Immunoprecipitation (IP) Protocol

(For scientific research purposes only, not for clinical diagnosis!)

Preparation of Lysate from Cells

1.1 Cell cultureAdherent cells:The cells should be about 80% - 90% confluentin a 100 mm cell culture dish,or the number of cells in the culture bottle was2-5×10^7,Suspension cells:Centrifuge to collect cells (2-5×10^7).

1.2 Add enough cold IP buffer.(Protease and phosphatase inhibitors should be added before use), vibrate. Maintain constant agitation for 10 min in ice bath, use pipette to mix it up. Then gently transfer the cell suspension into a 1.5ml microcentrifuge tube. Continue splitting for 20 min in ice bath.

1.3 Centrifuge at 12,000 rpm, 4°C for 10 min. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant and place in a fresh tube kept on ice,then determination with the BCA kit.

1.4 Denature some lysis solution for Input.

Tissue Protein Extraction

2.1 Wash the sample with cold PBS for twice or three times, then homogenize with an electric homogenizer. Volumes of lysis buffer must be determined in relation to the amount of tissue present.

2.2 Gently transfer the cell suspension into a 1.5ml microcentrifuge tube.Maintain constant agitation for 30 min in ice bath, use pipette to mix it up.

2.3 Centrifuge at 12,000 rpm ,4°C for 10 min. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant and place in a fresh tube kept on ice.Use BCA method to determine protein concentration.

2.4 Denature some lysis solution for Input.

Protocol for IP

3.1 Add 1.0μg IgG and 20μL Protein A/G PLUS-agarose to the IgG group.Add 20μl Protein A/G PLUS-agarose to the experimental group. Shaken at 4 ℃ for 1 h.

3.2 Centrifuge at 2000g ,4°C for 5 min. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant

3.3 Incubate 1-10μL(0.2-2μg)antibody coupled beads in 250 μl cell lysis supernatants, rotate overnight at 4 °C.

3.4 Mix the lysates with 80 μl Protein A/G PLUS-agarose rotating at 4 °C for 2 h.

3.5 Centrifuge at 2000g, 4°C for 5 min. gently remove the supernatant.

3.6 Wash the immunoprecipitation complex with 1 ml of precooled IP lysate (without any inhibitors) for 4 times. Centrifuge at 2000g ,4°C for 10 min , and the supernatant should be carefully discarded each time

3.7 After the last washing ,try best to remove the supernatant. Elute protein with 80μl 1x SDS protein gel sample loading buffer, boil for 10 min in boiling water. Centrifuge at 1000 g, 4 ℃, for 5 min. then aspirate the supernatant.

Detection

4.1 Load 50μl protein sample into the wells of the SDS-PAGE gel, along with molecular weight marker.

4.2 Determine those sample by the dying method with coomassie brilliant blue. Extraction of protein from gel for mass spectrometry.