INSTRUCTIONS

P-PER® Plant Protein Extraction Kit

89803

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<td>P-PER® Plant Protein Extraction Kit, contains sufficient reagents to extract protein from either 20 × 80 mg of fresh/frozen plant tissue or 20 × 20 mg of dried plant tissue</td>
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Kit Contents:
- P-PER® Reagent A, 20 ml, contains a preservative-free HEPES-based buffer, pH 7.0
- P-PER® Reagent B, 225 µl, contains a protein stabilizer
- P-PER® Reagent C, 20 ml, contains an organic extraction solution
- Polypropylene Mesh Bags, 20 each

Storage: Upon receipt store at room temperature.

Introduction

The P-PER® Plant Protein Extraction Kit uses a reagent-based method for rapid (as few as 10 minutes) recovery of soluble proteins from plant tissue samples. The resulting protein extract is compatible with many downstream applications, including SDS-PAGE, 2-D gel electrophoresis, Western blotting, activity assays and affinity purification. The P-PER® Kit includes three components: an organic solution to lyse plant cells, an aqueous buffer to gently solubilize proteins and a protein-stabilizing reagent. The P-PER® Kit has been tested on leaves, roots, flowers and seeds from various species, including Arabidopsis, tobacco, spinach, peas and soybeans. Extremely fibrous tissue samples, such as woody stems, may require additional mechanical grinding by devices not included in the kit.

Important Product Information

- Protein can be extracted from fresh, frozen and dry plant tissue. Proteins extracted from samples that have been powdered by freeze/grinding with liquid nitrogen may no longer be active (i.e., useable in functional assays).
- Use of protease inhibitors is critical. Add the necessary amount of an appropriate protease inhibitor cocktail (e.g., Halt™ Protease Inhibitor Cocktail, Product No. 78410) to Reagent A before making the P-PER® Working Solution.
- To inhibit oxidases and trap quinines, add sodium metabisulfite or another antioxidant to Reagent A before making the P-PER® Working Solution.
- For plant tissues rich in phenolic compounds, add a polyphenol adsorbent such as polyethylene glycol (PEG) or polyvinyl polypropylene (PVPP) to Reagent A before making the P-PER® Working Solution.
- Lysis and protein extraction may be performed at room temperature or 4°C. For use at 4°C, pre-chill P-PER® Reagents A, B and C before making the working solution; however, avoid long-term storage of reagents at 4°C.
- To avoid protein aggregation, dialyze, buffer-exchange or add stabilizers to the prepared protein extract. For long-term storage at -20°C, add ethylene glycol or glycerol to a final concentration of 10-20%.
- Use the Pierce BCA Protein Assay – Reducing Agent Compatible (Product No. 23250) to estimate protein concentration in the protein extract. The composition of the P-PER® Extraction Solution may interfere with standard protein assays.
- Prepare the P-PER® Working Solution just before use. For best results, do not store the working solution for more than one day.
Procedure Summary

1. Prepare P-PER® Working Solution.
2. Place tissue sample between mesh screens.
3. Add P-PER® Working Solution.
5. Withdraw the lysate.
6. Add lysate to centrifuge tube.
7. Centrifuge to partition organic and aqueous layers.
8. Recover protein extract (i.e., lower, aqueous layer).

Additional Materials Required
- Polypropylene or glass centrifuge tubes and pipettes (avoid polystyrene)
- Protease inhibitors, anti-oxidants, polyphenolic adsorbent and other additives (see the Important Product Information Section)
- Centrifuge suitable for the tubes being used
- Fume hood (see Note in step 1 of the Procedure)

Procedure for Plant Protein Extraction
The following procedure is for a single extraction from 80 mg of fresh or frozen plant tissue or from 20 mg of dry plant tissue such as seeds. This procedure can be effectively scaled for extractions from various amounts of plant tissue provided the ratio of P-PER® Working Solution (WS) to tissue weight is maintained.

