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CELL AND TISSUE LYSATE PREPARATION

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Recipes for all solutions (highlighted) in **bold** are included at the end of the protocol.

1.

Cultured cells:

a. Pre-cool a refrigerated centrifuge to 4°C. Pellet the cultured cells by centrifugation for 5 minutes at 1000 x g (approximately 2000 rpm) at 4°C. Wash 3 times with ice-cold **1X PBS** and then add chilled **RIPA buffer** with protease inhibitor. In general, add 100 µl **RIPA buffer** for approximately every 10^6 cells present in the pellet (count cells before centrifugation). Reduce the volume of **RIPA buffer** accordingly if a higher protein concentration is required. Vortex to mix and keep on ice for 30 min, vortexing occasionally. Go to step 3, lysis and storage.

2.

Tissues:

a. Dissect the tissue of interest and wash briefly with chilled **1X PBS** to remove any blood if necessary, cut the tissue into smaller pieces whilst keeping it on ice. Transfer the tissue to a homogenizer and add **RIPA buffer** with protease inhibitor. In general, add 500 µl **RIPA buffer** for approximately every 10 mg of tissue. Homogenize thoroughly and keep the sample on ice for 30 min. Vortex occasionally. Go to step 3, lysis and storage.

Tip 1

Add phosphatase inhibitors to lysis buffers for extraction of phosphorylated proteins.

3.

Lysis and storage:

a. Sonicate the sample to break the cells or tissue up further and to shear DNA. Adjust sonication time to your type of sample: 1 min for cell lysates and 2–5 min for tissue lysates at a power of about 180 watts (in rounds of 10 seconds sonication/10 seconds rest for each cycle). Keep the sample on ice during the sonication.

Tip 2

The addition of DNase for DNA digestion is not recommended as this introduces protein contamination from the enzyme.

b. Centrifuge at 10,000 x g (approximately 9700 rpm for rotors of a 9.5 cm radius) for 20 minutes at 4°C to pellet cell debris, and then transfer the supernatant to a fresh microfuge tube without disturbing the pellet.

c. Determine protein concentration of the lysate by Bradford or BCA protein assay.

d. Samples can be frozen at -80°C for long-term storage, or be used for immediate Western blotting or immunoprecipitation.

e. For Western blotting, mix sample with **4X SDS sample buffer** to a final dilution of 1X. Heat the mixture to 95°C for 5 minutes before loading onto an SDS-PAGE gel.

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Solutions

1X PBS	For 1000 ml
10 mM Na_2HPO_4	1.42 g
1.8 mM KH_2PO_4	0.24 g
137 mM NaCl	8 g
2.7 mM KCl	0.2 g
Adjust pH to 7.4	
Add ddH_2O to 1000 ml	

RIPA buffer	For 1000 ml
50 mM Tris•HCl, pH 7.4	50 ml
150 mM NaCl	8.76 g
1% Triton X-100 or NP-40	10 ml
0.5% Sodium deoxylcholate	5 g
0.1 % SDS	1 g
1 mM EDTA (0.5 M stock)	2 ml
10 mM NaF	0.42 g
Add ddH_2O to 1000 ml	
Add PMSF to a final concentration of 1 mM and any other protease inhibitors immediately before use.	

4X SDS sample buffer	For 1000 ml
12% SDS	120 g
25% Glycerol	250 ml
150 mM Tris•HCl (pH 7.0•1M stock)	150 ml
0.03% Bromophenol Blue	300 mg
20% β-mercaptoethanol	200 ml
Add ddH_2O to 800 ml, aliquot and store at -20°C.	
20% β-mercaptoethanol, (or 500 mM DTT replaced), should be added freshly before use.	

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