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Minute™ Plant Cytosolic and Nuclear Protein Isolation Kit

Cat. No. PF-045

Description

The kit is designed for rapid fractionation of fresh/frozen soft plant tissues, especially leaves. Unlike other commercial kit and Lab protocols, of which large amount of starting material (a few grams) and lengthy processing time are required, this kit only uses 100-200 mg starting material. By using a specialized filter cartridge coupled with our proprietary buffer system, four subcellular fractions (cytosol, nuclei, chloroplasts and organelles) can be obtained in about 1 hour. The subcellular fractions can be used in numerous applications that include but not limited to protein trafficking, nuclear protein extraction and nucleic acid purification/sequencing. The highly enriched nuclei can be used for proteomics, flow cytometry analysis, DNA sequencing, and protein-protein/protein-DNA interaction studies. The subcellular fractionation protocol includes grinding tissue to release plant cells followed by disruption of the cell membrane by rapidly passing through the filter. Different subcellular fraction is obtained by differential centrifugation using only a table top centrifuge. This kit has been tested for use with many species such as Arabidopsis, Spinach, Peas, and Rape.

Kit Components (20T) :

1.	Buffer A	30 ml
2.	Buffer B	1 ml
3.	Buffer C	25 ml
4.	Filter Cartridges with Collection Tubes	20
5.	Pestle for 1.5 ml Eppendorf tubes	2
6.	Protein Extraction Power	2g

Additional Materials Required but Not Provided

- Table-top micro centrifuge (***Perform all centrifugation steps at 4-8^{o}C)***
- 1 X PBS
- 1 X PBS with 5% BSA (for FACS),
- 1.5ml Eppendorf tubes.

Shipping and Storage: Ship at ambient temperature and store at 4^{o}C.

Protocol

Note: Read the protocol carefully before starting. It is recommended to add proteinase inhibitors to aliquot of buffer A and PBS prior to use. The protocol can be scaled down to as low as 50 mg starting material. Pre-chill A and B on ice.

1. Place 100-150 mg fresh/frozen young plant leaf in the filter with collection tube. Fold and roll the leaf and insert it into the filter. Add 100 µl buffer A to the filter**.** Punch the leaf in the filter repeatedly with a 200 µl pipette tip for about 100-200 times to reduce the volume (this step takes about 2-3 min).
2. Grind the tissue with a pestle (flat end) using gentle twisting force for about 100-200 times (about 2-3 min). (Note: the pestle is reusable. For cleaning, rinse it with water and dry with paper towel).

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3. Add 300 µl buffer A to the filter and stir the sample with a 200 µl pipette tip for a few times. Cap the filter and centrifuge at 1,500 X g for 5 min (optional: after centrifugation, transfer 200 µl supernatant back to the same filter and re-grind again, this can increase final yield). Transfer the supernatant to a fresh 1.5 ml microfuge tube and incubate on ice if isolation of cytosolic and microsomal fractions is desired (see 4A below). Resuspend the pellet in 1 ml cold PBS by pipetting up and down and centrifuge at 1,200 X g for 5 min. Pour out the supernatant and resuspend the pellet in 1 ml buffer A and resuspend the pellet as described above and add 20 µl Buffer B to the tube and vortex vigorously for 10-20 seconds. Be sure the pellet is fully resuspended. See **4B** below for isolating intact nuclei.

4A. For isolation of cytosolic and microsomal fractions:

Centrifuge the supernatant from step 3 at 4,000 X g for 10 min (the pellet contains mainly broken chloroplasts).
Transfer the supernatant to a fresh 1.5 ml microfuge tube and centrifuge at 16,000 X g for 30 min (the supernatant is cytosolic fraction and the pellet contains microsomal fraction (organelles and plasma membrane) with majority of nuclei and chloroplasts depleted).

4B. For isolation of nuclei:

a. Incubate 1 ml resuspended pellet from step 3 on ice for 5 min. Centrifuge the tube at 1,200 X g for 5 min.
b. Remove supernatant completely and resuspend the pellet in 200 µl cold PBS by pipetting up and down for about 30-40 times.
c. Add 1.2 ml buffer C to a fresh 1.5 ml microfuge tube and carefully overlay well resuspended pellet from step b on top of buffer C by slowly expel the liquid against the wall of the tube.
d. Centrifuge at 1,200 X g for 5 min. After centrifugation, a white/light green pellet and a green upper phase should be seen clearly. Remove the supernatant completely and wash the pellet (isolated nuclei) with 0.5 ml cold PBS. The nuclei can be resuspended in any buffer desired depending upon downstream applications. For example: For protein extraction, the nuclei can be lysed using 50-100 µl detergent-containing buffer for Western blot, ELSA or IP (see table and tech note below). To determine protein concentration, BCA assay is recommended. If flow cytometry analysis is desired resuspend the pellet in 0.2-0.5 ml PBS with 5% BSA. For nucleic acid extraction the pellet can be resuspended in tris or suitable nuclear lysis buffer,

Tech Notes:

1. Though this kit works the best for young plant leaves, it also works for other soft tissues such as seeds, shells and soft stems. For non-leaf soft tissues, cut them into 1 X 1 mm or smaller pieces and follow the protocol. The purity of nuclei from non-leaf tissue is generally not as good as those from leaves. In most cases, the major contaminants are plastids rather than chloroplasts.
2. If isolated nuclei show significant green color, Resuspend the nuclei in 0.5 ml PBS plus 20 µl buffer B and incubate on ice for 10 min. Centrifuge at 1,000 X g for 5 min and collet washed nuclei.
3. The amount of starting material specified in protocol works for most samples. Don't use more than 200 mg/sample. More is not better here.
4. To extract protein from isolated nuclear pellet, add 50 µl protein extraction reagent to the tube followed by addition of 40-50 mg protein extraction powder to the bottom of the tube (the use of the powder is optional if WA-009 is used, see table below). Homogenize the pellet using the pestle (round end) with twisting force for 100-200 time. Add another 50 µl reagent to the tube. Centrifuge at 8,000 Xg

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for 5 min. The supernatant is extracted nuclear protein solution. The typical protein yield is 20-50 µg/sample. If more proteins are needed combined two or more isolated nuclear preps prior to protein extraction.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	1 D or 2D electrophoresis and Western blotting, trypsin digestion. For 2DE the extracted protein should be precipitated by TCA prior to loading the gel.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, gel shift assay, enzymatic activity determination and other applications.