# Omnimabs® Anti-DYKDDDDK IP (Co-IP) Kit (Gel Method)

## Packaging List

| Name | Catalog Number | Packaging |
|---|---|---|
| Anti-DYKDDDDK IP (Co-IP) Kit (Gel Method) | OM643419 | 40T/Kit |

## Product Overview

The Omnimabs® Anti-DYKDDDDK Immunoprecipitation (Co-IP) Kit (Gel Method) contains reagents sufficient for 40 reactions, with 25μL gel used per reaction. It enables efficient antigen immunoprecipitation (IP) and immunoprecipitation (Co-IP) experiments. The Omnimabs® Anti-DYKDDDDK agarose gel provided in the kit allows for efficient and rapid antigen separation. The immunoprecipitation (Co-IP) kit is equipped with optimized pre-made buffers, providing the best reaction conditions for immunoprecipitation experiments and enhancing the stability of the experiments. This product can be widely used for immunoprecipitation reactions of antigens in samples such as cell lysates, cell supernatant, serum, and ascites.

## Kit Composition:

| Anti-DYKDDDDK 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).

## Immunoprecipitation:

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

1. Add 25 µL of Omnimabs® Anti-DYKDDDDK agarose gel to a 1.5 mL centrifuge tube.

2. Add 500 µL of pre-cooled PBS to the gel and mix gently.
3. 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.
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. 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.
5. Add the prepared DYKDDDDK-tagged protein sample to the centrifuge tube containing the gel. Incubate at room temperature with continuous mixing for 1 - 2 hours, or at 4°C for 2 - 4 hours.
6. Centrifuge at 1000 rpm for 5 minutes to collect the gel and remove the unbound sample. Save for analysis.
7. Add 1000 µL of IP Lysis/Wash Buffer to the centrifuge tube and gently mix for 5 - 10 minutes. Collect the gel 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 gel by centrifugation 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 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. Please read this operating manual carefully before conducting the experiment.
2. 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.
3. The agarose gel should be shaken thoroughly before use. The gel should be stored in the storage solution to prevent drying.
4. Low-temperature Transportation: Anti-DYKDDDDK 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 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 gel used**:

   **Suggestion**: Increase the amount of gel 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 gel**:

   **Suggestion**: Add 50 - 350 mM NaCl to the IP Lysis/Wash Buffer. Increase the elution strength and number of times.

4. **Gel adheres to the tube wall easily**:

   **Suggestion**: Use low-adsorption consumables for gel operation.