3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Plant Total Protein Extraction Kit
Mini 10–250 mg tissue samples

Catalog Number **PE0230**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Plant Total Protein Extraction Kit is designed specifically for use in plant bioscience to extract a qualitative sample of all proteins from any type of plant species or tissue. The protocol does not require any ultracentrifugation or aqueous polymer two-phase partitioning (APTP). The kit includes two reagents, a plant specific protease inhibitor cocktail and a new chaotropic reagent with increased solubilizing power to extract more hydrophobic proteins (Protein Extraction Reagent Type 4). Routine use of the reagent in sequential protein extraction of a plant tissue sample with fresh reagent removes nearly all protein from the tissue. The protease inhibitor cocktail is a mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, metalloproteases, aspartic, and aminopeptidases. It contains pepstatin A, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), E-64, leupeptin, 1,10-phenanthroline, and bestatin. The cocktail has been demonstrated to be highly effective in preventing protein degradation during the extraction process.

Following removal of polyphenolics, tannins, and other interfering substances, ground plant tissue, fresh or frozen, is resuspended in the chaotropic reagent. Plant debris is pelleted by centrifugation and the protein extract is collected. The end result is a qualitative total protein sample, ready for downstream proteomic analysis.

Components

This kit contains sufficient material for the preparation of 20 separate extracts from plant tissue samples of 10–250 mg.

Protein Extraction Reagent Type 4 (Catalog Number C0356) 1 bottle of powder that reconstitutes to a final volume of 23 ml.	1 bottle
Protease Inhibitor Cocktail for plant cell and tissue extracts (Catalog Number P9599) Packaged in amber vials.	1 × 1 ml

Reagents and Equipment Required But Not Provided

- High purity water (Catalog Number W4502)
- Methanol (Catalog Number 494437)
- Micropipettes
- Acetone (Catalog Number 179124)
- Liquid nitrogen
- Mortar and pestle
- Graduated cylinder
- Freezing vials (Catalog Number V5381)
- Centrifuge and centrifuge tubes

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Working Reagents should be made fresh just prior to use as described below. Unused kit component solutions can be frozen for future use.

Protein Extraction Reagent Type 4 - Add 15 ml of high purity water to the contents of the bottle. The solution will become cold to the touch and may require warming to 20–25 °C for complete solubilization. The final volume of the solution will be 23 ml. **Do not allow the temperature of the solution to rise above 30 °C, as cyanates, which are detrimental to proteins, may begin to form.** Aliquot any unused solution in 1 ml volumes and freeze at –20 °C for future use.

The Reagent Type 4 Working Solution should be prepared fresh just prior to use by addition of the Protease Inhibitor Cocktail for plant cell and tissue extracts (10 µl/ml). Prepare enough Reagent Type 4 Working Solution for the amount of plant tissue to be extracted (each mg of plant tissue requires 4 µl of Reagent Type 4 Working Solution).

The Protease Inhibitor Cocktail for plant cell and tissue extracts is supplied ready-to-use and should be added to appropriate solutions at a 1:100 dilution (10 µl/ml). Prepared solutions containing the cocktail should be used the same day. Store the cocktail at –20 °C for further use.

Methanol Solution - Dilute the Protease Inhibitor Cocktail for plant cell and tissue extracts 1:100 with methanol (Catalog Number 494437). Store the Methanol Solution at –20 °C. Prepare enough Methanol Solution for the amount of plant tissue to be extracted.

Determine the tare mass of each freezer vial. Label each vial with the tare mass. This is important for determining the tissue mass latter in the procedure.

Storage/Stability
The kit components are stable for at least 12 months as supplied when stored at –20 °C.

Procedure
This procedure was developed using *Arabidopsis* leaf tissue as the plant tissue type. Either fresh or frozen tissue may be used with this procedure. Other plant species and tissue types may be used with this procedure. The amount of starting material may be adjusted to fit the scale of the extraction. Use this procedure as a guideline.

It may be necessary to add non-specific nucleases to reduce the viscosity of the samples due to the presence of high molecular weight DNA.

1. Grind 10–250 mg of *Arabidopsis* leaf tissue in liquid nitrogen to a fine powder.
   Note: Keep tissue frozen at all times, do not allow the tissue to thaw. When grinding, the tissue should remain a gray/green powder. If the powder begins to turn dark green the tissue is thawing. High fibrous tissue may require extended grinding.
2. Transfer tissue samples to a 2 ml v-bottom freezing vial (Catalog Number V5381) held at –20 °C. Immediately proceed to step 3 to minimize any protein degradation.
3. Add 1.5 ml of the prepared Methanol Solution. Briefly vortex the sample (15–30 seconds) and place at –20 °C. Allow the mixture to incubate for 5 minutes at –20 °C with periodic vortexing.
   Note: The –20 °C temperature is essential to keep the proteins precipitated and prevent solubilization in the Methanol Solution.
4. Centrifuge the suspension at 16,000 × *g* for 5 minutes at 4 °C to pellet proteins and plant tissue debris.
5. Remove the supernatant with a pipette. Be careful not to disturb the pellet. Discard the supernatant.
6. Repeat steps 3–5 two additional times.
   Notes: Larger quantities of plant tissue (200–250 mg) or tissues known for high phenolics and tannin content may require an additional extraction with the Methanol Solution.

