Plant Total Protein Extraction Reagent

Catalog# BWR1013

Size: 50-100 assays

Lot # Check on the product label

Introduction

The Plant Total Protein Extraction Reagent is designed specially for extracting total proteins from fresh or frozen tissues of various plants (including root, stem, leaf and fruit). It is complicated and hard to extract proteins from plants for their formations, phenolics, polysaccharide, pigment, secondary metabolites, etc. With optimal reagents and protocol, this reagent can efficiently extract the soluble and hydrophobic proteins from various plants with the best active and detection status, and availably remove interfering substances including polyphenol, polysaccharide, chinone, pigment, lipid, secondary metabolites, etc. And the obtained proteins can be used for enzyme assay, unidirectional and two-dimensional protein electrophoresis, Western blot and co-immunoprecipitation assays.

Kit Components

Components	Size	Storage Instruction
Reagent A	30 ml	Store at RT for one year.
Reagent B	220 ml	Store at RT for one year.
Reagent C	1.5 ml	Store at RT for one year.

Protocol

1. **Frozen tissue homogenization:** Weight 50mg of such sample. Grind the liquid nitrogen stored frozen plant tissue to a fine powder in a frozen mortar. (**Note:** Keep tissue frozen at all times, do not allow the tissue to thaw. When grinding, the tissue should remain a gray or green powder. If the powder begins to turn dark green, the tissue is thawing. High fibrous tissue may require extended grinding.) Transfer tissue samples (powder) to a EP tube.
2. **Fresh tissue homogenization:** Weight 100mg of such sample. Completely break up the tissue pellet by grinding under liquid nitrogen condition. A pellet pestle may be required to break up the large pieces. Transfer tissue samples (powder) to a EP tube.
3. Add 1ml of Reagent A, shake for 5 min to mix thoroughly. Centrifuge at 12,000 × g for 5 min to pellet proteins and plant tissue debris. Discard the pellet.
4. Carefully transfer the supernatants to another tube, process it in the boiling water for 3 min.
5. Cool to room temperature, transfer to a new 10-15ml of tube, add the 8 times volume of -20℃ precooling Reagent B and mix thoroughly.

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6. Put it at -20℃ for at least 60 min to precipitate the protein completely.
7. Centrifuge at 12,000 × g for 15 min, remove the supernatants and keep the protein precipitation.
8. Dry in the air, then, add 50μl of Reagent C and shake to dissolve the protein precipitation completely.
9. Process the protein solution in the boiling water for 3 min.
10. Centrifuge at 12,000 × g for 5 min at room temperature, analyze the supernatants directly (Protein assay and SDS-PAGE).

Troubleshooting Guide

Problem	Cause	Solution
Low protein yield	Insufficient tissue homogenization	Grind tissue completely. Do not allow tissue to thaw. Fibrous tissue such as maize leaf, stems and some roots may require additional grinding.
	Old or dry tissue	Use young healthy plant tissue for protein extractions.
	Low protein content in the tissue	Some plant tissue does not contain large quantities of protein, such as fruit. Adjustments may be made by decreasing the volume of the extraction reagent or by increasing the amount of tissue.
Poor quality protein obtained.	Protein degradation	All steps should be operated on ice. And make certain the protease inhibitor cocktail for plant extracts is included in the appropriate solutions. Do not allow the tissue to thaw while grinding or before extraction.
	Formation of protein complexes	Many plant metabolites will complex with proteins. If these complexes form they are nearly impossible to break apart. These complexes will interfere with many down stream applications.
	Obtained proteins contain impurity residues, such as polyphenol or pigment, etc.	Remove the impurity residues by repeat albumen precipitation

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