Cell Lysis and Protein Extraction Procedures  
(Mammalian Tissue)

Use the cell lysis kit (mammalian) or the protocol below, which involves freezing tissue samples (for example, biopsy samples) in liquid nitrogen. Use liquid nitrogen and a mortar and pestle to grind the samples while they are still frozen. Break up any larger pieces beforehand (for example, wrap the frozen tissue sample in aluminum foil and crush with a hammer).

Reagents

- 2-D sample solution

1. Chill a mortar with liquid nitrogen, then grind small tissue pieces in the presence of liquid nitrogen to a fine powder.

2. Immediately after grinding, transfer 60 mg tissue powder to a microcentrifuge tube containing 1.0 ml of 2-D sample solution.

3. Optional: sonicate the sample on ice 5 times, for 2 sec each time.

4. Pause between sonication steps to avoid overheating.

5. Incubate the sample at room temperature for 30 min. Vortex from time to time.

6. Centrifuge at 35,000 × g for 30 min at room temperature.

7. Perform a protein assay to determine the protein concentration of the supernatant, which should be 5–10 mg/ml.

8. Dilute the supernatant with 2-D sample solution and incubate for 20 min at room temperature.

2-D sample solution (50 ml)

- 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 40 mM DTT,
- 0.2% (w/v) ampholytes (pH 3–10)
- Urea/thiourea stock solution 48 ml
- CHAPS 2.0 g
- Ampholytes, pH 3–10 250 μl
- DTT 0.31 g
- Bromophenol blue (1%) 10 μl
- Distilled or deionized H2O to 50 ml

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