Animal Co-Immunoprecipitation (ChIP)Kit

Catalog#JKR23001A

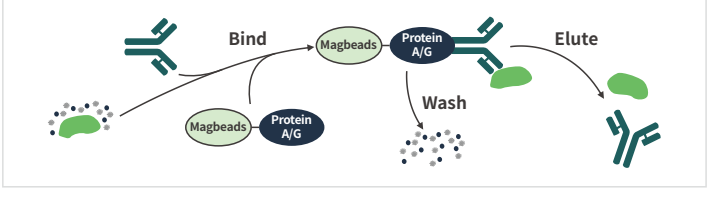
Instruction Manual (For Two Groups)

Sufficient reagents for 6T Co-IP assays perkit.
Store at -20 & 4°C

Table of Contents

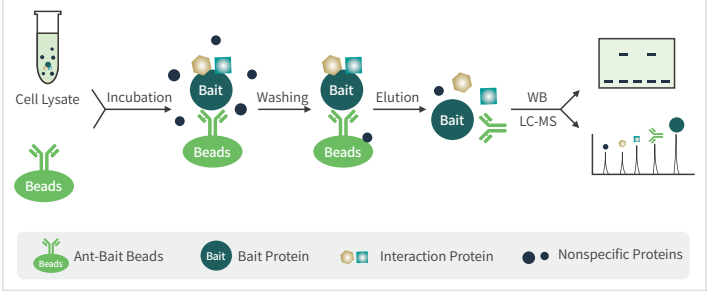
1. Experimental Principle

Co-Immunoprecipitation (Co-IP) is a method based on the specific interaction betweenantigens and antibodies, used to study protein-protein interactions. After antibodies bind to their corresponding proteins in the lysate, they are incubated with Protein A/G-coupled Sepharose or Magnetic Beads. The Protein A/G bead-antibody-target protein complex is then obtained via centrifugation or a magnetic stand. Under high temperature and reducing agents, the antigen and antibody dissociate, and the supernatant—containing antibodies, target proteins, and a small amount of contaminating proteins—is collected. The proteins are subsequently identified by Western Blot or mass spectrometry (MS). Its schematic diagram is as follows:

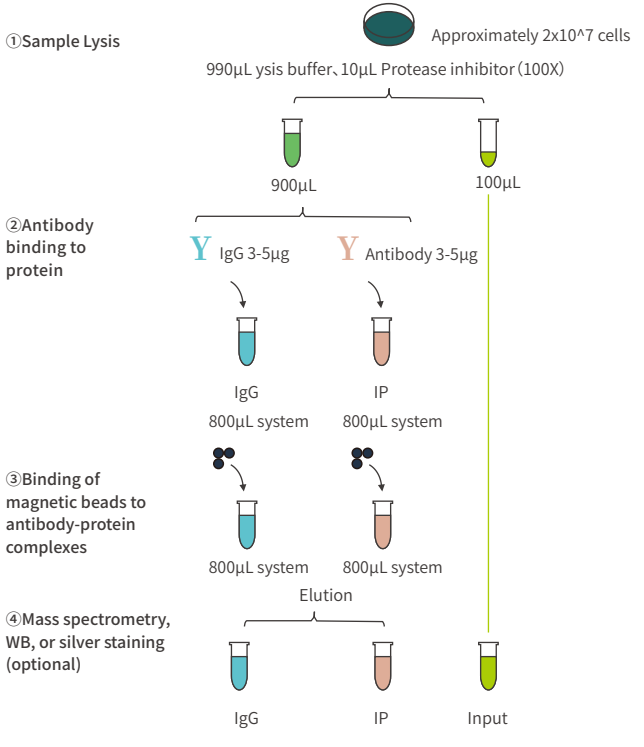


2. Technical Roadmap

2.1 Experimental Flowchart



2.2 Control Setup Flowchart



3. Kit Components

Component	Volume(6T)	Storage Temperature
Lysis buffer	9mL	4°C
Protein A/G Magnetic Beads	200μL	4°C
Incubation buffer	4.5mL	4°C
Wash buffer	20mL	4°C
Elution buffer	700μL	Protect from light at 4°C
Protease inhibitor (100X)	35μL	-20°C
Normal Rabbit IgG (1mg/mL)	30μL	-20°C
Normal Mouse IgG (1mg/mL)	30μL	-20°C

Special reminder 1: Reagents and consumables such as PBS, 6X loading buffer, and magnetic rack need to be prepared by the user.
Special reminder 2: 6T refers to 6 single-group (1 IP group or 1 IgG group) immunoprecipitation experiments; the subsequent steps include 1 IgG group and 1 IP group, consuming 2T of reagent.

