Protein A Immunomagnetic Beads

Introduction

TargetMol's Protein A Immunomagnetic Beads utilize bio-nano surface technology to directionally and densely coat Protein A onto the surface of superparamagnetic microspheres. They are suitable for immunoprecipitation (IP) of proteins, protein complexes, protein–nucleic acid complexes, and other antigens. The recombinant Protein A used in this product is especially suitable for binding mouse IgG2a, IgG2b, IgA, rabbit IgG, and human IgG1, IgG2, and IgG4. This product is compatible with immunoprecipitation reactions involving cell lysates, cell culture supernatants, serum, ascitic fluid, and other biological samples.

Product Features

- Low nonspecific adsorption
- High efficiency, high yield, and low reagent consumption
- Flexible and user-friendly operation
- Reliable results with high reproducibility

Product Information

Protein A Immunomagnetic Beads	Specification
Particle Size	2 μm
Human IgG Binding Capacity（Antibody Capacity）	0.5~0.6 mg/mL
Bead Concentration	10 mg/mL
Storage Solution	1×PBS，containing 0.1%（w/v）BSA，0.1%（V/V）proclin-300
Compatible Antibody Species	Broad species compatibility
Recommended Applications	IP, Co-IP, ChIP

Instructions

User-Supplied Reagents

Reagent	Suggested Composition
Washing Buffer (1×)	TBST: 50 mM Tris-HCl，150 mM NaCl, 0.1%(v/v) Tween-20，pH7.4
Acidity Elution Buffer	0.1 M Glycine，0.1% (v/v) Tween-20，pH2.5
Neutralization Buffer	1 M Tris-HCl, pH 9.0

1. Antigen Sample Preparation

Select an appropriate lysis buffer to process cell samples. Prepare cell lysates following standard procedures, keep the lysates on ice for immediate use, or store at –20 °C for long-term storage.

2. Immune Complexes Preparation

The amount of sample and incubation time depend on the specific antibody–antigen system and may require optimization for maximum yield. This protocol is suitable for reactions using 2~10 μg of affinity-purified antibody and can be scaled up as needed.

1) In a centrifuge tube, mix the cell lysate for each sample with 2~10 μg of immunoprecipitating antibody. It is recommended to use 500~1500 μg of total protein per immunoprecipitation reaction.
2) Dilute the antibody–sample mixture with Washing Buffer to a final volume of 300~500 μL.
3) Mix gently using a rotator or by inverting the tube manually. Incubate at room temperature for 1–2 hours or at 4 °C for 2~4 hours to allow formation of the antigen–antibody complex.

3. Magnetic Bead Pretreatment

1) Vortex the immunoprecipitation magnetic beads for 1 minute to fully resuspend. Transfer 25~50 μL of bead suspension into a 1.5 mL microcentrifuge tube.
2) Add 500 μL of Washing Buffer into microcentrifuge tube to wash the beads. Gently invert the tube several times to resuspend the beads. Place the tube in a magnetic separator for 1 minute, then remove and discard the supernatant. Repeat the washing step twice.

4. Antigen Precipitation Reaction

1) Add the antigen–antibody complex from Step 2 into the tube containing the pretreated magnetic beads. Mix gently.
2) Incubate on a rotator or gently invert at room temperature for 1–2 hours or at 4 °C for 2–4 hours. Perform magnetic separation and retain the supernatant for further analysis, keeping it on ice.
3) Wash the bead–antibody–antigen complex by adding 1000 μL of Washing Buffer. Gently pipette to disperse the beads, then perform magnetic separation and discard the supernatant. Repeat the wash two more times.

5. Antigen Elution

Two elution methods are provided below. Choose the appropriate method based on your downstream analysis.

1) Denaturing Elution: Suitable for SDS-PAGE analysis. Add 25 μL of 1× SDS-PAGE Loading Buffer (user-supplied) to the tube, mix well, and heat at 95 °C for 5 minutes. Then perform magnetic separation or centrifuge (13,000 g, room temperature, 10 min), and collect the supernatant for SDS-PAGE.
2) Acidic Elution: Add 100 μL of Acidity Elution Buffer to the tube. Incubate on a rotator at 37 °C for 5–10 minutes. Then perform magnetic separation or centrifugation, and collect the supernatant. To neutralize the eluate, add 50 μL of Neutralization Buffer to 100 μL of eluate to adjust the pH to neutral.

Storage

Store at 4℃ for 2 years

Precautions

1. Avoid freezing the magnetic beads. Beads should be stored in the storage solution to prevent drying.
2. To minimize bead loss, magnetic separation time should be no less than 1 minute each time.
3. Before withdrawing beads from the storage tube, ensure thorough resuspension by vortexing. Avoid bubble formation during handling.
4. It is recommended to use high-quality pipette tips and reaction tubes to reduce bead and solution loss due to surface adhesion.
5. To ensure optimal experimental performance, choose antibodies with high specificity for immunoprecipitation reactions.
6. Users may analyze the supernatant collected during the antibody and antigen binding steps to evaluate the binding efficiency between the antibody, antigen, and beads.

7. In IP experiments, binding affinity between different antibodies and antigens may vary. If the buffer system provided in this kit does not yield satisfactory results, users are encouraged to optimize or formulate alternative buffers.
8. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
9. Please wear a lab coat and disposable gloves.

Appendix: Comparison of the Binding Affinities of Protein A and Protein G to Antibodies of Different Species and Isotypes

Species	Antibody Classes	Protein A	Protein G
Human	IgA	Binding varies	-
	IgD	-	-
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	-	++++
	IgG4	++++	++++
	IgM	Binding varies	-
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	Binding varies	-
Rat	IgG1	-	+
	IgG2a	-	++++
	IgG2b	-	++
	IgG3	-	++
Cow	IgG	++	++++
Goat	IgG	-	++
Sheep	IgG	-	++
Horse	IgG	++	++++
Rabbit	IgG	++++	+++
Pig	IgG	+++	+++
Guinea Pig	IgG1	++++	++
	IgG2	++++	++
Hamster	IgG	+	++
Rhesus	IgG	++++	++++
Avian yolk	IgY	-	-
Dog	IgG	++	+
Koala	IgG	-	+
Alpaca	IgG	-	+

Note: “+++++”=strong binding，“+++”=medium binding，“+”=weak binding，“-”=no binding.