



Immunoprecipitation/IP Kit- Anti-Isovaleryl-CoA dehydrogenase / IVD Immunomagnetic Beads

**Catalog Number: MB14206-
MM04**

Please read this instruction manual carefully before using the product

Product Contents

| Contents | Package 1 | Package 2 | Storage |
|---|--|--|---------------------|
| Anti-Isovaleryl-CoA dehydrogenase / IVD Immunomagnetic Beads ^{1,3} | 1 mL | 5 mL | 2-8°C for 12 months |
| NP40 Cell Lysis Buffer | 4 mL | 22 mL | -20°C for 12 months |
| 5×TBST (pH7.4) | Required but not supplied | | |
| 1×TBST (pH7.4) | Required but not supplied | | |
| ddH ₂ O | Required but not supplied | | |
| Alkaline Elution Buffer | 3 mL | 15 mL | 2-8°C for 12 months |
| Acidity Elution Buffer | 3 mL | 15 mL | 2-8°C for 12 months |
| Neutralization Buffer | 2 mL | 8 mL | 2-8°C for 12 months |
| Magnetic Separator | Not included (refer related product MAGS001) | One MAGS001 included in China ² | |

[1] The IP KIT contains anti-Isovaleryl-CoA dehydrogenase / IVD 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-Isovaleryl-CoA dehydrogenase / IVD Immunomagnetic Beads, conjugated with Anti-Isovaleryl-CoA dehydrogenase / IVD antibody, are used for immunoprecipitation (IP) of Isovaleryl-CoA dehydrogenase / IVD proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing Isovaleryl-CoA dehydrogenase / IVD proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound Isovaleryl-CoA dehydrogenase / IVD proteins are dissociated from the Immunomagn Antigen Immunomagnetic Beads

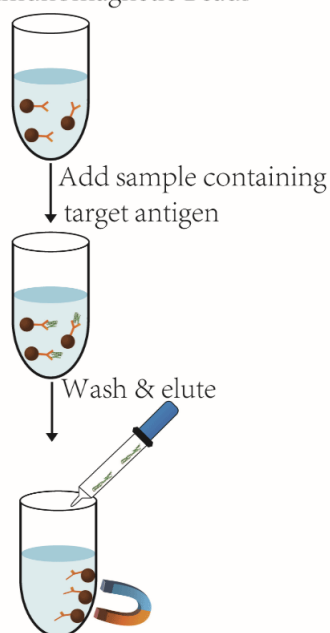


Fig. 1 Immunoprecipitation (IP) Protocol

Antibody Information

Antibody: Isovaleryl-CoA dehydrogenase / IVD Antibody, Mouse MAb(14206-MM04)

Immunogen: Recombinant Human Isovaleryl-CoA dehydrogenase / IVD protein (Catalog#14206-H07B)

Clone ID: 04

Isotype: Mouse IgG2b

Specificity: Human Isovaleryl-CoA dehydrogenase / IVD

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 Isovaleryl-CoA dehydrogenase / IVD (rh Isovaleryl-CoA dehydrogenase / IVD; Catalog#14206-H07B; AAH17202.1; His30-His423). The IgG fraction of the cell culture supernatant was purified by Protein A affinity chromatography.

Protocol

The protocol (Fig. 1) uses 50 μL Anti-Isovaleryl-CoA dehydrogenase / IVD Immunomagnetic 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 \times 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 \times 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 \times TBST buffer and incubate at room temperature for 30 min with mixing.
6. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbound sample and save for analysis.
7. Add 300 μL of 5 \times 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 ddH₂O 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°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 |

| Problem | Possible Cause | Solution |
|--|--|---|
| 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 | | |