Anti-HA Immunoassay (co) Precipitation Kit (G2)

Cat No: AKIT-Anti-HA

Description

The Anti-HA immunoprecipitation kit (G2) contains enough reagents to complete 40 reactions using 25 µL magnetic beads per reaction. It can efficiently complete antigen immunoprecipitation (IP) and immunocoprecipitation (Co-IP) experiments.

The Anti-HA magnetic bead (G2) provided in the kit enables rapid and convenient magnetic separation of antigens. 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

Anti-HA magnetic beads (G2)	1 mL
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
Magnetic rack	Double row of 16 holes

Operation Process

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 µL
100 mm x 60 mm	100 ~ 300 µL
6-well plate	100 ~ 200 µL

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 µL 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 µg/mL and place it on ice for future use (or place it at -20°C for long-term storage).

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 25 µL (0.25 mg) of Anti-HA magnetic bead (G2) into 1.5 mL centrifuge tube.

2. Add 500 µL pre-cooled PBS to the magnetic bead and mix gently.

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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 µL 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 protein samples containing HA labels 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 µL 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 µL SDS-PAGE Sample Loading Buffer (1×) 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 µL 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 µL Neutralization buffers per 100 µL 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:** Anti-HA magnetic bead (G2), 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**.

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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 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.

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Ordering Information

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