Omnimabs® Protein A IP(Co-IP) Kit (Gel Method)

Packaging List

Name	Catalog Number	Package
Protein A IP(Co-IP) Kit(Gel Method)	OM643437	40 Tests/Kit

Product Overview

The Omnimabs® Protein A Immunoprecipitation (IP) and Co-Immunoprecipitation (Co-IP) Kit (Gel Method) contains sufficient reagents for 40 reactions, with each reaction using 25 µL of gel. It can efficiently complete antigen immunoprecipitation (IP) and immunoprecipitation assays, with the antibody amount less than 10 µg. The Omnimabs® Protein A agarose gel provided in the kit enables rapid and convenient antigen separation. 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 Agarose Gel	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

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 Gmount 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 the even distribution of gel, mix the gel in the bottle by repeated inversion or gentle vortexing before use.

i. Add 25 μL of Omnimabs® Protein A agarose gel to a 1.5 mL centrifuge tube.

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

iii. Place the centrifuge tube in the centrifuge and centrifuge at 1000 rpm for 5 minutes to collect the gel at the bottom 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. Place the centrifuge tube in the centrifuge and centrifuge at 1000 rpm for 5 minutes to collect the gel at the bottom of the centrifuge tube. Remove the supernatant.

v. Add the antigen sample/antibody mixture to the centrifuge tube containing the gel, keep mixing and incubate at room temperature for 1 - 2 hours, or at 4°C for 2 - 4 hours.

vi. Centrifuge at 1000 rpm for 5 minutes to collect the gel, remove the unbound sample, and save for analysis.

vii. Add 1000 μL of IP Lysis/Wash Buffer to the centrifuge tube, gently mix for 5 - 10 minutes. Collect the gel 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 gel by centrifugation 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 gel by centrifugation 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. 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.
3. The agarose gel should be fully shaken before use. The gel should be stored in the storage solution to prevent drying.
4. Low-temperature transportation: Protein A agarose gel, 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.
5. 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 antibody cannot bind to the antigen.
   Suggestion: Select another specific antibody or an antibody that recognizes a different epitope of the antigen.

   iii. 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 gel used is insufficient.
   Suggestion: Increase the amount of gel 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 gel.
Suggestion: Add 50 - 350 mM NaCl to the IP Lysis/Wash Buffer. Increase the elution strength and number of times.

4. Gel easily adheres to the tube wall:

Suggestion: Use consumables with a low adsorption rate for gel operation.