   After removing the supernatant from the final extraction with the Methanol Solution, invert the tubes over a clean paper towel to remove any visible Methanol Solution. The pellet does not need to be dry to proceed to step 7; however, any visible Methanol Solution remaining on the tissue pellet should be avoided.
7. Add 1.5 ml of acetone (Catalog Number 179124) pre-chilled to –20 °C. Briefly vortex the sample (15–30 seconds) and place at –20 °C. Allow the mixture to incubate for 5 minutes at –20 °C.
8. Centrifuge the suspension at 16,000 × *g* for 5 minutes at 4 °C to pellet proteins and plant tissue debris.
9. Remove the supernatant with a pipette. Be careful not to disturb the pellet. Discard the supernatant.
10. Allow the pellet to air dry for 5–10 minutes at room temperature or use a SpeedVac® for 30–60 seconds to remove any residual acetone.
    Note: Larger quantities of plant tissue (200–250 mg) or tissues known for high phenolics and tannin content may require an additional extraction with pre-chilled acetone.
11. After drying the sample, weigh the freezer vial and subtract the predetermined tare mass of the vial to determine the plant tissue mass.

12. To the plant tissue pellet add 4 µl of Reagent Type 4 Working Solution for each mg of plant tissue. Completely break up the tissue pellet by vortexing. A pellet pestle may be required to break up the large pieces. Allow the solution to incubate for 15 minutes with intermittent vortexing or continuous gentle mixing at ambient temperature. Note: The temperature of the solution should not be allowed to rise above 30 °C. Also, the temperature should also not fall below 15 °C, or the urea and thiourea will precipitate out of solution.
13. Centrifuge the suspension at 16,000 × *g* for 30 minutes to pellet plant tissue debris.
14. Remove the supernatant (total protein sample) by pipette and place in a clean, labeled tube. Be careful not to disturb the pellet.
15. The total protein sample is now ready for SDS-PAGE or other proteomic analysis.

Procedure for IEF and 2D gel electrophoresis

Plant tissues with high levels of salts may require desalting or dialysis after extraction prior to IEF. For isoelectric focusing (IEF) or 2D gel applications, reduction and alkylation of the protein sample is highly recommended. Reduction with tributylphosphine and subsequent alkylation with iodoacetamide improves resolution, allows greater sample loads, and therefore, improves visualization of low abundant proteins.

It is also suggested to determine the protein concentration of the sample. The protein concentration may be determined by use of the Bradford Reagent (Catalog Number B6916).

1. Reduce the protein sample by adding tributyl-phosphine (TBP, Catalog Number T7567). TBP is an uncharged reducing agent for the reduction of disulfide bonds. The 200 mM TBP stock solution is stored under argon in a flame sealed ampule. TBP is added to a final concentration of 5 mM (25 µl of the 200 mM TBP stock solution per ml of protein sample) and incubated at ambient temperature for 1 hour.
2. Alkylate the protein sample with Alkylating Reagent Iodoacetamide (Catalog Number A3221). Iodoacetamide is an alkylating reagent for cysteine and histidine residues in proteins. Iodoacetamide eliminates artifacts of disulfide formation during electrophoresis, resulting in less streaking and better resolution. Add iodoacetamide to a final concentration of 15 mM (30 µl of a 0.5 M solution of iodoacetamide per ml of protein sample) and incubate for 1.5 hours at room temperature.
3. Centrifuge the final reduced and alkylated protein sample at 16,000–20,000 × *g* for five minutes at room temperature to pellet any insoluble material.
4. The protein sample is now ready for loading onto IEF gels. The protein concentration of the sample may need to be adjusted by further dilution with Reagent Type 4 Working Solution prior to loading.

Troubleshooting Guide

Problem	Cause	Solution
Low protein yield	Insufficient tissue disruption	Grind tissue in liquid nitrogen to a fine powder. Do not allow tissue to thaw. Fibrous tissue such as maize leaf, all stems, and some roots may require additional grinding. Addition of grinding aids, such as clean high quality sand, may be used as a last resort.
	Old or dry starting tissue	Use young healthy plant tissue for protein extractions. Avoid old or dry tissue.
	Low protein content in starting tissue	Some plant tissue does not contain large quantities of protein. Adjustments may be made by decreasing the volume of the extraction reagent or by increasing the amount of tissue. One may do additional extractions and combine them; however, a concentration step may be required with this approach.
Poor quality protein recovered.	High phenolics or tannin concentration	Additional methanol and/or acetone washes may be required for large quantities of tissue such as pine needle or cotton leaf. If there is any green color remaining in the methanol or acetone washes, repeat washes until colorless.
	Protein degradation	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 with methanol.
	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.
	Large molecular weight contaminants	Make certain the final protein extract is centrifuged for 30 minutes at 16,000 × *g* to pellet any contaminants. Be careful not to disrupt the pellet in any of the steps of the procedure.

References

1. Herbert, B.R., Electrophoresis, **19,** 845-851 (1998).
2. Ferro, M., et al. Electrophoresis, **21**, 3517-3526 (2000).
3. Loomis, W.D., Meth. Enzymol., **31**, 528-545 (1974).
4. Molloy, M.P., et al., Electrophoresis, **19,** 837-844 (1998).

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