A benchmark study of MAGicBeads SerA and other brands of magnetic beads

MAGIC BEADS

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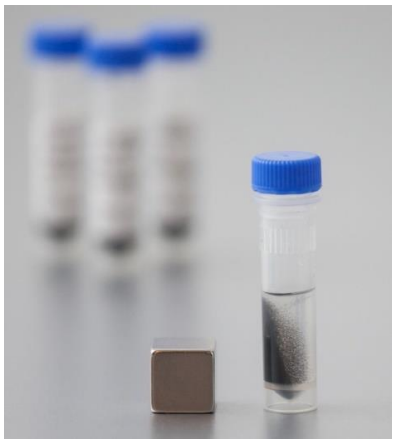
Introduction

Protein A from *Staphylococcus aureus* is known to bind the Fc portion of immunoglobulins from a variety of species and subclasses, and in some cases to the Fab region, but the affinity is strongest for immunoglobulin G from human, rabbit and mouse IgG_2. Hence, protein A coupled to beads is perhaps the most commonly used tool for numerous antibody-based applications such as affinity purification, immunoprecipitation and depletion. Protein A coupled magnetic beads are becoming a popular research tool, as they provide a rapid and convenient method to conduct such experiments in a small scale, using simple magnetic separation instead of more advanced or time-consuming techniques like chromatography or centrifugation. In this study, we compare MAGicBeads SerA with corresponding magnetic beads from other companies. In conclusion, MAGicBeads SerA offers the highest antibody selectivity and binding capacity for a magnetic bead available on the market.



Binding capacity for human IgG

A comparative benchmark analysis was performed in order to determine the binding capacity of different protein A magnetic beads for human immunoglobulin G (IgG) from serum. The performance of MAGicBeads SerA was compared with corresponding magnetic beads from GE Healtcare (Protein A Mag Sepharose™ XTRA), Millipore (PureProtome™ Protein A magnetic beads), Invitrogen (Dynabeads® Protein A) and Spherotec (SPHERO Protein A magnetic particles) (see Table 1). Beads from 250 µl of each brands bead solution, were overloaded with IgG, by mixing the beads with 250 µl of human serum for 1 hour at room temperature. The load of IgG was above the capacity for each separation medium. Beads were then washed three times with 500 µl of PBS and eluted three times with 250 µl of 60 mM citrate, pH 3.0. The three elution fractions were pooled. The binding capacity was determined by calculating the amount of eluted IgG using A_{280} measurements. Each sample was performed in duplicates. Binding capacity for MAGicBeads SerA was 6.2 mg IgG/ml of bead solution, which is considerably higher than corresponding products from the other companies (Fig 1).

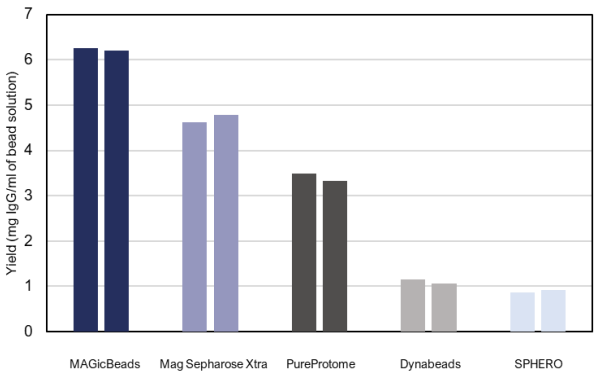


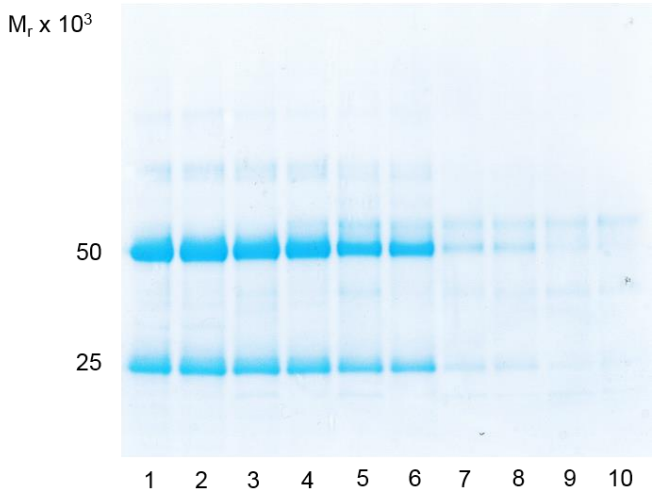
Fig 1. Antibody binding capacity determination for different protein A magnetic beads. Samples were run in duplicate.

Selectivity for human IgG

The eluted human IgG fractions were further analyzed by SDS-PAGE under reducing conditions on a NUPAGE 4-12 % bis-Tris gel stained with coomassie blue in order to determine the purity. 5 µl of eluted pool from each purification run was loaded onto the gel.
Purity of the isolated human IgG using the MAGicBeads SerA was very high and considerably higher than for the corresponding products from Millipore, Invitrogen and Spherotec, where a significant band at approximately 60 kDa, probably originating from albumin, can be observed (Fig 2.).

