values for two typical flow rates.

Column: BabyBio A 1 ml (pre-packed column)
Medium: WorkBeads Protein A
Sample: 1 mg/mL human polyclonal IgG (Octagam from Octapharm) in PBS (20 mM Na-phospate, 150 mM NaCl, pH 7.4) (PBS SmartBuffer from Medicago; Art. No. 09-9500-100)
Binding buffer: PBS

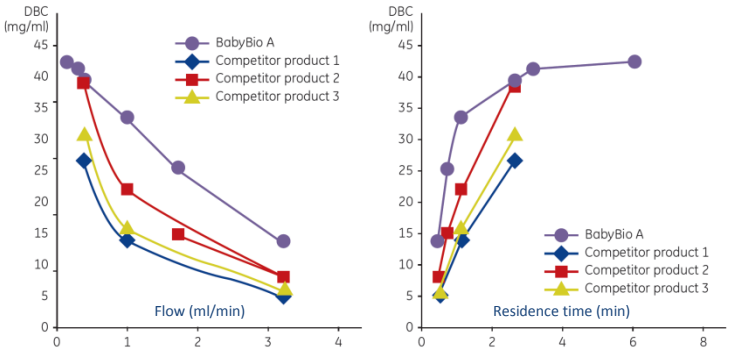

Figure 1. Dynamic binding capacity for human serum IgG at different flow rates by frontal analysis of 1 mg/ml IgG in PBS, pH 7.4. Competitor values are obtained from corresponding data sheets and are based on experiments under similar conditions.

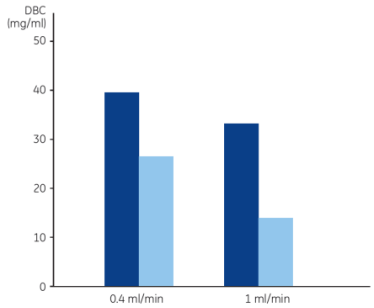

Figure 2. Dynamic binding capacity for human serum IgG on BabyBio A 1 ml (dark blue) and corresponding 1-ml column from a competitor (light blue) (values from data sheet). Data extracted from Fig. 1.

A monoclonal IgG expressed in CHO cells was purified on BabyBio™ A 1 ml . Cell supernatant was dilued in PBS buffer before application (Fig. 3A). Step elution was done at pH 2.7. The purification results in high purity as judged from analysis by SDS-PAGE (Fig. 3B), and was homogeneously monomeric as determined by size-exclusion chromatography (Fig. 3C).

Column: BabyBio A 1 ml (pre-packed column)
Medium: WorkBeads Protein A
Flow: 1 ml/min
Sample: 10 mL clarified supernatant from CHO cells diluted 1:11 in PBS Smart buffer
Binding buffer: PBS
Elution buffer: 100 mM Gly-HCl, pH 2.7
Elution: Step gradient

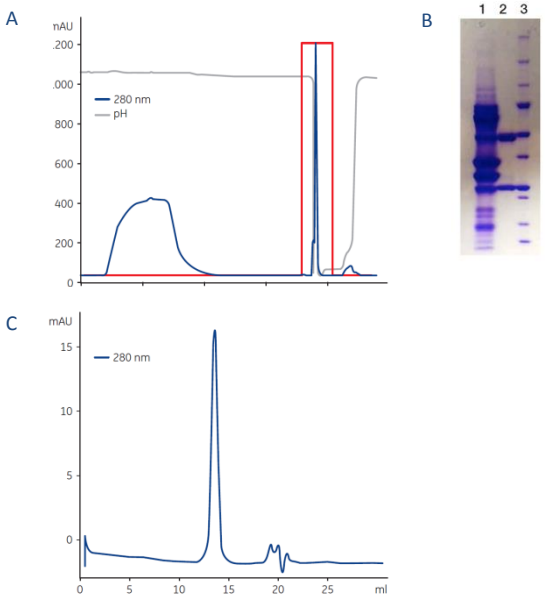

Figure 3. A) Purification of a monoclonal IgG from CHO cell supernatant. Analysis of the purified MAb by B) SDS-PAGE (1. Sample, 2. Purified MAb, 3. Mr markers; 250, 150, 100, 75, 50, 37, 25, 21. 6.9 kD) and by C) Size-exclusion chromatography (SEC).

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