



## Basic Immunoassay (co) Precipitation Kit PRODUCT DATA SHEET

### Basic Immunoassay (co) Precipitation Kit

**Cat No: AKIT-Basics**

#### Description

Basic immunoprecipitation kit contains enough reagents to complete 40 reactions. It can efficiently complete antigen immunoprecipitation (IP) and immunocoprecipitation (Co-IP) experiments.

The immunoprecipitation kit is equipped with optimized prefabricated buffer, which provides the best reaction conditions for the immunoprecipitation experiment and enhances the stability of the immunoprecipitation experiment. This product can be widely used in the immunoprecipitation reaction of antigens in cell lysate, cell secreted supernatant, serum, ascites and other samples.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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#### Kit Composition

IP Lysis/Wash Buffer	100 mL
SDS-PAGE Sample Loading Buffer (5 ×)	10 mL
Phosphatase inhibitor cocktail (50 ×)	2 mL
PMSF (100 ×)	1 mL
Elution Buffer	5 mL
Neutralization Buffer	1 mL

#### Operation Process

**(Take Protein A/G magnetic beads as an example)**

##### Adherent cell sample:

1. Remove the medium and wash the cells twice with PBS.
2. Cells were collected into 1.5 mL EP tube and IP Lysis/Wash Buffer was added proportioned, and add corresponding inhibitor such as PMSF. After mixing, the cells were placed on ice for 5 ~ 20 min (mixed several times).



3. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

**Petri dish IP Lysis/Wash Buffer Recommended volume:**

Petri dish size/surface area	Volume of IP Lysis/Wash Buffer
100 mm x 100 mm	500 ~ 1000 $\mu$ L
100 mm x 60 mm	100 ~ 300 $\mu$ L
6-well plate	100 ~ 200 $\mu$ L

**Suspended cell sample:**

1. Cells were collected at 4°C, 500 ~ 1000 xg, 10 min, and the supernatant was discarded.
2. Wash the cells with PBS once, that is, suspend the cell mass with PBS at 4°C, 500 ~ 1000 xg, 5 min, collect the cells, discard the supernatant.
3. Resuspend cells with pre-cooled IP Lysis/Wash Buffer. The 500  $\mu$ L IP Lysis/Wash Buffer was used for every 50 mg cells. At the same time, add the corresponding inhibitors such as PMSF, mix well and put on the ice for 5 ~ 20 min (mix several times during the period).
4. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

**Serum sample:**

It is recommended to dilute a serum sample with IP Lysis/Wash Buffer to a final concentration of target protein of 50 to 150  $\mu$ g/mL and place it on ice for future use (or place it at -20°C for long-term storage).

**Preparation of immune complex**

**Note: The amount and incubation time required for the sample are dependent on each specific antibody antigen system and may need to be optimized for maximum yield.**

The following protocol is for 2 ~ 10  $\mu$ g affinity purified antibodies, which can be scaled up as required.

1. In a centrifuge tube, the cell lysate of each sample was combined with 2 ~ 10  $\mu$ g immunoprecipitation antibody. The recommended total protein amount for each immunoprecipitation reaction is 500 ~ 1500  $\mu$ g.
2. Dilute the antibody and prepared sample to 300 ~ 500  $\mu$ L from IP Lysis/Wash Buffer.
3. Incubate at room temperature for 1 ~ 2 h, or 4°C for 2 ~ 4 h to form immune complexes.

**Immunoprecipitation:**

**Note: In order to ensure the uniform distribution of magnetic beads, mix the magnetic beads in the bottle by repeatedly reversing or slightly vortexing before use.**



