

Protein Sample Preparation

Protocol

Bulletin 6196

Mammalian Tissue

Use the MicroRotor™ cell lysis kit (mammal). Alternatively, use the protocol here, which involves freezing mammalian tissue samples (for example, biopsy samples) in liquid nitrogen at -196°C .

- 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 lysis buffer.
- 3 Optional: sonicate the sample on ice 5 times, for 2 sec every time. Pause between sonication steps to avoid overheating.
- 4 Incubate the sample at room temperature for 30 min. Vortex from time to time.
- 5 Centrifuge at $35,000 \times g$ for 30 min at room temperature.
- 6 Perform a protein assay to determine the protein concentration of the supernatant, which should be 5–10 $\mu\text{g}/\mu\text{l}$.
- 7 Dilute supernatant with 2 \times SDS-PAGE sample buffer (to a final 1 \times concentration), and incubate for 20 min at room temperature

TIPS

Use liquid nitrogen and a mortar and pestle to grind the samples while they are still frozen. Mill any larger pieces beforehand (for example, wrap the frozen tissue sample in aluminum foil and crush with a hammer).

REAGENTS

Mammalian Tissue

- Lysis buffer
- SDS-PAGE sample buffer (2 \times)

EQUIPMENT

- Mortar and pestle

PRODUCT LINKS

- MicroRotor cell lysis kit (mammalian)
- RC DC protein assay
- SDS-PAGE sample buffer (2 \times)

BIO-RAD

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).



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