Immunoprecipitation (IP) Protocol

-Instruction

Immunoprecipitation (IP) is a method of purification and enrichment of target proteins depending on antigen- antibody specific reactions. Antibody combines with target proteins in cell lysate or expression supernatant. And then the antibody can react with protein A/G or sepharose beads coupled with secondary antibody or magnetic beads. Protein complexes can be separated by centrifugation. Wash the complex clean by buffer and suspend the complex in sample loading buffer. Follow the experimental operation of sample preparation in WB protocol by boiling the complex for 10min. Finally, you can collect the supernatant by centrifugation, which include antibodies, target proteins and some other proteins. The supernatant is a protein sample for WB experiment. We can make a conclusion according to the result of the WB experiment.

-Solution and reagent

RIRA buffer (10mM Tris-HCl pH7.6, 1mM EDTA, 0.1% SDS, 0.1% NaDOC, 1% TritonX-100)

Protein inhibitors

PBS (refer to WB protocol)

PBST (refer to WB protocol)

0.2M Glycine-HCl (pH 1.5-2.5)

Tris-HCl (pH 9.4)

6×Sample loading buffer (refer to WB protocol)

-Sample preparation

Preparation for sample of cell lysates

1. Scrap the cells in ice PBS and gather into a tube. Centrifuge at 2500rmp to collect cells.
2. Add ice RIRA buffer into the cell (10^7 cells per 1 ml) which contains protease inhibitors. Suspend the cells in the buffer and sonicate the cells by ultrasound with proper power.
3. Keep the buffer on ice for 20-30min.
4. Centrifuge for 20min at 12000rmp, 4℃. Transfer the supernatant into a new tube and detect the protein concentration by BCA assay. The sample can be frozen at -80℃. But the fresh sample is recommended.

Preparation for sample of tissue

1. Solve animal and take the tissues you need. Wash the blood away.

2. Grind the tissue by liquid nitrogen, and then sonicate it by ultrasound. It may be longer than cells. Then follow the same process as the sample of cell lysates.

-Immunoprecipitation

1. 0.5-1mg total protein is used as an IP reaction. Take 500ul cell lysates which contain 0.5-1mg proteins into a tube.
2. Add 3-5ug primary antibody and incubate at 4℃ overnight to form antigen-antibody complex.
3. Add 50ul magnetic beads into the complex solution at 4℃ for 2-4h.
4. Centrifuge the complex by 2500rmp at 4℃ to collect the antigen-antibody-bead complex.

-Wash and Elution or Thermal denaturation

Wash

There two methods that are used for washing magnetic beads, acid elution or thermal denaturation. The former is suitable for proteins at any size and it has a lower background. The latter is more convenient, but protein G or A will be boiled to the supernatant and interfere the results.

Wash the antigen-antibody-bead complex by a series of washing buffers as follow:

a. 1ml RIPA buffer

b. 1ml PBST buffer

c. 1ml PBS buffer

each buffer for twice and centrifuge by 2500rmp at 4℃

Acid elution

1. Elute the pellet twice with 50μl 150 mM glycine-HCl (pH 1.5-2.5) elution buffers for twice.
2. Add 2μl Tris-HCl (pH 9.4) to neutralize the elution buffer.
3. Add 20μl 6×sample loading buffer and boil the solution

Thermal denaturation

1. Add sample loading buffer according to the volume of the antigen-antibody-bead complex
2. Boil the Antigen-antibody-bead complex at 100℃ for 10min.
3. Transfer the supernatant into a new tube.

-Detection

The result of IP reaction is detected by WB. Load the protein sample into the SDS-PAGE gel referring to the WB protocol. Light chain interference and heavy chain interference are common in the detection. If the weight of target protein is close to 55kD, we recommend a light chain antibody as a secondary

antibody. If the weight of target protein is close to 25kD, we recommend HRP-protein A/G as a secondary antibody.