ITSI BIOSCIENCES
FAST, ACCURATE, EASY- TO-USE

PROTEIN ISOLATION FROM PLANT TISSUE

Important: K-0025-20 is a validated kit and procedure for isolation of proteins from plant cells and plant tissue. Exercise extreme caution when working with proteins and protect your protein sample from breakdown and contamination by wearing gloves and placing tubes on ice. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants such as keratin. Do not let samples stand at room temperature longer than it is necessary to prepare samples for analysis or storage.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT:

Item	Size	Catalog #	Storage
Buffer 1	20 mL	K-0025-20.1	-20°C
Buffer 2	10.0 mL	K-0025-20.2	-20°C
Buffer 3	5 mL	K-0025-20.3	-20°C
Protease inhibitor	1.0 ml	K-0025-20.4	-20°C
Procedure			

MATERIALS REQUIRED but Not supplied:

1. Refrigerated centrifuge
2. Ice bucket
3. Adjustable pipette (Use recently calibrated adjustable pipettes to ensure accuracy)
4. Homogenizing device
5. Vortex
6. Reducing agent (DTT)

PROCEDURE:

- Prepare enough of the working lysis buffer for the day.
- Thaw all solutions. For every 10ml of working Lysis Buffer add 2ml of **Buffer 1**, 1.0ml of **Buffer 2**, 0.5ml of **Buffer 3**, 0.1ml of **Protease Inhibitor** and bring the volume up to 10ml with reagent grade water. Place the working buffer on ice. Buffer 3 is viscous and may have to be warmed briefly.
- Before beginning step 1 add 1.5 mg DTT to every 10ml of working lysis buffer.

1. Place the fresh or frozen tissue on ice and add about 2ml of working lysis buffer per gram of tissue.
2. Homogenize the tissue using a mechanical motor driven device, or by hand with mortar and pestle until no chunks of tissue are visible. Take care to keep the sample on ice during this process.
3. Transfer the homogenate to microcentrifuge tubes.
4. Incubate the homogenized sample on ice for about 30 minutes. Vortex at least 3 times during the incubation.
5. Centrifuge the sample at 15,000xg for 10 minutes at 4°C.
6. Transfer the supernatant from the tubes and place in a new microcentrifuge tube.
7. Steps 5 & 6 should be repeated if some of the pelleted material is accidently transferred to the new tube.
8. Store at −80°C if not analyzed immediately.
9. To ensure good resolution on an SDS-PAGE gel or a 2D gel the resulting lysate should be cleaned up. ITSI recommends the ToPREP precipitation kit.

STORAGE:

ITSI recommends that protein lysates be stored at −80°C until analyzed.

General Safety Information:
Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with water and follow established guidelines/procedures in your laboratory. **WARNING: Intended for research use only, not for use in human, therapeutic or diagnostic applications. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**

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