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IMMUNOPRECIPITATION

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Keep samples as cool as possible by carrying out the steps below on ice or in a 4°C cold room. Recipes for all solutions highlighted **bold** are included at the end of the protocol.

1.

Sample preparation:

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. See "Cell and Tissue Lysate Preparation" for Proteintech recommended lysis protocols.

Tip 1

High concentrations of detergents interfere with immunoprecipitation (IP). Lyse cells with as small a volume volume of **RIPA lysis buffer** as possible before diluting the lysates with 1x PBS to the desired final volume.

Tip 2

Use sufficient lysate: for each IP aim to use between 1–3 mg total protein. Lysates of 0.2–0.5 ml, containing a total of 1–3 mg protein, are ideally suited to a single IP. Measure the total protein amount by protein assay, such as Bradford or BCA assay.

Tip 3

Make sure protease inhibitors are present in the lysate buffer. The concentration of protease inhibitor should be 1.5–2 times that of a typical lysate preparation protocol for Western blotting.

2.

Pre-clear the lysate (optional):

a. Resuspend Protein A or G sepharose bead slurry by gently vortexing the storage bottle. Quickly add 50 µl of 50% bead slurry per 0.5–1 mg of total protein to the microfuge tube containing your lysate.

Tip 4

Carefully cut the end of your pipette tip at a 45° angle using a sharp blade to facilitate pipetting the bead slurry. To maintain suction, only a very small section of pipette tip need be removed.

b. Incubate on a rotary mixer for 30 minutes at 4°C.

c. Centrifuge at 1000 rpm for 3 minutes at 4°C and transfer the supernatant to a fresh tube.

Tip 5

Pre-clearing with Protein A or G sepharose beads is recommended for tissues abundant in IgG.

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3. Immunoprecipitation (method 1):

a. Add an appropriate amount (1–4 µg) of primary antibody to the whole (or pre-cleared) lysate. Optimal antibody concentration should be determined by titration. Set up a negative control experiment with control IgG (corresponding to the primary antibody source). Gently rock the incubations at 4°C for 2–4 h or overnight.

b. Add 50 µl Protein A or G sepharose bead slurry to capture the immunocomplex. Gently rock the mixture at 4°C for 1–4 h.

c. Centrifuge the IP mixture at 1000 rpm for 30 seconds at 4°C and discard the supernatant.

d. Wash the beads 3–4 times with 1 ml 1x TBST with 1x Protease inhibitor, centrifuge and discard the supernatant as in step 6. Keep about 80 µl supernatant after the last centrifuge.

e. Resupend the pellet with 20 µl **5x SDS Sample Buffer**, gently vortex for several seconds. Heat at 95–100°C for 5 min and centrifuge at 10,000gX g (approximately 9700 rpm for rotors of a 9.5 cm radius) for 3 minutes.

4. Immunoprecipitation (method 2 : according to Proteintech IP Kit):

a. Transfer the whole (or pre-cleared) lysate containing 1–3 mg of total protein to a Spin Column with End caps in place.

b. Add 50 µl Protein A or G sepharose bead slurry to capture the immune-complex. Gently rock the mixture at 4°C for 1–4 h.

c. Centrifuge the IP mixture at 1000 rpm for 30 seconds at 4°C and discard the supernatant.

d. Take off the End caps, the supernatant is released from the Spin Columns bottom. If necessary, resuspend the beads mixture to enhance the flow velocity.

e. Wash the beads 5 times with 1 ml 1x TBST with 1x Protease inhibitor. If necessary, centrifuge the Spin Columns at 500 rpm for 30 seconds at 4 °C and collect the supernatant with Collection Tubes and discard it.

f. Place Spin Columns in a fresh microfuge tube and pool the elutions. Elute the pellet with 40 µl Elution buffer and centrifuge at 10000 rpm for 1 min at 4 °C, once again.

g. Add 10 µl Alkali neutralization buffer and 25 µl **5X Sample buffer** to the elutions, heat at 95–100°C for 5 min.

5. Western blotting analysis:

a. Load supernatants onto an SDS-PAGE gel, alternatively, transfer the supernatant carefully to a fresh, well-labeled microfuge tube and store at -80°C for later use. (For method 2, directly store at -80°C for later use).

b. Separate IPs by SDS-PAGE and transfer proteins to PVDF membrane. Probe with appropriate antibodies.

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5. Western blotting analysis continued:

Tip 6

For detection of immunoprecipitated proteins by Western blotting, without or reduced detection of non-specific artifacts (such as the heavy and light chains of the immunoprecipitating antibody), detect primary antibodies using HRP-conjugated anti-rabbit light chain-specific(L) antibody and HRP-conjugated Protein A instead of traditional HRP-conjugated secondary antibodies. (Protein A has higher affinity to intact antibodies compare with the denatured antibodies).

Solutions

RIPA lysis buffer	For 1000 ml
50 mM TrisHCl, pH 7.4 (1 M stock)	50 ml
150 mM NaCl	8.76 g
1% Triton X-100	10 ml
0.5% Sodium Deoxylcholate	5 g
0.1 % SDS	1 g
10 mM NaF	0.41 g
1 mM EDTA (0.5 M stock)	2 ml
Add ddH_2O to 1000 ml	
Adjust to pH 7.4	
Add PMSF to 1 mM and other protease inhibitors immediately prior to use.	

5X SDS sample buffer	For 50 ml
250 mM Tris HCl (pH 7.0) (1M stock)	12.5 ml
35% Glycerol	17.5 ml
10% SDS	5 g
0.02% Bromophenol Blue	10 mg
10% ß-mercaptoethanol	5.0 ml
Add ddH_2O to 50ml, aliquot and store at -20°C.	

Related Proteintech Products

Product Name	Catalog Number	Size	Applications
Normal mouse IgG control	B900620	100 µl	IP; IHC/ICC
Normal rabbit IgG control	30000-0-AP	100 µl	IP; IHC/ICC
Normal rabbit IgG control	SA00001-18	100 µl	IP; WB
HRP-conjugated mouse anti-rabbit IgG(L) Specific	SA00001-7	100 µl	IP; WB

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