4. Procedure

4.1 Total protein extraction

4.1.1 Cell samples

1) Washing: Wash the sample (approximately 2×10^7 cells) twice with 1 mL of pre-cooled PBS, and aspirate the PBS as much as possible after the last wash;
2) Lysis: Add 990 μL of Lysis buffer and 10 μL of Protease inhibitor (100X) according to the cell count, fully lyse on ice for 30 min, inverting to mix every 5 min;
3) Sonication: Sonicate for 5 min using an ultrasonic cell disruptor at 20% power, with 3 s on and 3 s off, in an ice bath;
4) Centrifugation: Centrifuge at 4°C, 12000 rpm for 10 min, and collect the supernatant.

4.1.2 Tissue Samples

1) Grinding: Take fresh or cryopreserved tissue (approximately 0.3g), place it in a sterilized, pre-cooled mortar, and grind with liquid nitrogen until powdered;

2) Lysis: Mix 990 μL Lysis buffer and 10 μL Protease inhibitor (100X) as lysis solution, pipette 800 μL into a mortar, continue grinding on ice for 5-10min until the sample becomes a fine homogenate, transfer to a new EP tube, then add the remaining 200 μL lysis solution to the mortar to collect residual sample, and also transfer to the same EP tube;
3) Fully lyse the EP tube containing the sample homogenate on ice for 30 min, inverting to mix every 5 min;
4) Sonication: Sonicate using an ultrasonic cell disruptor for 8-10min at 20% power, with 3s on and 3s off, in an ice bath;
5) Centrifugation: 4°C, 12000 rpm, 10 min, collect the supernatant, then add Lysis buffer to the supernatant to bring the volume to 1 mL, mix well.

Note: The entire protein extraction process is performed on ice to reduce protein degradation caused by high temperature; avoid bubble formation during sonication to minimize protein degradation. Store total protein at -20°C.

4.2 CoIP

4.2.1 Antibody-bound protein

1) Prepare two 1.5 mL EP tubes labeled as IP and IgG; add 3-5 μg of target antibody to the IP tube, and add 3-5 μg of species-matched IgG to the IgG tube;
2) Take 100 μL of the total protein solution from step 4.1, label it as Input, and store at -20°C for later use. Add 450 μL of protein solution to the IgG and IP tubes respectively, then supplement the volume to 800 μL with Incubation buffer. Incubate with gentle mixing overnight at 4°C (approximately 16 h).

4.2.2 Magnetic Bead Preparation

1) Remove the Protein A/G Magnetic Beads from the 4°C refrigerator, invert several times to mix the beads and solution thoroughly. Transfer 30 μL each into two new 1.5 mL EP tubes, labeled as IgG and IP;
2) Add 0.5 mL of pre-chilled Wash buffer to both the IgG and IP tubes to resuspend the magnetic beads, place them on a magnetic stand for 1 min to separate the beads from the solution, carefully aspirate and discard the supernatant with a pipette, and repeat this step 3 times.

4.2.3 Magnetic Bead and Complex Binding

1) Add the two sets of incubated mixtures to the corresponding washed magnetic bead tubes, and incubate with gentle mixing at room temperature for 2 h;
2) Place the two tubes on a magnetic stand for 1 min to separate the beads from the solution, and carefully aspirate and discard the supernatant with a pipette.

3) Add 0.5 mL of pre-chilled Wash buffer to both the IgG and IP tubes, place them on a magnetic stand for 1 min to separate the beads and solution, carefully aspirate and discard the supernatant with a pipette, and repeat this step 3 times.

