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IMMUNOPRECIPITATION PROTOCOL

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

SAMPLE PREPARATION

Non-Denaturing:

1. Place cell culture dish on ice and wash the cells with ice cold PBS.
2. Drain PBS and add ice cold lysis buffer.
3. Scrape cells off the dish using a cell scraper and gently transfer the cell suspension into a microcentrifuge tube.
4. Maintain agitation for 30 minutes at 4°C.
5. Centrifuge in a micro centrifuge at 4°C. The time and force of centrifugation may vary depending on the cell type.
6. Gently remove the tubes from the centrifuge and place them on ice.
7. Aspirate the supernatant ad place in a fresh tube on ice.

Denaturing:

1. Add 100uL denaturing lysis buffer to the cells.
2. Mix well by vortexing vigorously for 2 to 3 seconds.
3. Transfer the cell suspension to a microcentrifuge tube.
4. Heat samples to 95°C for 5 minutes to denature.
5. Dilute the suspension with 0.9mL of non-denaturing lysis buffer. Mix gently.
6. Fragment the DNA by passing the lysed suspension 5-10 times through a needle attached to a syringe.
7. Incubate on ice for 5 minutes

Tissue lysates:

1. Dissect the tissue quickly with clean tools. If possible, do so on ice to prevent degradation.
2. Place tissue in microcentrifuge tubes and snap freeze by immersing in liquid nitrogen.
3. Add 300uL of lysis buffer for approximately 5mg of tissue and homogenize with an electric homogenizer.
4. Rinse twice with another 300uL of lysis buffer per rinse and maintain constant agitation for 2 hours at 4°C.
   Note: If denaturing is required, follow steps 2-5 from the denaturing protocol above.
5. Centrifuge for 20 minutes at 12000rpm at 4°C in a microcentrifuge. Aspirate the supernatant and place in a fresh tube kept on ice.

PRE-CLEARING THE LYSATES

1. Add 50uL of normal serum to 1 mL of lysate and incubate for 1 hour on ice.
2. Add 100uL of bead slurry to the lysate and incubate for 30 minutes at 4°C.
3. Spin in microcentrifuge at 14,000 x g for 10 minutes at 4°C.
4. Discard bead pellet and keep supernatant for immunoprecipitation.

IMMUNOPRECIPITATION WITH ANTIBODY IN SOLUTION

1. While on ice, add 10-50ug cell lysates to a microcentrifuge tube plus the recommended amount of antibody.
2. Incubate the sample with the antibody for a few hours at 4°C with gentle agitation.
   Note: The length of incubation depends on the amount of protein and the affinity properties of the antibody.
3. Prepare the Sepharose beads.
4. Mix the slurry well and add 70-100uL of the beads to each of the samples, while on ice.

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5. Incubate the beads mixture for 4 hours at 4°C with gentle agitation.
6. Centrifuge tube after incubation and wash 3 times with lysis buffer.
7. Add 25-50 uL 2x loading buffer and boil for 5 minutes at 100°C.
8. Centrifuge and transfer the supernatant to a new tube for Western blotting.

BLOCKING AND INCUBATION

1. Incubate cells with blocking buffer for 30 minutes to block unspecific binding of the antibody.
2. Incubate the cells with the primary antibody overnight at 4°C (in the dark if using a fluorescent primary).
3. Remove the solution and wash cells 3 times with TBS for 5 minutes each.
   Note: Skip to step 15 if using a primary conjugated antibody.
4. Incubate the cells with the secondary antibody for 1 hour at room temperature in the dark.
5. Remove the solution and wash 3 times with TBS for 5 minutes each in the dark.

COUNTERSTAINING AND MOUNTING

1. Incubate the cells with DAPI for 1 minute.
2. Rinse with TBS.
3. Mount coverslip with a drop of mounting medium.
4. Store in the dark at -20°C or 4°C.