Immunoprecipitation (IP) Detection
(Magnetic Beads Coupled Antibody)

I. Experimental Reagents:

(1) Antibody conjugated magnetic beads
(2) Cell lysis buffer for IP (without inhibitors) (RM00022)
(3) Protease Inhibitor Cocktail (RM02916)
(4) 1×PBS buffer (RM00012)
(5) 5×SDS-PAGE Loading Buffer(RM00001) or non-reduced 5×SDS sample buffer (dilute with deionized water to working concentration prior to use)
(6) Elution buffer: 0.1-0.2M glycine, pH: 2.5-3.1
(7) Neutralization buffer: 1M Tris-base, pH: 10.4

II. Experimental steps:

1. Sample processing

(1) Adherent cell culture

a. Remove the culture medium from adherent cells and wash the cells once with PBS or serum-free culture medium. Centrifuge at 300g for 5 minutes, discard the supernatant and collect the pellet.
b. Add 1ml Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 10^7 cells and mix well by gently pipetting to ensure sufficient contact between the lysis buffer and cells. Rotate at 2-8°C for 15 minutes at 20 rpm.
c. Sonicate at low temperature for 1 minute.
d. Centrifuge the tube at 14,000 × *g*, at 4°C for 10 minutes, carefully transfer the supernatant to a new tube.

(2) Suspension cell culture

a. Centrifuge suspension cells at 300g for 5 minutes, discard the supernatant, collect the pellet.
b. Add 1ml Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 10^7 cells and mix well by gently pipetting to ensure sufficient contact between the lysis buffer and cells. Rotate at 2-8°C for 15 minutes at 20 rpm. After lysis, there should be no obvious precipitate.
c. Sonicate at low temperature for 1 minute.
d. Centrifuge the tube at 14,000 × *g*, at 4°C for 10 minutes, carefully transfer the supernatant to a new tube.

(3) Tissue sample

a. Cut the tissue into small pieces.
b. Take tissue that has been frozen for more than 30 minutes in liquid nitrogen or an ultra-low temperature freezer, quickly grind it with liquid nitrogen. Limit the grinding time within 1-2 minutes to avoid protein degradation. Add 1ml Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 100-200mg tissue.
c. Rotate at 4°C for 15 minutes to ensure complete lysis.

[Alternatively,
b. Add 1ml Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 100-200mg tissue.
c. Homogenize with a glass homogenizer or tissue grinder at low temperature until fully lysed, limit the process within 1-2 minutes to avoid protein degradation.]

d. Sonicate at low temperature for 2 minutes.
e. Centrifuge the tube at 14,000 × *g*, at 4°C for 10 minutes, carefully transfer the supernatant to a new tube.

2. Magnetic Beads Pre-treatment:
(1) Invert or vortex the Magnetic Beads to mix well (no separation in the solution).
(2) Transfer 30-40µl Magnetic Beads to a new EP tube, add 500µl of pre-cooled Cell lysis buffer for IP. Use 1ml-pipette to gently mix 10 times at a steady speed. Place the tube in a magnetic separation rack for 2 minutes, discard the supernatant. Repeat washing step twice for a total of 3 washes.

3. Protein Binding:
(1) Add the antigen-containing sample (usually 300µl, total protein amount 200-500µg or purified protein amount 20µg) to the pre-treated Magnetic Beads, mix well and incubate at 4°C with gentle agitation for 2 hours or overnight.
(2) Place the tube in magnetic separation rack for 2 minutes, discard the supernatant.
(3) Add 500µl pre-cooled Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail), use 1ml-pipette to gently mix 10 times at a steady speed. Place the tube in magnetic separation rack for 2 minutes, discard the supernatant. Repeat washing step 3 times for a total of 4 washes.

4. Antigen Elution:
(1) Denaturing Elution:
This method is suitable for SDS-PAGE detection analysis.
a. After removing the supernatant from magnetic beads, add 35µl of 1X SDS-PAGE Loading Buffer, mix well, and heat at 95℃ for 10 minutes.
b. Place the tube in magnetic separation rack. Collect the supernatant for SDS-PAGE detection.

[Alternatively,
a. Remove the tube from magnetic separation rack, add 35 µl of non-reduced 1X SDS sample buffer, mix well, let it stand at room temperature for 10 minutes. Place the tube back in magnetic separation rack, collect the supernatant.
b. Add 10X DTT, heat at 95°C for 10 minutes, and perform SDS-PAGE detection.]

(2) Non-denaturing Elution:
a. After removing the supernatant from magnetic beads, add 50µl elution buffer, mix

well, and incubate at room temperature for 5 minutes.
b. Place the tube in magnetic separation rack for 2 minutes, collect the supernatant into a new EP tube.
c. Add neutralization buffer to adjust the pH to 7.0-8.0. This sample can be used for subsequent functional analysis.