Omnimabs® Anti-DYKDDDDK IP(Co-IP) Kit (Magarose)

Packaging List

Name	Catalog Number	Package
Anti-DYKDDDDK IP(Co-IP) Kit (Magarose)	OM643431	40 Tests/Kit

Product Overview

The Omnimabs® Anti-DYKDDDDK Immunoprecipitation (IP) and Co-Immunoprecipitation (Co-IP) Kit (Magarose) contains sufficient reagents for 40 reactions, with each reaction using 25 µL of magnetic beads. It can efficiently complete antigen immunoprecipitation (IP) and immunoprecipitation assays. The Omnimabs® Anti-DYKDDDDK agarose magnetic beads provided in the kit enable rapid and convenient magnetic separation of antigens. The immunoprecipitation kit is equipped with optimized pre-made buffers, providing the best reaction conditions for immunoprecipitation experiments and enhancing the stability of the immunoprecipitation assay. This product can be widely used for immunoprecipitation reactions of antigens in samples such as cell lysates, cell culture supernatants, serum, and ascites.

Kit Components:

Anti-DYKDDDDK Agarose Magnetic Beads	1 mL
IP Lysis/Wash Buffer	100 mL
SDS-PAGE Sample Loading Buffer (5x)	10 mL
Phosphatase Inhibitor Cocktail (50x)	2 mL
PMSF (100x)	1 mL
Elution Buffer	5 mL
Neutralization Buffer	1 mL
Magnetic Rack	Double-row 16-well

Operating Procedure

Adherent Cell Samples:

i. Remove the culture medium and wash the cells twice with PBS.

ii. Collect the cells into a 1.5 mL EP tube, add IP Lysis/Wash Buffer in proportion, and add corresponding inhibitors such as PMSF at the same time. Mix well and let stand on ice for 5 - 20 minutes (mix several times during this period).

iii. Centrifuge at 4°C, 12,000 - 16,000 xg for 10 minutes to collect the supernatant, and place it on ice for subsequent experiments (or store at -80°C for long-term storage).

Recommended volume of IP Lysis/Wash Buffer for culture dishes:

Culture Dish Size/Surface Area	IP Lysis/Wash Buffer Volume
100 mm x 100 mm	500 - 1000 L
100 mm x 60 mm	100 - 300 L
6-well Plate	100 - 200 L

Suspension Cell Samples:

i. Collect the cells at 4°C, 500 - 1000 xg for 10 minutes, and discard the supernatant.

ii. Wash the cells once with PBS, that is, resuspend the cell pellet with PBS, collect the cells at 4°C, 500 - 1000 xg for 5 minutes, and discard the supernatant.

iii. Resuspend the cells with pre-cooled IP Lysis/Wash Buffer. Use 500 µL of IP Lysis/Wash Buffer for every 50 mg of cells. Add corresponding inhibitors such as PMSF at the same time, mix well and let stand on ice for 5 - 20 minutes (mix several times during this period).

iv. Centrifuge at 4°C, 12,000 - 16,000 xg for 10 minutes to collect the supernatant, and place it on ice for subsequent experiments (or store at -80°C for long-term storage).

Serum Samples:

It is generally recommended to dilute the serum sample with IP Lysis/Wash Buffer to a final

concentration of the target protein of 50 - 150 μg/mL, and place it on ice for standby (or store at -20°C for long-term storage).

Immunoprecipitation:

Note: To ensure the even distribution of magnetic beads, mix the magnetic beads in the bottle by repeated inversion or gentle vortexing before use.

i. Add 25 μL of Omnimabs® Anti-DYKDDDDK agarose magnetic beads to a 1.5 mL centrifuge tube.

ii. Add 500 μL of pre-cooled PBS to the magnetic beads and mix gently.

iii. Place the centrifuge tube in the magnetic rack to collect the magnetic beads to one side of the centrifuge tube. Remove the supernatant.

iv. Add 200 - 500 μL of IP Lysis/Wash Buffer to the centrifuge tube. Invert the centrifuge tube several times or gently vortex and mix for 1 minute. Collect the magnetic beads using the magnetic rack. Remove the supernatant.

v. Add the protein sample labeled with the DYKDDDDK tag to the centrifuge tube containing the magnetic beads, keep mixing and incubate at room temperature for 1 - 2 hours, or at 4°C for 2 - 4 hours.

vi. Collect the magnetic beads using the magnetic rack, remove the unbound sample, and save for analysis.

vii. Add 1000 μL of IP Lysis/Wash Buffer to the centrifuge tube, gently mix the magnetic beads for 5 - 10 minutes. Collect the magnetic beads and discard the supernatant. Repeat the washing two more times.

viii. Denaturing elution: Add 80 - 100 μL of SDS-PAGE Sample Loading Buffer (1×) to the centrifuge tube, and heat the sample in a 100°C water bath or metal bath for 10 minutes. Separate the magnetic beads by magnetic rack and retain the supernatant containing the target antigen.
Note: If it is necessary to maintain the activity of the protein, the following elution method can also be used. Low pH elution: Add 100 μL of Elution Buffer to the centrifuge tube. Keep mixing and incubate the centrifuge tube at room temperature for 5 - 10 minutes. Separate the magnetic beads by magnetic separation and retain the supernatant containing the target antigen. Add 20 μL of Neutralization Buffer to every 100 μL of the eluate to neutralize the low pH.

Precautions

1. Before performing the experiment, please read this operating manual carefully.
2. Do not centrifuge, dry or freeze the magnetic beads at high speed, as these operations will cause the magnetic beads to aggregate and reduce the binding ability.

3. The affinity of different types of antibodies to antigens in IP experiments is different, and the binding of antibodies to antigens will also be affected by the IP Lysis/Wash Buffer. Therefore, the operation details can be optimized or the buffer can be screened and prepared for the experiment.
4. The magnetic beads should be fully shaken before use. The magnetic beads should be stored in the storage solution to prevent drying.
5. Low-temperature transportation: Anti-DYKDDDDK agarose magnetic beads, IP Lysis/Wash Buffer, Elution Buffer, and Neutralization Buffer are stored at 4°C, and PMSF, Phosphatase Inhibitor Cocktail, and SDS-PAGE Sample Loading Buffer are stored at -20°C.
6. This product is for scientific research use only.

Problem Solving

1. **Antigen not immunoprecipitated**:
    i. The amount of antigen in the sample is too low to be detected.
    Suggestion: Verify the expression and/or lysis efficiency of the protein in the lysate by SDS-PAGE or Western blotting; if necessary, increase the sample amount.
    ii. The components in the IP Lysis/Wash Buffer interfere with the binding of the antigen to the antibody.
    Suggestion: Use other buffers for immunoprecipitation and rinsing (for example, TBS containing 0.5% CHAPS).
2. **Low amount of protein obtained**:
    i. The protein is degraded.
    Suggestion: Add protease inhibitors.
    ii. The amount of magnetic beads used is insufficient.
    Suggestion: Increase the amount of magnetic beads used to capture the immunocomplex.
    iii. The amount of the target protein in the sample is insufficient.
    Suggestion: Increase the amount of the antigen sample.
3. **Multiple non-specific bands**:
    i. Non-specific proteins bind to the magnetic beads.
    Suggestion: Add 50 - 350 mM NaCl to the IP Lysis/Wash Buffer. Increase the elution strength and number of times.