Plant Co-Immunoprecipitation (Co-IP) Kit

Catalog#JKR23001

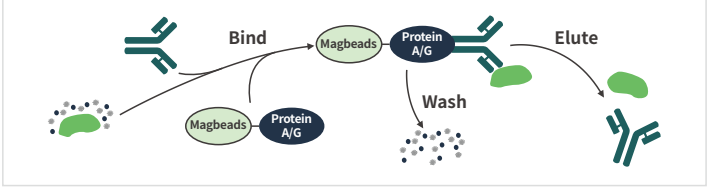
Instruction Manual (For Two Groups)

Sufficient reagents for 6T Co-IP assays per kit.
Store at -20 & 4℃

Table of Contents

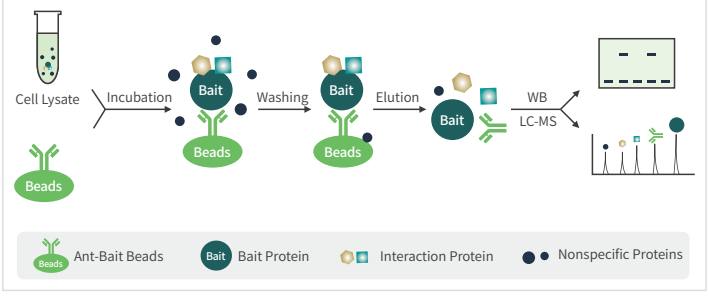
1.Experimental Principle

Co-Immunoprecipitation (Co-IP) is a method based on the specific interaction between antigens 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. Proteins are subsequently identified by Western Blot or mass spectrometry (MS). Its schematic diagram is as follows:

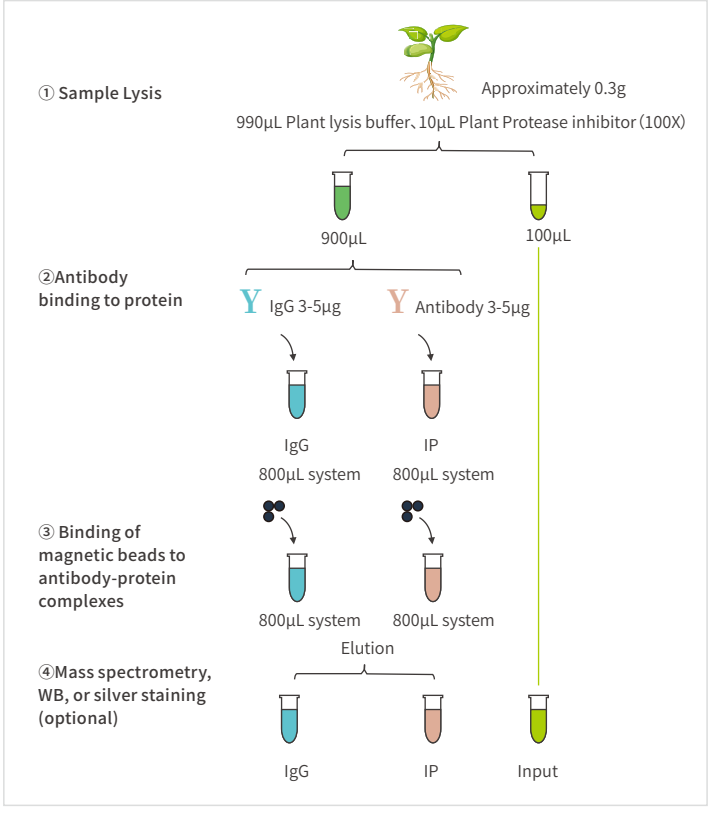


2.Technical Roadmap

2.1 Experimental Flowchart



2.2 Control Setup Flowchart



3.Kit Components

Component	Volume (6T)	Storage Temperature
Plant lysis buffer	9mL	4°C
ProteinA/G Magnetic Beads	200μL	4°C
Incubation buffer	4.5mL	4°C
Wash buffer	20mL	4°C
Elution buffer	700μL	4°C, protect from light
Plant protease inhibitor (100 X)	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.Operating Steps

4.1 Total protein extraction

4.1.1 Plant samples

1) Grinding: Take fresh young tissue or cryopreserved tissue (approximately 0.3g), place it in a sterilized and pre-cooled mortar, and grind with liquid nitrogen until powdered;
2) Lysis: Mix 990 μL Plant Lysis buffer and 10 μL Plant Protease inhibitor (100X) as the lysis solution, pipette 800 μL of the lysis solution into the mortar, continue grinding on ice for 5-10 min until the sample forms 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 similarly transfer it to the EP tube;
3) Fully lyse the EP tube containing the sample homogenate on ice for 30 min , inverting and mixing once every 5 min ;
4) Ultrasonication: Sonicate using an ultrasonic cell disruptor for 12–15 min at 20% power, with 3 sec of sonication and 3 sec of intervals, performed in an ice bath;
5) Centrifugation: 4°C, 12000 rpm, 10 min, collect the supernatant, then add Plant Lysis

buffer to the supernatant to bring the total volume to 1 mL, mix well.

Note: Perform the entire protein extraction process on ice to reduce protein degradation caused by high temperature; avoid bubble formation during sonication to minimize protein degradation. Store the total protein after lysis 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 the target antibody to the IP group, and add 3-5μg of the same species 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 total protein solution to the IgG and IP tubes respectively, then supplement the volume to 800 μL with Incubation buffer. Incubate with gentle mixing overnight (approximately 16 h) at 4°C.

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 30μL 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 three 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 1min to separate the beads from the solution, carefully aspirate and discard the supernatant with a pipette, and repeat this step three 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 x SDS Loading buffer from the Input group, boil for 10 min, and store at -20°C for future use.

4.3 WB (Optional)

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

4.4 Silver Staining (Optional)

1) Take 20 μL each of the IgG, IP, and Input group samples obtained in step 4.2.4 to 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 at room temperature on a decolorizing shaker for 5 min, discard the deionized water, add fixation solution to cover the gel, cover with a lid, and shake at room temperature on a decolorizing shaker 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) Color Development : 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 verification, no desired target band is found?

A: Caused by various reasons:

1) It is possible that the sample was 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) It may be due to low antibody concentration 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 an antibody suitable for IP or WB.
4) Some IP antibodies are not bound to the magnetic beads; in this case, choose magnetic beads suitable for IP.
5) If the Tag is not exposed on the surface of the fusion protein conformation, change the Tag fusion expression site.
6) The salt or alkalinity of the lysis buffer is too high; use a lysis buffer with lower salt or alkalinity. Inappropriate antibody selection; replace the antibody.

Q2: Validation by WB after CoIP shows that although the target band is visible, the background is high:

A: Caused by multiple factors:

1) High background due to non-specific protein binding. To avoid non-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 due to non-specific adsorption on the transfer membrane; wear gloves during experimental operations, use tweezers to handle, and avoid touching the membrane transfer surface.
4) If there may be large protein complexes that are not fully dissolved in the prepared sample, perform brief sonication after sample preparation (3 times, 5 sec each), then centrifuge and take the supernatant for subsequent experiments.
5) If washing is insufficient, perform multiple washes and consider increasing the concentration of NaCl and detergent in the washing 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, possibly leading to high background, select an appropriate antibody, considering monoclonal antibodies.
8) If using too many cells or tissues for lysis results in high background, reduce the sample amount, recommending 100-500 μg of cell lysate.
9) Protein degradation may also result in high background; try to use freshly prepared samples whenever possible.

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