Minute™ Plant Microsomal Membrane Extraction Kit

Catalog number: MM-018

Description

Isolation of microsomal membranes from plant tissues is a common laboratory procedure. Microsomal fraction of plant cell lysate is the focus of interest in many plant research projects. Microsomal fraction is believed to be enriched for plasma membranes, endoplasmic reticulum, Golgi apparatus, vacuolar membranes and other components of membrane system. Traditional method for microsomal fraction isolation involves so called differential pelleting protocol where a series of centrifugation steps are required to obtain various membrane fractions. Traditional protocol for microsomal fraction isolation requires large amount of starting material and employs tedious ultracentrifugation steps. MM-018 offers a simple, rapid and user friendly approach for microsomal membrane extraction using small amount of starting material (200 mg). Water soluble cytosolic proteins are removed during the procedures and water insoluble microsomal fraction, especially plasma membrane fraction, is extracted with optimized buffers in a table-top microcentrfuge. The procedure is simple, rapid and no special instrument required. Native microsomal proteins can be isolated from plant tissue in about one hour without ultracentrifugation. The protein yield is in the range of 100-200 µg/sample.

Applications

Microsomal membranes extracted with this kit can be used for many downstream applications such as SDS-PAGE analysis, Western blotting, IP. ELISA, enzyme activity assays, proteomics and membrane trafficking analysis.

Kit components

1. 25 ml buffer A
2. 10 ml buffer B
3. Filter cartridge with 2.0 ml collection tube (50)
4. Plastic rods (4)

Storage: Store the kit at -20ºC.

Additional Materials Required

Table-Top Microcentrifuge with a maximum rpm of 14,000-16,000. 1 X PBS.

Important Product Information

Prior to plasma membrane isolation addition of protease inhibitor cocktail to buffer A is recommended. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use.

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Protocol

1. Pre-chill buffers and the filter cartridge in collection tube on ice.

2. Place 200 mg fresh plant tissue in the filter. **For plant leaf**, fold or roll the leaf and insert it into the filter. Punch the leaf in the filter repeatedly with a 200 µl or 1 ml pipette tip for 60 times to reduce the volume. **For seeds and soft stems**, cut them with a sharp blade into smaller pieces and place in the filter cartridge(s).

3. Add 300 µl cold buffer A to the filter (**Important:** quickly pipette the buffer after **shaking the bottle vigorously for a few seconds**). Grind the tissue with a plastic rod provided for 2-3 min (about100 times) with twisting force (Note: The plastic rod is reusable. For cleaning, rinse it thoroughly with distilled water and dry it with paper towel).

4. Cap the filter and centrifuge in a microcentrifuge at 14,000 rpm at 4°C for 20 min. Discard the filter and remove the supernatant completely.

5. Resuspend the pellet in 300 µl cold buffer B by pipetting up and down or vortexing. Centriduge at 12,000 rpm for 10 min at 4°C. Transfer supernatant to a pre-chilled 2.0 ml microfuge tube.

6. Add 1 ml cold 1 X PBS to the tube, invert a few times to mix the contents well. Centrifuge the tube at 14,000-16,000 rpm for 30 min at 4°C. Remove the supernatant and dissolve the pellet (microsomal fraction) in 50-100 µl detergent containing buffers of your choice.

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