Anti-Cyclophilin A/PPIA Magnetic Beads Immunoprecipitation (IP) Kit

Catalog Number: MB203608-T44

Please read this instruction manual carefully before using the product

Product Contents

	Contents	Package 1 (20 Tests)	Package 2 (100 Tests)	Storage
1	Anti-Cyclophilin A/PPIA Magnetic Beads[1 3]	1 mL	5 mL	2-8℃ for 12 months
2	NP40 Cell Lysis Buffer[2]	4 mL	22 mL	-20℃ for 12 months
3	5×TBST (pH7.4)	Required but not supplied		
4	1×TBST (pH7.4)	Required but not supplied		
5	ddH_2O	Required but not supplied		
6	Alkaline Elution Buffer	3 mL	15 mL	2-8℃ for 12 months
7	Acidity Elution Buffer	3 mL	15 mL	2-8℃ for 12 months
8	Neutralization Buffer	2 mL	8 mL	2-8℃ for 12 months
9	Magnetic Separator	One Simple Magnetic Separator (Cat# MAGS001)		

[1] The IP KIT contains anti-Cyclophilin A/PPIA Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).
[2] Using NP-40 cell lysate buffer in the kit is required, otherwise, the magnetic beads may be precipitated.
[3] Immunomagnetic Beads kits are shipped at ambient temperature in which immunomagnetic beads are provided in liquid buffer.

Product Description

The Anti-Cyclophilin A/PPIA Immunomagnetic Beads, conjugated with Anti-Cyclophilin A/PPIA antibody, are used for immunepreciptation (IP) of Cyclophilin A/PPIA proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing Cyclophilin A/PPIA proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound Cyclophilin A/PPIA proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

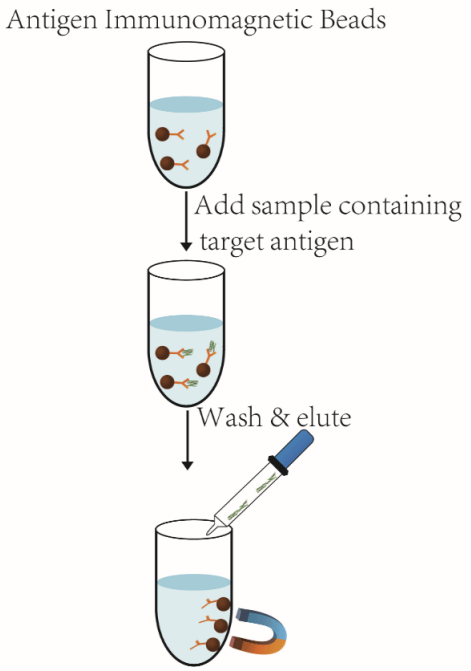

Fig. 1 Immunoprecipitation (IP) Protocol

Antibody Information

Antibody:	Cyclophilin A/PPIA Antibody, Rabbit PAb, Antigen Affinity Purified (Cat# 203608-T44)
Immunogen:	E. coli-derived Human Cyclophilin A/PPIA fragment
Isotype:	Rabbit IgG
Specificity:	Human Cyclophilin A/PPIA
Preparation:	Produced in rabbits immunized with E. coli-derived Human Cyclophilin A/PPIA fragment, and purified by antigen affinity chromatography.
Applications:	IP, Minimum Protein Purification
Alternative Names:	PPIA

Protocol

The protocol (Fig. 1) uses 50 µL Anti-Cyclophilin A/PPIAImmunomagnetic Beads, but this can be scaled up or down as required.

Cell Lysis

Cells may be lysed using any standard cell lysis protocol in accordance with your starting materials. **We suggest using NP40 Cell Lysis Buffer (supplied with kit).**

