Immunoprecipitation (IP)

I. Preparation of Solutions and Reagents

1. PBS Buffer:

Prepare by dissolving PBS powder in ddH_2O.

2. 5× SDS Loading Buffer:

Weigh 0.606 g Tris and dissolve in 5 mL ddH_2O. Adjust the pH to 6.8 using 1 M HCl.

Add 1 g SDS and stir until completely dissolved. Then add 5 mL glycerol and 0.025 g bromophenol blue. Mix thoroughly and bring the final volume to 10 mL with ddH_2O. Aliquot into 1 mL/tube and store at –20°C. Before use, add 100 µL β-mercaptoethanol to each tube.

II. Experimental Procedure

A. Sample Preparation

1. Collect the cells and wash them twice with PBS.
2. Add cell lysis buffer (containing protease inhibitors). Use a cell scraper to detach the cells and transfer them into 1.5 mL EP tubes (keep on ice).

Add 1 mL of lysis buffer per 10 cm culture dish.

Add 400–500 µL of lysis buffer per well of a 6-well plate.

3. Transfer the EP tubes to a vertical mixer and rotate at 4°C for 15 minutes to ensure complete cell lysis.

B. Beads Pretreatment

1. Add an appropriate amount of Protein A/G beads to a microcentrifuge (EP) tube. Add 1 mL of PBS buffer, gently shake to mix, and centrifuge at 4000 rpm for 1 min at 4 °C. Discard the supernatant. Repeat the wash once.
2. Add 1 mL of cell lysis buffer (without protease inhibitors), gently shake to mix, and centrifuge at 4000 rpm for 1 min at 4 °C to replace the PBS with lysis buffer. Discard the supernatant and keep the beads on ice for subsequent use.

C. Sample–Antibody–Beads Binding

1. Centrifuge the lysed cells at 12,000 rpm, 4°C for 15 minutes. Transfer 50 µL of the supernatant to a new EP tube for subsequent Western blot analysis. Add the remaining supernatant to an EP tube containing beads.
2. Add the IP antibody targeting the protein of interest (refer to the product website for recommended dilution).
3. Place the EP tube on a vertical rotator and incubate with gentle rotation at 4°C for 3–4 hours.
4. Centrifuge at 4,000 rpm, 4°C for 1 minute and discard the supernatant.
5. Add 1 mL of cell lysis buffer (without protease inhibitors) and mix on a vertical rotator at 4°C for 10 minutes. Centrifuge at 4,000 rpm, 4°C for 1 minute and discard the supernatant. Repeat this washing step 3 times.

D. Elution by Heat Denaturation

1. Leave approximately 50 µL of bead pellet in each EP tube, then add an appropriate amount of SDS loading buffer. Heat the tubes in a 100 °C metal bath for 10 minutes to elute the target protein from the beads.
2. Centrifuge at 4,000 rpm for 1 minute at 4 °C, and collect the supernatant. The supernatant contains the target protein and can be used for subsequent experiments.

III. Experimental Notes

1. The beads described in this protocol refer to agarose beads (Protein A/G Agarose, C0132). Low-speed centrifugation can be used to separate the target protein from the beads. However, if using Protein A/G magnetic beads (C0104B), centrifugation is not applicable. Instead, a magnetic rack should be used to separate the target protein from the magnetic beads. Specifically, place the mixture of magnetic beads and protein onto a magnetic rack and let it stand for 1–2 minutes until the beads are fully attracted to the tube wall. Then, carefully remove the supernatant.
2. PBS buffer should be pre-chilled at 4°C before use.

IV. Recommended Experimental Products

A. Immunoprecipitation Beads:

Protein A/G Agarose, C0132

Protein A/G Immunomagnetic Beads, C0104B

Magrose Beads Protein A/G, C0146

Protein A/G Immunoprecipitation Kit, C0104

B. Cell Lysis Buffer

RIPA Lysis Buffer (Strong), C0045

RIPA Lysis Buffer (Medium), C0046

RIPA Lysis Buffer (Weak), C0047

C. Protease Inhibitor

Protease Inhibitor Cocktail (EDTA-Free, 100× in DMSO), C0001

Protease Inhibitor Cocktail, MS-safe (10×)，C0055

Protease and Phosphatase Inhibitor Cocktail, MS-safe (10×), C0057

D. Antibody for IP Assays

Recommended Product: https://www.targetmol.com/all-antibodies

TargetMol US

www.targetmol.com sales@targetmol.com 1-781-999-5354

36 Washington Street, Wellesley Hills,MA 02481 USA

TargetMol EU

www.targetmol.com sales@targetmol.com +43(0)676/7860258

Hafenstraße 47-51，4020 Linz，Austria