Protein A Magnetic Beads

OM62570

Product Description

The Protein A Magnetic Beads are typically used for isolating antibodies from serum, cell culture supernatant or ascites and for immunoprecipitation and co-immunoprecipitation of antigens from cell or tissue extracts. Protein A Magnetic Beads contain a recombinant Protein A with five Fc-binding domains per protein. These domains can bind to antibodies from many different species, including mouse, human, rabbit, pig, dog and cat.

Product Features

Composition	Recombinant Protein A
Magnetization	Superparamagnetic
Particle size	200 nm
Concentration	10 mg/mL
Binding Capacity	≥ 0.6 mg human IgG/mL of bead
Application	IP , CoIP, ChIP
Storage Condition	Store at 4°C for 2 years.

Protocol

1. Cell lysis

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of Cell Extraction Buffer or NP-40 Cell Lysis Buffer.

2. Preparation of Magnetic Beads

2.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

2.2 Transfer 25-50 µL of Protein A Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

2.3 Add 400 µL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Hereinafter referred to as magnetic separation). Remove and discard the supernatant. Repeat this step for 2 times.

3. Binding of Antibody

3.1 Dilute antibody (Ab) to the final concentration of 5-50 µg/mL with binding/wash buffer. The optimal amount of Ab may be adjusted as required.

3.2 Add 400 µL of diluted Ab to the Protein A Magnetic Beads. Rotate tube for 30 minutes at room temperature or 2 hours at 4°C.

3.3 Perform magnetic separation. Transfer the supernatant into a new tube for further analysis, if desired. The supernatant is the non-binding fraction.

3.4 Add 400 µL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 4 times.

4. Immunoprecipitation of Target Antigen

4.1 Remove the tubes from the magnetic separator and add your sample containing the antigen (Ag) (typically 5-50 µg in 400 µL binding/wash buffer) and gently pipette to resuspend the Protein A Magnetic Beads-Ab complex.

4.2 Incubate with rotation for 30 minutes at room temperature or 2 hours at 4°C to allow Ag to bind to the Protein A Magnetic Beads-Ab complex.

4.3 Perform magnetic separation. Remove and discard the supernatant.

4.4 Wash the Magbeads-Ab-Ag complex 5 times using 400 µL binding/wash buffer for each wash. Perform magnetic separation between each wash, remove supernatant and resuspend by gentle pipetting.

4.5 Resuspend the Protein A Magnetic Beads-Ab-Ag complex in 400 µL binding/wash buffer and transfer the bead suspension into a clean tube. This is recommended to avoid co-elution of the proteins bound to the tube wall.

5. Elution

This is a non-denaturation elution method.

5.1 Perform magnetic separation and remove the supernatant. Add 400 µL of binding/wash buffer into the tube and rotate for 5 minutes. Perform magnetic separation for 1 minute and remove the supernatant. Then add 25-50 µL elution buffer into the tube with magnetic beads-Ab-Ag complex, rotate for 5 minutes.

5.2 Perform magnetic separation, collect the supernatant.

5.3 The final solution can be used as samples for denaturing SDS-PAGE. Or the elution can be adjusted to neutral pH with neutralization buffer immediately and used for further analysis.

Troubleshooting

Q1: How to improve the efficiency of antibody binding to magnetic beads?
A1: The binding efficiency of magnetic beads to antibodies is related to the species and subtype of the antibody. Please confirm the affinity of the type of antibody with the affinity of Protein A ligand. If the affinity of the subtype of the antibody is lower, increase the incubation time of the antibody and the magnetic beads (30 to 120 min) and the pH of the binding buffer(8-9), and reduce the ionic strength (25~100 mM NaCl).

Q2: How to improve the specificity of magnetic beads in immunoprecipitation?
A2: The antibody can be incubated with the sample to form an antibody-antigen complex, and the complex can be captured with Protein A magnetic beads. This method can increase the binding efficiency of the antibody to the antigen and reduce the binding time of the magnetic beads with the sample, thereby increasing the specificity of the precipitated product. This method is also recommended for CoIP &ChIP.

Q3: How to solve the phenomenon that the magnetic beads are easy to adhere to the tube wall?
A3: Recommend to use a low adsorption tube for magnetic bead operation. In addition, the addition of 0.01% to 0.1% (v/v) of nonionic detergent (such as NP-40, Tween-20 or Triton X-100) into the buffer can effectively reduce the adhesion of the magnetic beads to the tube.

Q4: How to solve agglomeration of the magnetic beads during use?
A4: If the magnetic beads are agglomerated during use, it is generally difficult to oscillate and break up that tends to uneven distribution. The reason is that the beads are placed in the magnetic field for too long and the beads are firmly bonded together. After treated with ultrasonic water bath for 2 minutes, the magnetic beads can be dispersed. However it should be noted the ultrasonic treatment time.

Appendix : Binding strength of Protein A to different species of Ig·s and their subclasses.

Species	Antibody Subtype	Protein A
Human	Total IgG	+++++
	IgG1, IgG2	+++++
	IgG3	-
	IgG4	+++++
	IgM	+
	IgD	-
	IgA	+
	IgA1, IgA2	+
	IgE	+++
	Fab	+
	ScFv	+
Mouse	Total IgG	+++++
	IgM	-
	IgG1	+
	IgG2a ,IgG2b	+++
	IgG3	+++
Rat	Total IgG	+++
	IgG1	+
	IgG2a	+
	IgG2b	+
	IgG2c	+++++
Cow	Total IgG	+++
	IgG1,IgG2	+++
Goat	Total IgG	+++
	IgG1,IgG2	+++
Sheep	Total IgG	+++++
	IgG1, IgG2	+++++
Horse	Total IgG	+++++
	IgG(ab),IgG(c)	+
	IgG(T)	+
Rabbit	Total IgG	+++++

Guinea Pig	Total IgG	+++
Hamster	Total IgG	+++
Pig	Total IgG	+++++
Donkey	Total IgG	+++
Cat	Total IgG	+++++
Dog	Total IgG	+++++
Monkey	Total IgG	+++
Chicken	Total IgG	-
Notes:	+ weak binding	+++ medium binding
	+++++ strong binding	- no binding