Minute™ Plant Endosome Enrichment Kit

Catalog Number PE-050

Description

An understanding of endomembrane processes and their biological implication is important for understanding plant growth and development. Endosomes are a heterogeneous collection of organelles in the sorting and delivery of internalized material from cell surface and the transport of materials from Golgi to lysosome or vacuole. In plant cells, two clearly defined endosomal compartments have been identified: the trans-Golgi network (early endosome equivalent) and the multivesicular body (late endosome equivalent). Traditionally, methods for isolation and purification of these two compartments are tedious and time-consuming involving sucrose gradient centrifugation. In some cases, sucrose gradient isolation is coupled with immunoaffinity purification and larger amount of starting materials are usually required (10-50 g). We have developed this kit using a spin-column-based format coupling with selective precipitation for enrichment of endosomal compartment. This approach is easy and rapid and only small amount of starting material is required.

Kit components

1. Buffer A	10 ml
2. Buffer B	10 ml
3. Buffer C	10 ml
4. Plastic rod	2
5. Filter and collection tube	20

Important Note

1. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microcentrifuge. Add proteinase/phosphatase (if protein phosphorylation is involved) inhibitor cocktails to aliquot of buffer A.
2. Chill buffers on ice prior to use.

Protocol

1. Place 200-250 mg fresh/frozen young plant leaf or seedling in a filter with collection tube. Fold and insert it into the bottom of 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 1-2 min).
2. Grind the tissue with the plastic rod provided using gentle twisting force for about 200 times (about 2-3 min). (Note: the rod is reusable. For cleaning, rinse it with dd H2O and dry with paper towel).
3. Add 400 µl buffer A to the filter and stir the sample with a 200 µl pipette tip for a few times. Cap the filter and Centrifuged at 10,000 X g for 5 min. This step is to remove chloroplasts, nuclei and other larger plastids. After centrifugation, transfer all supernatant to a fresh 1.5 ml microfuge tube.
4. Discard the filter and centrifuge at 16,000 X g for 10 min at 4°C. Transfer 500ul supernatant to a fresh 1.5 ml microfuge tube.

5. Add 500 µl buffer B to the tube, vortex briefly to mix well and incubate on ice for 5 min. Centrifuge at 2800 X g for 5 min (this is to remove majority of rubisco that is the major source of cross-contamination in plant fractionation). Transfer all supernatant to a fresh tube and add 500 ul buffer C to the tube. Mix well by vortexing briefly. Incubate on ice for 20 min.
6. After incubation, spin the tube at 10,000 X g for 5 min. Remove supernatant completely. Add 1.5 ml cold ddH20 to the tube slowly without disturbing the pellet (this is to remove residual reagent). Pour out the water completely and resuspend the pellet in 100-200 ul phosphate buffered saline (PBS) or a buffer of your choice by pipetting up and down repeatedly (This is enriched endosome fraction). The choice of buffer is depending upon downstream applications (see table below).

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.