

Plant Tissue Extraction Kit

(Catalog #BN00549; 50 assays; Store at -20°C)

I. Introduction:

Assay Genie's Plant Protein Extraction Kit provides an effective way of extracting soluble protein and other biomolecules and metabolites from all kinds of dry and fresh plant tissues without the use of liquid nitrogen. The kit includes optimized Extraction Buffer to extract plant protein and metabolites, Protease Inhibitor Cocktail to prevent protein degradation and Homogenization Pestles and Tubes to homogenize the plant tissue. The extracted protein can be used in a variety of downstream applications such as Western Blotting, 2-D gel electrophoresis, immunoprecipitation assay, and enzymatic activity assays etc. and extracted small molecules can be used in plant metabolic studies. This easy-to-use and rapid procedure ensures consistent protein yield ~ 2-9 mg/ml depending on the sample type.

II. Application:

- Extraction of plant soluble protein and metabolites for various downstream applications such as Western Blotting, 2-D gel electrophoresis, immunoprecipitation assay, enzymatic activity assay and quantitation of metabolites etc.

III. Sample Type:

- Fresh or frozen plant tissues, dry plant tissues such as seeds

IV. Kit Contents:

Components	BN00549	Cap Code	Part Number
Plant Extraction Buffer	25 ml	NM	BN00549-1
Protease Inhibitor Cocktail (200X)	1 ml	Orange	BN00549-2
Homogenization Pestles	3	-	BN00549-3
Homogenization Tubes (1.5 ml)	3	-	BN00549-4

V. User Supplied Reagents and Equipment:

- Blades and tweezers
- Micro centrifuge
- Multi-well spectrophotometer (ELISA reader)
- BCA Protein Assay Kit (Cat # BN01034)

VI. Storage Conditions and Reagents Preparation:

Store kit at -20°C, protect from light. Homogenization Pestles and tubes can be stored at room temperature or -20°C. Thaw reagents before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Plant Total Protein Extraction Protocol:

- Extraction:** Add 5 µl of Protease Inhibitor Cocktail to 995 µl of Plant Extraction Buffer before use and keep on ice to chill. Clean the tissue with dH₂O and remove excess water. Weigh the fresh or frozen tissue (~50-100 mg) and cut into small pieces with blade. Transfer the tissue pieces into provided 1.5 ml Homogenization Tube and add ~200-400 µl ice cold Plant Extraction Buffer with Protease Inhibitor Cocktail. Homogenize the tissue on ice by rotating the pestle in the provided 1.5 ml Tube for 60 times. Keep the Tube on ice for 10 min. Centrifuge at 10,000 x g, 4°C for 5 min. and collect the supernatant.

Notes:

- Thaw the frozen plant tissue on ice before cutting into small pieces.
 - To soften the dry plant tissue such as seeds, roots etc. completely soak the tissue in dH₂O and incubate at 4°C for 1-2 days.
 - Homogenization Pestles and Tubes are reusable. After each use, rinse 3 times with dH₂O and air-dry.
- Protein Quantitation:** Measure the protein concentration using BCA Protein Assay Kit (Cat #BN01034 or equivalent). Keep the plant tissue homogenate on ice for immediate use, or store at -80°C for future use.

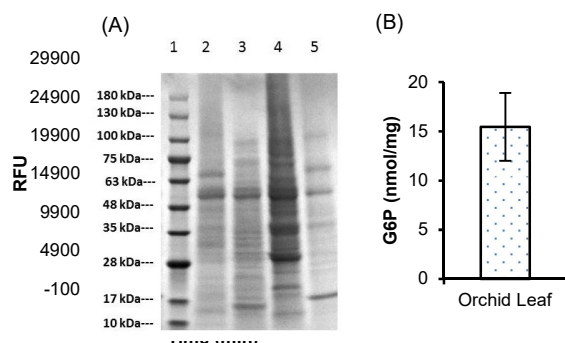


Figure: (A) SDS-PAGE of Plant tissue lysates: 25 µl of lysate from lettuce (20 µg, lane 2), green Pepper (20 µg, lane 3), avocado (40 µg, lane 4) and orchid leaf (10 µg, lane 5) was loaded onto a 4-20% SDS-PAGE and following electrophoresis stained with coomassie blue. Plant lysates were prepared following the kit protocol. Lane 1: Marker (B) Measurement of Glucose-6-Phosphate in Orchid Leaf lysate (5 µg) using G6P Fluorometric Assay Kit (Cat #BN00911). (C) Measurement of glucose-6-phosphate dehydrogenase activity in orchid leaf lysate (0.6 µg) using G6PDH Activity Assay Kit (Cat #BN00964).

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