Table 1. Experimental conditions for antibody binding capacity determination using protein A magnetic beads

Supplier	MAGic Bioprocessing	GE Healthcare	Millipore	Invitrogen	Spherotec
Separation medium	MAGicBeads SerA	Protein A Mag Sepharose™ Xtra	PureProtome™ Protein A magnetic beads	Dynabeads® Protein A	SPHERO™ Protein A (PAMS-40-5)
Bead solution volume	250 µl	250 µl	250 µl	250 µl	250 µl
Sample	250 µl of human serum diluted with 250 µl phosphate buffered saline pH 7.5				
Load	Overloaded	Overloaded	Overloaded	Overloaded	Overloaded
Binding conditions	Protein A magnetic beads where incubated with sample for one hour at room temperature using an end-over-end mixer				
Binding and washing buffer	Phosphate buffered saline (10mM phosphate, 140mM NaCl, 2.7mM KCl), pH 7.5				
Elution conditions	Human IgG were eluted 3 times with 250 µl of 60mM citrate, pH 3.0				



Lane

1-2: Eluted pool, MAGicBeads SerA
3-4: Eluted pool, Protein A Mag Sepharose Xtra
5-6: Eluted pool, PureProtome Protein A magnetic beads
7-8: Eluted pool, Dynabeads Protein A
9-10: Eluted pool, SPHERO Protein A magnetic particles

Fig 2. Purity benchmark study. The purified human IgG from the binding capacity test, were analyzed by SDS-PAGE stained with coomassie blue under reducing conditions. 5 µl of IgG fraction from each purification run were loaded on the gel.

MAGicBeads SerA - Kinetic study of human IgG binding

In a second test, the dependency of the human IgG binding capacity on reaction time for MAGicBeads SerA was investigated. This test was conducted in order to determine the time of binding necessary to reach saturation. 500 µl of bead solution was mixed with human serum (500 µl serum with 500 µl of PBS). The beads were allowed to bind IgG for 5, 10, 20, 30, 60, and 90 min, before washing and elution. The graph shows the amount of purified IgG obtained per ml of bead solution at the different binding times (Fig 3.).

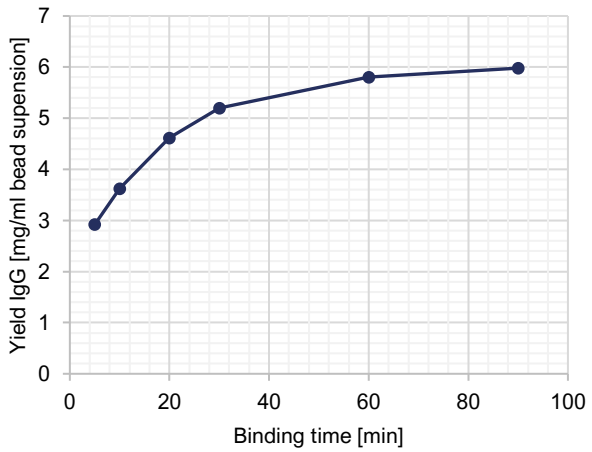
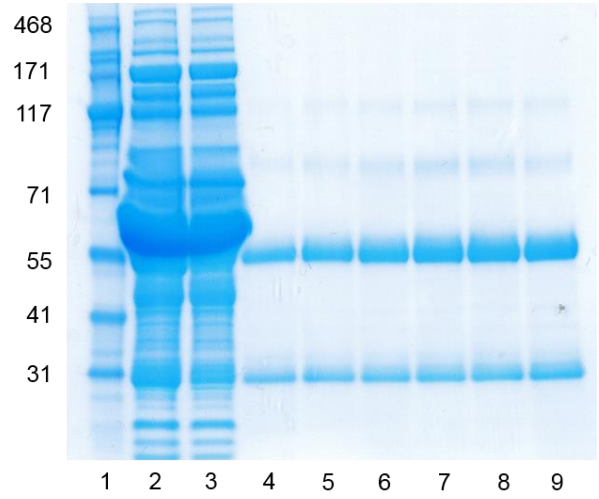


Fig 3. Dependency of antibody bindning capacity on the time of bindning for MAGicBeads SerA.



Lane

1: Protein molecular weight marker, kDa
2: Human serum input
3: Human serum depleted, 90 min binding
4: Eluted pool, 5 min binding
5: Eluted pool, 10 min binding
6: Eluted pool, 20 min binding
7: Eluted pool, 30 min binding
8: Eluted pool, 60 min binding
9: Eluted pool, 90 min binding

Fig 4. Purity assessment of human IgG obtained at different binding times from purification in serum, using MAGicBeads SerA. SDS-PAGE stained with coomasie blue under reducing conditions.

Binding saturation was reached in the range of 60-90 min binding with a total capacity close to 6 mg IgG/ml bead solution, which is in good agreement with the results obtained from the benchmark binding capacity test. Already after 5 min binding, 3 mg IgG/ml bead solution was purified from the human serum sample. Purity of all human IgG fractions was high, as analyzed by SDS-PAGE under reducing conditions on a NUPAGE 4-12 % bis-Tris gel stained with coomassie blue (Fig 4.).

Conclusions

The benchmark study performed at our laboratory, show that MAGicBeads SerA has the highest binding capacity for human IgG, as compared to the corresponding products from GE Healthcare, Millipore, Invitrogen, and Spherotec. The purity of the purified human IgG, obtained with MAGicBeads SerA, was also considerably higher than the products from Millipore, Invitrogen and Spherotec. A kinetic study using the MAGicBeads SerA product showed that 50% binding saturation was reached already after 5 minutes reaction in human serum resulting in 3 mg IgG/ml bead solution.