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Antibodies | ELISA kits | Proteins

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) in **bold** are included at the end of the protocol.

Sample Preparation

Cell lysis is the breaking down of the cell membrane and the separation of proteins from the non-soluble parts of the cell. Lysate buffers contain different detergents that help to release soluble proteins. Dependent on the location of the protein of interest, a different lysate buffer is needed to obtain a high yield and purity of the protein.

Cell & Tissue Lysate Preparation Tips:

a. Use protease inhibitors in the lysis buffer
b. The concentration of proteinase inhibitor(s) should be 1.5–2 times that used for Western blotting lysates. Proteintech usually uses **RIPA buffer**, which enables efficient cell lysis and protein solubilization, while avoiding protein degradation and interference with the protein's immunoreactivity and biological activity.
c. Optimize your lysate concentration
d. Commonly used amounts for IP: 0.2–0.5 ml lysate containing 1–4 mg total protein. Measure the total protein amount by protein assay, such as Bradford or BCA assay.
e. High concentrations of detergent interfere with IP.
f. Try to lyse cells with a small volume of **RIPA** and then dilute the lysates with **PBS** to the final volume. Depending on the experiment, the detergent strength is crucial for the IP results (e.g., Triton-X, Tween, SDS, CHAPS).
g. Keep cells/tissue on on ice and use ice-cold buffers.

Cultured Cells:

h. Pre-cool a refrigerated centrifuge to 4°C. Pellet the cultured cells by centrifugation at 4°C, for 5 minutes at 2000 rpm.
i. Wash 3 times with ice-cold 1X **PBS** and then add cold **RIPA buffer** with protease inhibitors.
j. Add 100 µl **RIPA buffer** for approximately every 10^6 cells present in the pellet (count cells before centrifugation).
k. Reduce the volume of **RIPA buffer** accordingly if a higher protein concentration is required.
l. Vortex to mix and keep on ice for 30 minutes. Vortex occasionally.

Tissues:

m. Dissect the tissue of interest and wash briefly with cold 1X **PBS**.
n. Cut the tissue into smaller pieces while keeping it on ice.
o. Transfer the tissue to a homogenizer and add **RIPA buffer** with protease inhibitors.
p. Add 500 µl **RIPA buffer** for approximately every 20–50 mg of tissue.
q. Homogenize thoroughly and keep the sample on ice for 30 minutes. Vortex occasionally.

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Lysis & Storage

a. Sonication: high power, 1–2 minutes, then keep the sample on ice for 20 minutes.
b. Centrifuge at 10,000 rpm for 20 minutes at 4°C to pellet cell debris.
c. Transfer the supernatant to a fresh microfuge tube without disturbing the pellet. Determine protein concentration by protein assay, such as Bradford or BCA assay.
d. Samples can be frozen at -80°C for long-term storage, or be used for immediate immunoprecipitation.

Pre-Clear The Lysate (Optional)

Pre-clearing is a step that decreases binding of non-specific proteins, lipids, carbohydrates, or nucleic acids. This step is performed by incubation of the lysate with the solid support (e.g., agarose or magnetic beads) in the absence of the capture antibody.

Tip 1

Pre-clearing with Protein A or G agarose beads is recommended for tissues adundant with IgG.

a. Resuspend Protein A or G agarose bead slurry by gently vortexing the storage bottle.
b. Transfer beads and wash with 1X **PBS** 4~5 times before use. Quickly add 50 µl of 50% bead slurry per 1–4 mg of total protein to the microfuge tube containing the lysate.
c. Carefully cut the end of your pipette tip to facilitate pipetting and homogenization.
d. Incubate on a rotary mixer for 30 minutes at 4°C.
e. Centrifuge at 1000 rpm for 3 minutes at 4°C and transfer the supernatant to a fresh tube.

Immunoprecipitation Workflow

a. Add 300 µl **incubation buffer** and an appropriate amount of primary antibody to the whole (or pre-cleared) lysate. Optimal antibody concentration should be determined by a previous titration experiment.
b. Gently rock the mixture at 4°C for 4 hours or overnight.
c. Set up a negative control with control IgG corresponding to the primary antibody source.
d. Add Protein A or G agarose slurry (50 µl) to capture the immunocomplex. Gently rock the mixture at 4°C for 4 h.
e. Centrifuge the mixture at 500–1000 rpm for 30 seconds at 4°C and discard the supernatant.
f. Wash the beads 3–4 times with 1X TBS or 1X **PBS** with 0.2% Tween 20 (or another detergent depending on its stringent and protein of interest).
g. Centrifuge and discard the supernatant. Keep about 60 µl of supernatant after the last centrifuge.
h. Resuspend the pellet with 20 µl 4X **SDS Sample Buffer**, gently vortex for several seconds, and load on the gel.

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Buffer Optimization

Binding Buffer:

The majority of bindings to protein A or G work well in physiological conditions. Some bindings to protein A or G can be enhanced by adapting the pH value (e.g., Protein G binds best to IgG at pH 5.0).

Washing Buffer:

The washing step should not interfere with the desired protein bindings and should remove all unwanted protein bindings and debris.

Perform washing and obtain elutions using gravitational flow through filter columns loaded in microfuge tubes.

Most commonly used buffers:

- PBS
- TBS

Most commonly used detergents:

- NP40
- Triton-X
- CHAPS

Most commonly used additive:

- DTT (reduction of disulfid bonds)

Elution Buffer:

If the IP sample is further used for Western blotting, the sample can be directly diluted in a SDS-PAGE sample buffer containing reducing agents.

The most commonly used EB is glycine 0.1 M at pH 2.5–3.5 and 1% of SDS to disturb the bead-antibody-antigen interactions.

If the antibody-protein binding does not dissociate, or if the protein becomes denaturated, the pH can be changed.

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Buffer Solutions

RIPA Buffer
50 mM Tris•HCl, pH 7.4 (1 M stock)
150 mM NaCl
1% Triton X-100 or NP-40
0.5% Sodium deoxylcholate
0.1% SDS
1 mM EDTA (0.5 M stock)
10 mM NaF
Add ddH_2O to the final volume
Add PMSF to a final concentration of 1 mM and any other protease inhibitors immediately before use

1X PBS
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
137 mM NaCl
2.7 mM KCl
Adjust pH to 7.4
Add ddH_2O to the final volume

4X SDS Sample Buffer
12% SDS
25% Glycerol
150 mM Tris•HCl (pH 7.0•1M stock)
0.03% Bromophenol Blue
20% β-mercaptoethanol
Add ddH_2O to the final volume, aliquot, and store at -20°C
20% β-mercaptoethanol (or 500 mM DTT replace) should be freshly added before use

Elution Buffer (EB)
100 mM $NaHCO_3$
1% SDS

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Buffer Solutions

Incubation Buffer
2.7 mM KCl
1.5 mM KH_2PO_4
3.2 mM $Na_2HPO_4 \cdot 12H_2O$
137 mM NaCl
5 mM EDTA•2Na
10 mM NaF
Add ddH_2O to 1000 ml, adjust to pH 7.4

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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