4.2.4 Elution

Add 100 μL of Elution buffer to both the IgG and IP tubes, incubate in a boiling water bath for 10 min, place on a magnetic stand for 2 min, collect the supernatant as the eluate, mix with 20 μL of 6× SDS loading buffer for the Input group, boil for 10 min, and store at -20°C for later use.

4.3 WB (Optional)

Take 20 μL each of the IgG, IP, and Input group samples obtained from step 4.2.4 to perform WB detection.

4.4 Silver Staining (Optional)

1) Take 20 μL of the IgG, IP, and Input group samples obtained in step 4.2.4 and perform SDS-PAGE gel electrophoresis.
2) Fixation: Peel the post-electrophoresis gel from the glass plate, rinse it clean with water, place it in a clean 12 cm diameter glass dish, add deionized water to cover the gel, cover with a lid, shake on a destaining shaker at room temperature for 5 min, discard the deionized water, add fixation solution to cover the gel, cover with a lid, and shake on a destaining shaker at room temperature for 30 min.
3) Sensitization: Discard the fixative, add deionized water to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 5 min, repeat washing with water once, for a total of 2 times. Add sensitization solution to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 30 min.
4) Staining: Discard the sensitization solution, add deionized water to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 2 min, repeat washing with water once, for a total of 2 times. Add staining solution to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 20 min.
5) Staining: Discard the staining solution, add deionized water to cover the gel, cover the lid, shake at room temperature on a destaining shaker for 1 min, repeat the water wash once, for a total of 2 times. Add developing solution to cover the gel, shake at room temperature on a destaining shaker for about 2 min until the solution turns cloudy, discard the liquid, add fresh developing solution and continue developing until the target bands are clear, then photograph.

4.5 Mass Spectrometry (Optional)

Take 30 μL of IgG and IP histone samples for LC-MS detection.

5. Frequently Asked Questions

Q1: After CoIP followed by WB validation, no desired target band is observed?

A: This can be caused by various factors:

1) Possibly the sample is degraded by proteases; the corresponding strategy is to add protease inhibitors, perform all operations on ice below 4°C, and avoid repeated freeze-thaw cycles.
2) Possibly the antibody concentration is too low, resulting in faint bands; then adjust the IP or WB antibody concentration, and if necessary, set up a concentration gradient to determine the optimal concentration.
3) The antibody affinity is too low; select antibodies suitable for IP or WB.
4) Some IP antibodies did not bind to the magnetic beads; in this case, magnetic beads suitable for IP should be selected.
5) If the Tag is not exposed on the surface of the fusion protein conformation, the Tag fusion expression site needs to be changed.
6) The salt-alkalinity of the lysis buffer is too high; a lysis buffer with lower salt-alkalinity should be used.
7) Inappropriate antibody selection; replace the antibody.

Q2: After CoIP followed by WB verification, it was found that although the target band is visible, the background is very high:

A: Caused by multiple factors:

1) High background due to non-specific protein binding; to avoid primary specific protein binding, lyse cells in serum-free solution, pre-wash immunoprecipitation with protein A/G magnetic beads, and increase rinse frequency and salt/alkalinity (high salt or detergent) after immunoprecipitation.
2) Contamination of experimental instruments or reagents; use clean instruments and reagents.
3) High background from non-specific adsorption on the transfer membrane; wear gloves during experimental operations, use tweezers for handling, and avoid touching the membrane transfer surface.
4) If large protein complexes that are not fully dissolved may be present in the prepared sample, perform brief sonication after sample preparation (3 times, 5 sec each), then centrifuge and use the supernatant for subsequent experiments.
5) If washing is insufficient, perform multiple washes and consider increasing the concentration of NaCl and detergent in the wash solution.
6) If non-specific proteins may adsorb to the beads, perform preclearing to eliminate non-specific adsorption.
7) If the antibody itself has poor specificity, potentially leading to high background, select a suitable antibody, considering monoclonal antibodies.
8) If excessive cells or tissues are used for lysis, causing high background, reduce the sample amount; 100-500 μg of cell lysate is recommended.
9) Protein degradation may also result in high background; try to use freshly prepared samples whenever possible.

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