Immunoprecipitation efficiency for short time pulldowns using LOABeads™ Protein A

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Introduction

In this study, the recovery efficiency of immunoprecipitation of human ferritin using LOABeads Protein A 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 LOABeads Protein A (Table 1).

Table 1. Experimental conditions

Magnetic beads	LOABeads™ Protein A
Sample	Human ferritin standard
Bead volume	0.2 µl (10 µl 2% bead suspension)
Sample volume	50 µl + 450 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 anti-ferritin antibody (6 µg/ml) labeled with a $[Ru(bpy)_3]^{2+}$-quelate. Thereafter, 0.2 µl of the LOABeads Protein A 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, after which the 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.

The measurements of the initial concentration of ferritin in the standard solutions and the concentrations after the immunoprecipitation with LOABeads Protein A and anti-ferritin antibody are presented in Table 2. The recoveries of the ferritin immunocomplex through immunoprecipitation were significantly high for both standard solutions (Fig 1), given the short adsorption time.

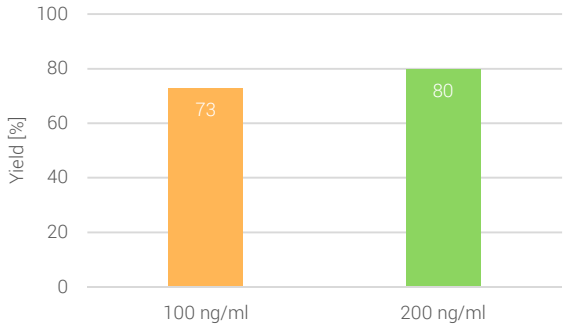

Fig 1: Recovery of immunoprecipitations of ferritin, using an anti-ferritin monoclonal antibody captured with LOABeads Protein A

Conclusions

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

References

[1] Konz T., Añón-Alvarez E., Montes-Bayón M., and Sanz-Medel A., (2013) Antibody Labeling and Elemental Mass Spectrometry (Inductively Coupled Plasma-Mass Spectrometry) Using Isotope Dilution for Highly Sensitive Ferritin Determination and Iron-Ferritin Ratio Measurements. *Anal Chem*, 85, 8334-8340

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

Table 2: Concentration of ferritin in standard solution before and after immunoprecpitation

Initial concentration				Final concentration			
Theoretical conc. [ng/ml]	Measured conc. [ng/ml]	SD	RSD [%]	Theoretical conc. [ng/ml]	Measured conc. [ng/ml]	SD	RSD [%]
100	95	7.9	8.4	100	23	2.3	8.8
200	194	14.5	7.5	200	39	3.8	9.7