

Protein Sample Preparation

Protocol

Bulletin 6197

Plant Leaves

To minimize the deleterious effects of endogenous plant compounds, use the MicroRotor cell lysis kit (plant) or the protocol here, which involves grinding the tissue in a mortar and pestle with liquid nitrogen.

- 1 Cool protein precipitation and wash solutions to -20°C . Chill a mortar with liquid nitrogen.
- 2 Place 3–4 leaves in the mortar, add liquid nitrogen, and grind the leaves in the liquid nitrogen to a fine powder.
- 3 Transfer leaf powder into 20 ml of protein precipitation solution and incubate for 1 hr at -20°C . Stir occasionally.
- 4 Centrifuge the solution at -20°C for 15 min at $35,000 \times g$. Discard supernatant, add wash solution and suspend the pellet. Incubate for 15 min at -20°C , stirring occasionally. Repeat until wash solution turns light green.
- 5 Centrifuge the solution at -20°C for 15 min at $35,000 \times g$ and discard supernatant. Resuspend pellet in 2 ml wash solution.
- 6 Transfer suspension into a shallow ceramic shell and cover with perforated parafilm. Put shell into a desiccator and apply a vacuum until the pellet (acetone powder) is dry.
- 7 Mix 3 mg of sample powder with 1 ml 1 \times SDS-PAGE sample buffer and incubate for 30 min at room temperature. Vortex from time to time.
- 8 Centrifuge the solution at -20°C for 15 min at $>16,000 \times g$. Collect and heat the supernatant for 3 min at 95°C .
- 9 Cool solution to room temperature and perform the protein assay.

TIPS

With plant leaves, precipitate proteins with 20% TCA in prechilled acetone (-20°C). To remove the plant phenols, rinse the pellet at least twice with cold acetone (-20°C) and air-dry samples in a vacuum.

REAGENTS

Plant Leaves

- Protein precipitation solution
- Wash solution
- SDS-PAGE sample buffer (1 \times)

EQUIPMENT

- Mortar and pestle

PRODUCT LINKS

- MicroRotor cell lysis kit (bacteria)
- MicroRotor cell lysis kit (yeast)
- 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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