Immunoprecipitation efficiency for short time pulldowns

MAGIC BEADS

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Introduction

In this study, the recovery efficiency of immunoprecipitation of human ferritin using MAGicBeads SerA was measured with a ruthenium based immunoassay [1]. The assay uses an anti-ferritin antibody labeled with a ruthenium chelate. This allows quantification of the ruthenium content and, thus, also the ferritin concentration. The method is sensitive and precise.

Immunoprecipitation

Two standard solutions of human ferritin at approximately 100 ng/ml and 200 ng/ml were prepared and subsequently divided into two aliquots each. One aliquot was used to directly determine the ferritin concentration using a precise ruthenium immunosorbent assay, based on flow injection analysis by inductively coupled plasma mass spectrometry (FIA-ICP-MS) [1]. The other aliquot was used for immunoprecipitation using MAGicBeads SerA (Table 1).

Table 1. Experimental conditions

Magnetic beads	MAGicBeads SerA
Sample	Human ferritin standard
Bead volume	0.2 µl (10 µl 2% bead suspension)
Sample volume	50 µl
Magnetic separator	Neodymium cube magnet
Binding conditions	PBS (15 mM phosphate, 150 mM NaCl, pH 7.4)

A 50 µl aliquot of the standard solution was mixed with 450 µl of a solution containing an antiferritin antibody (6 µg/ml) labeled with a $[Ru(bpy)_3]^{2+}$-quelate. Thereafter, 0.2 µl of the MAGicBeads SerA beads, prepared as a 2% bead suspension, were added and mixed for 10 minutes at room temperature, to collect and precipitate the immune complex. The magnetic beads were separated and the supernatant collected, whereafterthe beads were washed.

The concentration of ferritin remaining in the supernatant was determined through the same immunoassay and the recovery for the immunoprecipitation was calculated.

Table 2: Initial concentration of standard

Theoretical conc. [ng/ml]	Measured conc. [ng/ml]	SD	RSD [%]
100	95	7.9	8.4
200	194	14.5	7.5

Table 3: Concentration of standard after immunoprecipitation

Theoretical conc. [ng/ml]	Measured conc. [ng/ml]	SD	RSD [%]
100	23	2.3	8.8
200	39	3.8	9.7

The measurements of the initial concentration of ferritin in the standard solutions are presented in Table 2 and the concentrations after the immunoprecipitation with MAGicBeads SerA and anti-ferritin antibody are presented in

Table 3. The recoveries of ferritin immunocomplex through immunoprecipitation were significantly highfor both standard solutions (Fig 1), given the short adsorption time.

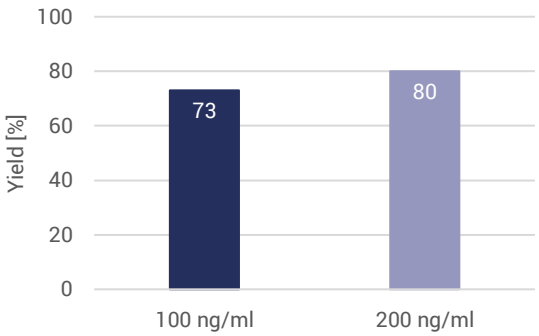

Fig 1. Recovery of immunoprecipitations of transferrin, using an anti-transferrin monoclonal antibody captured with MAGicBeads SerA

Conclusions

Immunoprecipitating 5 or 10 ng ferritin, using 0.2 µl MAGicBeads SerA and 2.7 µg anti-ferritin antibody for only ten minutes, yields excellent recoveries of the immunocomplex. With an extended adsorption time, MAGicBeads SerA can serve as an efficient alternative to traditional non-magnetic agarose beads coupled with protein A. An added benefit is the more efficient wash steps, thanks to magnetic separation, relative using a centrifuge to pellet the beads between washes..

References

[1] Konz T, Añón-Alvarez E, Montes-Bayón M, and Sanz-Medel A. (2013) *Anal Chem*, **85**, 8334-8340

*This Application Note has been compiled by employees of MAGic Bioprocessing AB, using original data kindly provided by Dr. Montes-Bayón. The data has been obtained using a free sample and evaluation of MAGicBeads SerA. No payment for service or consultation have occurred. The final text has been approved by Dr. Montes-Bayón.