Sino Biological Inc.
Biological Solution Specialist

Immunoprecipitation/IP Kit-
Anti-CD3d/CD3 delta Immunomagnetic Beads

Catalog Number: MB10981-MM12

Please read this instruction manual carefully before using the product

Product Contents

Contents	Package 1	Package 2	Storage
Anti-CD3d/CD3 delta Immunomagnetic Beads[1 3]	1 mL	5 mL	2-8℃ for 12 months
NP40 Cell Lysis Buffer	4 mL	22 mL	-20℃ for 12 months
5×TBST（pH7.4）	Required but not supplied		
1×TBST（pH7.4）	Required but not supplied		
ddH_2O	Required but not supplied		
Alkaline Elution Buffer	3 mL	15 mL	2-8℃ for 12 months
Acidity Elution Buffer	3 mL	15 mL	2-8℃ for 12 months
Neutralization Buffer	2 mL	8 mL	2-8℃ for 12 months
Magnetic Separator	Not included (refer related product MAGS001)	One MAGS001 included in China[2]	

[1] The IP KIT contains anti-CD3d/CD3 delta Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).
[2] The Magnetic Separator cannot be included for oversea customers due to shipment prohibition.
[3] Immunomagnetic Beads kits are shipped at ambient temperature in which immunomagnetic beads are provided in liquid buffer.

Product Description

The Anti-CD3d/CD3 delta Immunomagnetic Beads, conjugated with Anti-CD3d/CD3 delta antibody, are used for immunepreciptation (IP) of CD3d/CD3 delta proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing CD3d/CD3 delta proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound CD3d/CD3 delta proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

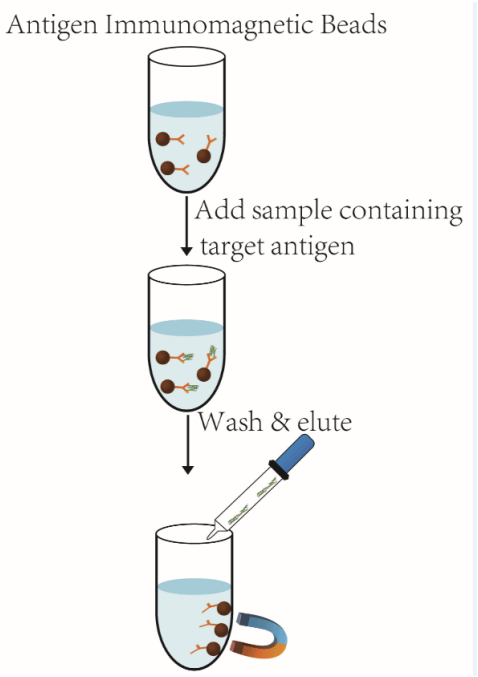

Fig. 1 Immunoprecipitation (IP) Protocol

Antibody Information

Antibody: CD3d / CD3 delta Antibody, Mouse MAb(10981-MM12)
Immunogen: Recombinant Human CD3d / CD3 delta Protein (Catalog#10981-H08H)
Clone ID: 12
Isotype: Mouse IgG1
Specificity: Human CD3d / CD3 delta
Guaranteed Applications: IP, Minimum Protein Purification
Preparation: This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, recombinant Human CD3d / CD3 delta (rh CD3d / CD3 delta; Catalog#10981-H08H; NP_000723.1; Met1-Ala105). The IgG fraction of the cell culture supernatant was purified by Protein A affinity chromatography.

Protocol

The protocol (Fig. 1) uses 50 µL Anti-CD3d/CD3 deltaImmunomagnetic 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 (~100 µg of protein in 100 µL) to the pre-washed Immunomagnetic Beads, add 400 µL of 1×TBST buffer and incubate at room temperature for 30 min 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 Protocols

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. Elution Using Sample Buffer

1. Add 100 µL of SDS-PAGE sample buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100^{o}C for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the antigen.

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 -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

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
		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 Buffer and load entire elution fraction on