Note: Add protease inhibitors, anti-oxidants, polyphenolic adsorbents and other additives to Reagent A before making the WS. For example, add 10 µl of Halt™ Protease Inhibitor Cocktail (Product No. 78410) to 990 µl of Reagent A. Always use a sterile pipette tip when handling Reagent A.

1. Just before use prepare the WS by mixing 990 µl of Reagent A with 10 µl of Reagent B. Then add 750 µl of Reagent C and vortex mixture until a colloidal suspension forms. Because the two reagents rapidly separate into two phases, be sure to thoroughly mix the WS again immediately before adding to the tissue sample(s). For best results, do not store the working solution for more than one day.

Note: Reagent C has an unusual odor that some may find unpleasant. To minimize exposure to the odor, perform extraction procedure in a fume hood. Consult the material safety data sheet (MSDS) with regard to safety concerns.

2. Place plant tissue into the polypropylene mesh bag. The bag contains an inner layer screen mesh that is sealed on three sides. With the open end of the bag facing up, pull open the bag and place the tissue between the inner screens. For best results, place the tissue as low in the bag as possible.

Note: Each mesh bag can hold up to 500 mg of plant tissue, which requires approximately 11 ml of WS. For tissue samples > 500 mg, either split the sample into amounts < 500 mg in separate bags or use a large mechanical grinding device, such as a blender, with the appropriate amount of WS.

3. Pipette or pour the WS into the mesh bag or mechanical grinding device with the tissue.
4. Firmly hold the open end of the bag upright and lay the lower portion on a flat firm surface. Use a hard, round-tipped object, such as a capped marking pen, and from the outside of the bag, rub and massage the plant tissue making sure the WS and the tissue are thoroughly mixed and no visibly intact tissue remains. For hard seeds, such as soybeans, a ceramic pestle works well for smashing and mixing tissue into the WS.

5. Once a homogeneous mixture is achieved, allow liquid to settle to the bottom of the bag and then retrieve liquid with a pipette inserted into the clear strip edge of the bag. To retrieve the entire sample, rub the bag from one end toward the side with the clear strip, similar to moving toothpaste to one end of the tube.

6. Place mixture into a centrifuge tube and centrifuge for 5 minutes at 2,000-5,000 \( \times g \) to partition organic and aqueous phases. Filtration of the sample is not necessary.

7. Extracted, soluble protein will be in the lower aqueous layer. The upper organic layer may be discarded or assayed for waxes, fatty acids and other large chain hydrocarbons. To recover the protein-containing aqueous layer, insert a pipette tip through the organic layer into the aqueous layer and gently exert positive pressure to dispense any of the organic layer that entered the pipette tip as it passed through. Carefully remove the protein-containing aqueous layer and transfer it to a new tube.

Suggestions for Downstream Applications

- 1-D Gel Electrophoresis (SDS-PAGE): Dilute sample with sample buffer and electrophorese using standard conditions.
- 2-D Gel Electrophoresis: Dilute sample at least 1:10 with sample loading buffer to lower the concentration of salt in the WS (~133 mM). Alternatively, use PAGEprep® Advance Kit (Product No. 89888).
- Protein Assay: Use the Pierce BCA Protein Assay – Reducing Agent Compatible (Product No. 23250) to estimate protein concentration in the tissue extract. The composition of the P-PER® Extraction Solution may interfere with standard protein assays.

Related Thermo Scientific Products

- 78410 Halt™ Protease Inhibitor Cocktail Kit
- 78501 M-PER® Mammalian Protein Extraction Reagent, 250 ml
- 78248 B-PER® Bacterial Protein Extraction Reagent, 500 ml
- 78990 Y-PER® Yeast Protein Extraction Reagent, 500 ml
- 89826 Mem-PER® Membrane Protein Extraction Reagent Kit
- 78833 NE-PER® Nuclear and Cytoplasmic Extraction Kit

Patent pending on P-PER® Protein Extraction Technology