Immunoprecipitate Target Antigen

1. Add 50 µL of Immunomagnetic Beads into a 1.5 mL microcentrifuge tube.
2. Add 150 µL of 1× TBST buffer to the Immunomagnetic Beads and gently vortex to mix.
3. Place the tube into a Magnetic Separator to collect the beads against the wall side of the tube. Remove and discard the supernatant.
4. Add 1 mL of 1×TBST buffer to the tube. Invert the tube several times or gently vortex to mix for 1 min. Collect Immunomagnetic Beads with a Magnetic Separator. Remove and discard the supernatant.
5. Add the sample containing target protein (Cell lysate: 0.5-1mg; Recombinant protein: 5-25 µg) to the pre-washed Immunomagnetic Beads, add 1×TBST buffer until final volume to 200-500 µL, and incubate at 37℃ for 20-30 min (or at room temperature for 2-3h) with mixing.
6. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbounded sample and save for analysis.
7. Add 300 µL of 5×TBST buffer to the tube and gently mix. Collect the Immunomagnetic Beads and discard the supernatant. Repeat this wash twice.
8. Add 300 µL of ddH_2O to the tube and gently mix. Collect the Immunomagnetic Beads on a Magnetic Separator and discard the supernatant.

Elute Target Antigen.

A. Alkaline Elution

1. Add 100 µL of Alkaline Elution buffer to the tube.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the sample, add 50 µL of Neutralization Buffer for each 100 µL of eluate.

B. Acidity Elution

1. Add 100 µL Acidity Elution Buffer.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 15 µL of Neutralization Buffer for each 100 µL of eluate.

C. Denaturing Elution

1. Add 10 µL of 2×SDS-PAGE Sample Loading Buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100 ℃ for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the antigen.

General Test System of Sino Biological Inc. (for reference) :

	Recombinant Protein	**Cell Lysate**
Sample Quality	10µg add into 0.5mg cell lysate (without interfering proteins)	0.5mg
Final Volume	300µL	
Incubate Time	Room temperature, 2h	
Elute	Using 10 µL of 2×SDS-PAGE Sample Loading Buffer	

Reference Information

Related Products

Products	Cat No.
Magnetic Separator-1.5 (2 tubes)	MAGS001
Immunoprecipitation Kit -Immunomagnetic Beads Protein A Kit	BA10600
Immunoprecipitation Kit -Immunomagnetic Beads Protein G Kit	BG13103
Immunoprecipitation Kit -Immunomagnetic Beads Protein L Kit	BL11044
Immunoprecipitation Kit -Immunomagnetic Beads Protein A/G Kit	BAG001
Immunoprecipitation Kit -Anti-DYKDDDDK(Flag®) Tag Immunomagnetic Beads Kit	TB101274
Immunoprecipitation Kit -Anti-GFP Tag Immunomagnetic Beads Kit	TB13105
Immunoprecipitation Kit -Anti-Myc Tag Immunomagnetic Beads Kit	TB100029
Immunoprecipitation Kit -Anti-HA Tag Immunomagnetic Beads Kit	TB100028
Immunoprecipitation Kit -Anti-V5 Tag Immunomagnetic Beads Kit	TB100378
Immunoprecipitation Kit -Anti-GST Tag Immunomagnetic Beads Kit	TB11213
Magpoins™ His-Tag Immunoprecipitation Kit	TBN001

Trouble Shooting

Problem	Possible Cause	Solution
Little or no protein is detected	Protein degraded	Include protease inhibitors (PMSF) in the lysis buffer
		Use new lysate or lysate stored at -80° C
	No or minimal protein was expressed	Verify protein expression by SDS-PAGE or Western blot
		Analysis of the lysate using an positive control as a reference
Little or no protein is detected	No or minimal protein was expressed	Increase the amount of lysate used for IP/Co-IP
		Use a more sensitive detection system
Magnetic Beads aggregated	Magnetic Beads were frozen or centrifuged	Handle the Beads as directed in the instructions
	Buffer was incompatible with magnetic beads	
	Detergent was not added to the wash and bind solutions	
Failure to co-IP interacting protein	Wash conditions were too stringent for the weak or transient interaction	Reduce the number of washes
		Lower the ionic strength of the wash buffer
	Interacting protein was expressed at a low level	Apply additional protein sample
		Use a more sensitive detection system
	Buffer system was not optimal for the protein: protein interaction	Optimize the co-IP buffer
	Insufficient sample was loaded on the gel for Western blot detection	Elute sample in 30% acetonitrile 0.5% formic acid, then
		Bring the sample back up in SDS-PAGE Sample Loading Buffer and load entire elution fraction on