Omnimabs® Protein A IP (Co-IP) Kit

Packaging List

Name	Catalog Number	Package
Protein A IP (Co-IP) Kit	OM643428	40 Tests/Kit

Product Overview

The Omnimabs® Protein A Immunoprecipitation (IP) and Co-Immunoprecipitation (Co-IP) Kit 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, with the antibody amount less than 10 µg and no need for centrifugation. The Omnimabs® Protein A 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:

Protein A 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:

1. Remove the culture medium and wash the cells twice with PBS.
2. Collect the cells into a 1.5 mL EP tube, add IP Lysis/Wash Buffer in proportion, and add corresponding inhibitors such as PMSF. Mix well and let stand on ice for 5 - 20 minutes (mix several times during this period).
3. Centrifuge at 4°C, 12000 - 16000 xg for 10 minutes to collect the supernatant. Place 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:

1. Collect the cells at 4°C, 500 - 1000 xg for 10 minutes and discard the supernatant.
2. Wash the cells once with PBS, i.e., resuspend the cell pellet with PBS. Collect the cells at 4°C, 500 - 1000 xg for 5 minutes and discard the supernatant.
3. Resuspend the cells with pre-cooled IP Lysis/Wash Buffer. Use 500 µL IP Lysis/Wash Buffer for every 50 mg of cells. Add corresponding inhibitors such as PMSF, mix well and let stand on ice for 5 - 20 minutes (mix several times during this period).
4. Centrifuge at 4°C, 12000 - 16000 xg for 10 minutes to collect the supernatant. Place 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 target protein concentration of 50 - 150 µg/mL. Place on ice for standby (or store at -20°C for long-term storage).

Preparation of Immunocomplex:

Note: The amount of sample and incubation time required depend on each specific antibody-antigen system, so optimization may be required to obtain the maximum yield.
The following experimental protocol is for 2 - 10 µg of affinity-purified antibody and can be scaled up proportionally as needed.

1. In a centrifuge tube, combine the cell lysate of each sample with 2-10 µg of immunoprecipitation antibody. The recommended total protein amount for each immunoprecipitation reaction is 500 - 1500 µg.
2. Dilute the antibody and the prepared sample to 300 - 500 µL with IP Lysis/Wash Buffer.
3. Incubate at room temperature for 1 - 2 hours, or at 4°C for 2 - 4 hours to form the immunocomplex.

Immunoprecipitation:

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

1. Add 25 µL (0.25 mg) of Omnimabs® Protein A magnetic beads to a 1.5 mL centrifuge tube.
2. Add 500 µL of pre-cooled PBS to the magnetic beads and mix gently.
3. Place the centrifuge tube in the magnetic rack to collect the magnetic beads to one side of the centrifuge tube. Remove the supernatant.
4. Add 200 - 500 µL of IP Lysis/Wash Buffer to the centrifuge tube. Invert the centrifuge tube several times or gently vortex for 1 minute. Collect the magnetic beads using the magnetic rack. Remove the supernatant.
5. Add the His-tagged protein sample to the centrifuge tube containing the magnetic beads. Incubate at room temperature with continuous mixing for 1 - 2 hours, or at 4°C for 2 - 4 hours.
6. Collect the magnetic beads using the magnetic rack and remove the unbound sample. Save for analysis.
7. Add 1000 µL of IP Lysis/Wash Buffer to the centrifuge tube and gently mix the magnetic beads for 5 - 10 minutes. Collect the magnetic beads and discard the supernatant. Repeat the washing two more times.
8. 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 using the magnetic rack and retain the supernatant containing the target antigen.

Note: If it is necessary to maintain protein activity, the following elution method can also be used. Low pH Elution: Add 100 µL of Elution Buffer to the centrifuge tube. Incubate the centrifuge tube at room temperature with continuous mixing for 5 - 10 minutes. Separate the magnetic beads using a magnet 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. Please read this operating manual carefully before conducting the experiment.
2. Do not centrifuge, dry or freeze the magnetic beads at high speed. These operations will cause the magnetic beads to aggregate and reduce their binding ability.
3. The affinity of different types of antibodies to antigens in IP experiments is different, and the binding of antibodies to antigens is also affected by the IP Lysis/Wash Buffer. Therefore, you can optimize the operation details by yourself or screen and prepare buffers for the experiment.
4. The magnetic beads should be shaken thoroughly before use. The magnetic beads should be stored in the storage solution to prevent drying.
5. Low-temperature Transportation: Protein A 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 only.

Problem Solving

1. **Antigen not immunoprecipitated**:

   Insufficient antigen in the sample for detection:

   Suggestion: Verify the protein expression and/or lysis efficiency in the lysate by SDS-PAGE or Western blotting; increase the sample volume if necessary.

   Components in the IP Lysis/Wash Buffer interfere with the binding of the antigen to the antibody:

   Suggestion: Use other buffers for immunoprecipitation and washing (e.g., TBS containing 0.5% CHAPS).

2. **Low protein yield**:

   Protein degradation:

Suggestion: Add protease inhibitors.

Insufficient amount of magnetic beads used:

Suggestion: Increase the amount of magnetic beads used to capture the immune complex.

Insufficient amount of target protein in the sample:

Suggestion: Increase the amount of antigen sample.

3. **Multiple non-specific bands**:

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.

4. **Magnetic bead aggregation**:

Aggregation of magnetic beads in the low pH Elution Buffer is normal and does not affect the normal use of the magnetic beads:

Sugestion: Adjust the pH to neutral with IP Lysis/Wash Buffer, then resuspend the magnetic beads with Tris buffer (pH 7.5) containing 0.1% (v/v) Tween-20 and treat with an ultrasonic water bath to restore the uniform state of the magnetic beads. These treatments do not affect the antibody binding efficiency of the magnetic beads. Adding a non-ionic detergent (such as Tween-20 or Triton X-100) at a final concentration of 0.1% (v/v) can effectively prevent magnetic bead aggregation. **Note**: Ultrasonic treatment will also cause the antibodies captured by the magnetic beads in the sample solution to fall off, so this method should not be used after adding the sample and before elution.