Immuno-affinity purification of anti-rabbit IgG using MAGicBeads ACT

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MAGIC BEADS

Introduction

Affinity purification based on protein-protein interaction, is a technique used to enrich a specific protein of interest. In this study, we covalently couple rabbit IgG to MAGicBeads ACT magnetic beads and subsequently use these modified beads to purify goat anti-rabbit IgG from serum.

Coupling of antigen to magnetic beads

Rabbit IgG in PBS was incubated with MAGicBeads ACT (see Table 1). During incubation, the IgG concentration in the supernatant was monitored using A280. The supernatant was removed when the coupling was determined to be complete. Remaining reactive structures were blocked using ethanolamine (50 vol%) and the beads were treated according to the Product Manual. The 1 mg of rabbit IgG was coupled to the magnetic beads with a coupling efficiency of 94% within 1 hour (Fig 1).

Table 1. Experimental conditions for coupling

Magnetic beads	MAGicBeads ACT
Sample	Rabbit IgG (>95% purity)
Bead volume	0.1 ml (1 ml 10% bead suspension)
Sample volume	1 mg IgG in 1 ml PBS
Magnetic separator	MAGicSep LAB
Binding conditions	60 minutes in PBS (15 mM phosphate pH 7.4, 150 mM NaCl)
Coupling efficiency	94%

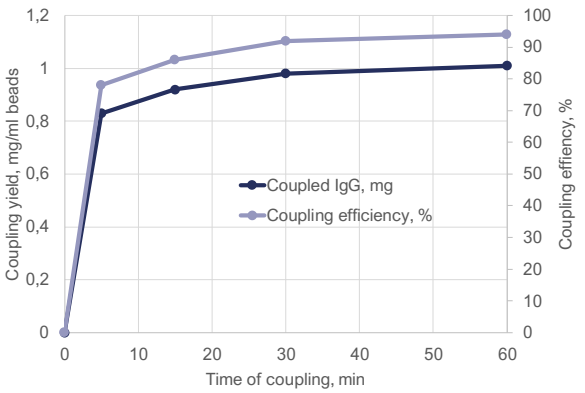

Fig 1. Coupling of rabbit IgG to MAGicBeads ACT is sufficiently completed within 1 hour.

Purification of anti-rabbit IgG

1 ml of goat serum, containing polyclonal antibodies raised against rabbit IgG, was added to 0.1 ml of the pre-washed settled LOABeads Rabbit IgG beads, manufactured as described in the previous section. Sample and beads were mixed for 60 minutes. Beads were thereafter washed with PBS and captured goat anti-rabbit IgG antibodies eluted using 0.5 ml citrate (60 mM, pH 3). Two subsequent purification runs were performed with the same beads. Between each run, beads were regenerated according to the Product Manual.

Table 2. Experimental conditions for purification

Magnetic beads	MAGicBeads ACT coupled with rabbit IgG
Sample	Goat serum containing anti-rabbit IgG
Bead volume	0.1 ml (1 ml 10% bead suspension)
Sample volume	1 ml
Magnetic separator	MAGicSep LAB
Binding conditions	60 minutes in PBS (15 mM phosphate pH 7.4, 150 mM NaCl)
Elution conditions	15 minutes in 0.5 ml citrate (60 mM, pH 3)
Yield	2 x 0.5 mg anti-rabbit IgG

Approximately 0.5 mg of polyclonal goat anti-rabbit IgG were purified in each run. Purified antibodies from the two separate enrichments showed the same high purity, as determined by SDS-PAGE under reducing conditions (Fig 2).

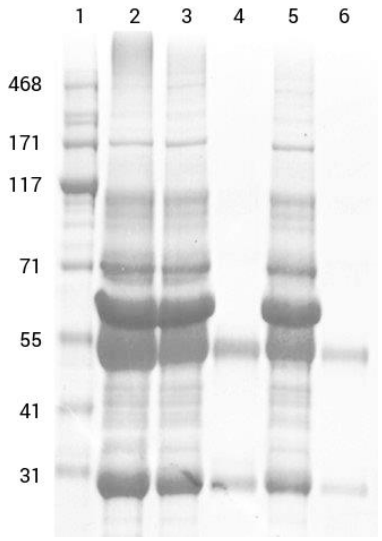

Fig 2. SDS-PAGE gel electrophoresis. Anti-rabbit IgG purified from goat anti-serum with MAGicBeads ACT. 1. Protein molecular weight, 2. Input goat serum, 3. Flowthrough goat serum first run, 4. Purified anti-rabbit IgG first run, 5. Flowthrough goat serum second run. 6. Purified anti-rabbit IgG second run.

Conclusions

The coupling of proteins to MAGicBeads ACT under mild and neutral conditions is fast and efficient. Purification of the polyclonal goat anti-rabbit IgG antibodies is also efficient, with a binding capacity of 5 mg anti-rabbit IgG per ml beads, yielding a total of 1 mg polyclonal antibody from 1 ml serum in two purification runs. The coupled beads can also be reused without losing capacity or specificity.