1. Add 20 ~ 50  $\mu$ L of Protein A/G magnetic bead into 1.5 mL centrifuge tube.
2. Add 500  $\mu$ L pre-cooled PBS to the magnetic bead and mix gently.
3. Put the centrifugal tube into the magnetic rack and collect magnetic beads to one side of the centrifugal tube. Remove the supernatant.
4. Add 200 ~ 500  $\mu$ L IP Lysis/Wash Buffer to the centrifuge tube. Reverse the centrifugal tube several times or mix with a slight vortex for 1 min. Use a magnetic rack to collect magnetic beads. Remove the supernatant.
5. Add the antigen sample/antibody mixture into the centrifuge tube fitted with magnetic beads and incubate at room temperature for 1 ~ 2 h, or 4°C 2 ~ 4 h.
6. Collect magnetic beads with a magnetic rack, remove uncombined samples, and store them for analysis.
7. Add 1000  $\mu$ L IP Lysis/Wash Buffer to the centrifuge tube and gently mix the magnetic ball for 5 ~ 10 min. Collect the beads and discard the supernatant. Repeat twice.
8. **Denaturing elution:** Add 80 ~ 100  $\mu$ L SDS-PAGE Sample Loading Buffer (1 $\times$ ) to the centrifuge tube and heat the sample in 100°C water bath or metal bath for 10 min. Magnetic beads are separated by magnetic rack to retain the supernatant containing the target antigen.

**Note:** The following elution methods can also be used to maintain protein activity.

**Low pH Elution:** Add 100  $\mu$ L Elution Buffer to centrifuge tube. Keep mixed and incubate centrifuge tube at room temperature for 5 ~ 10 min. Magnetic beads are magnetically separated to retain the supernatant containing the target antigen. Low pH is neutralized by adding 20  $\mu$ L Neutralization buffers per 100  $\mu$ L of wash out solution.

## Notes

1. Before performing the experiment, please read this operation manual carefully.
2. Do not centrifuge, dry or freeze magnetic beads at high speed, these operations will cause magnetic beads to gather and reduce the binding ability.
3. The affinities between different types of antibodies and antigen Lysis are different in IP experiments. The binding of antibodies to antigens is also affected by IP Lysis/Wash Buffer, so **you can do your own experiments by optimizing the operation details or screening and formulating buffers.**
4. Magnetic beads should be fully oscillated and uniform before use. Magnetic beads should be kept in storage solution to prevent drying.



5. Transported at low temperature, IP Lysis/Wash Buffer, Elution Buffer and Neutralization Buffer are **stored at 4°C**. PMSF, Phosphatase inhibitor cocktail and SDS-PAGE Sample Loading Buffer were **stored at -20°C**.

6. This product is for scientific research only.

## **Problem Solving**

### **1. No immunoprecipitation of antigen**

① The sample contains too little antigen to be detected

**Recommendation:** To verify the expression and/or cleavage efficiency of proteins in the lysate by SDS-PAGE or protein immunoblotting; If necessary, increase the sample size

② Antibodies cannot bind to antigens

**Recommendation:** Choose another specific antibody, or choose another antibody that recognizes a different epitope.

③ Ingredients in IP Lysis/Wash Buffer interfere with the binding of antigens to antibodies

**Recommendation:** Immunoprecipitation and rinsing with other buffers (e.g., TBS containing 0.5% CHAPS)

### **2. Low protein intake**

① The protein is degraded

**Recommendation:** Add protease inhibitor

② The amount of magnetic beads used is not enough

**Recommendation:** Increase the amount of magnetic beads used to capture immune complexes

③ The amount of target protein in the sample is not enough

**Recommendation:** Increase the amount of antigen sample

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

There are non-specific proteins that bind to the magnetic beads

**Recommendation:** Add 50 ~ 350 mM NaCl to IP Lysis/Wash Buffer. Increase elution intensity and frequency.

### **4. Magnetic beads gather**

The aggregation of magnetic beads in the low pH Elution Buffer is a normal phenomenon and does not affect the normal use of magnetic beads.

**Recommendation:** The magnetic beads can be restored to uniform state by using IP Lysis/Wash Buffer to neutral and then oscillating with Tris buffer (pH 7.5) containing 0.1% (v/v) Tweene-20 and treated



with ultrasonic water bath. None of the above treatments affect the antibody binding efficiency of the magnetic beads. A non-ionic detergent with a final concentration of 0.1% (v/v), such as Tween-20 or Triton X-100, can also be added to effectively prevent the accumulation of magnetic beads.

**Note:** Ultrasonic treatment will also remove antibodies captured by magnetic beads in the sample solution, so this method should not be used before elution of magnetic beads after sample addition.

#### **Ordering Information**

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