Minute™ Plasma Membrane Protein Isolation Kit for Plants

Catalog number: SM-005-P

Description

Plasma membrane (PM) protein accounts for a small fraction of total cellular protein in plants but performs a very critical role in plant physiology. Isolation and purification of PM protein from plant tissues have been traditionally done by sucrose density ultracentrifugation and aqueous two-phase partitioning. These methods, while relatively effective, require ultracentrifugation and large amount of starting material. The procedures are usually tedious and time consuming. To overcome the shortcomings, we have developed this PM isolation kit. Plant tissues are first sensitized by buffer A, homogenized, and pass through a specialized filter cartridge that allows homogenates to pass through with a zigzag path. The cell membranes are ruptured into a range of predefined size during the process. Native plasma membranes are separated from a mixture of un-ruptured cells, nuclei, cytosol and organelles by subsequent differential centrifugation and density centrifugation without using ultracentrifugation. Due to the use of same amount of starting material, defined centrifugal force and pre-defined duration in every experiment, the result is much more consistent with high degree of PM protein enrichment. The procedure can be completed in about 1h.

Applications

The kit is designed to rapidly isolate native membrane proteins from plant tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, membrane protein structure analysis, 2-D gels, enzyme activity assays and other applications.

Buffer Formulations: Proprietary

Kit components (50 preps)

1. 25 ml buffer A
2. 10 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5 4 plastic rods
6. Tissue dissociation beads

Storage: Store Buffer A and Buffer B at -20°C upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge.

The rpm use in following protocol is based on a Eppendoff 5415C table top microcentrifuge.

Important Information:

1. Read the entire procedures carefully. Thaw buffer A and buffer B completely, invert the bottles a few times and place them on ice. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4ºC in a cold room or in a refrigerated microcentrifuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. The use of protease inhibitor cocktails is optional.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Plasma Membrane Protein Isolation Procedures

Tissue homogenization: **Due to greater diversity in plant samples, it is critical to choose a proper homogenization method to achieve optimal results.**

1. Place the filter cartridges in collection tubs, and incubate on ice.

2. **A. For most soft plant tissues**, cut the fresh or frozen plant tissue (200-300mg) into small pieces (1-2 mm X 1-2 mm) and place them onto a filter cartridge. Punch the tissue repeatedly with a 1 ml pipette tip to reduce the volume. Weight out 50-80 mg tissue dissociation bead and place it on top of the tissue in the filter. Homogenize the tissue using the plastic rod provided for 1-2 min. Add 100 µl buffer A to the filter and continue to grind the tissue for a few minutes. After grinding add additional buffer A to the filter to the top and place the filter with cap open on ice for 5 min. Go to step 3.
   B. For tissues with high water contents such as tissue of many fruits, cut tissue (about 300mg) into small pieces and place in a filter cartridge. Punch the tissue repeatedly with a 1 ml pipette tip to reduce the volume. Centrifuge in a table top microcentrifuge at top speed for 1 min to remove excessive water and homogenize the tissues as described above. After homogenization and incubation for 5 min on ice go to step 3.

C. For samples with tough tissues such as roots and rice leaves, the homogenization step can be done outside of the filter by using to a mortar or similar homogenization device. After homogenization in proper amount of buffer A (about 300-400 µl), transfer the homogenates onto a filter cartridge and go to step 3.

3. Cap the filter cartridge and centrifuge at 14,000 rpm (16,000 X g) for 30 seconds.

4. Discard the filter and resuspend the pellet by vigorously vortexing for 10-20 seconds.

   Following procedures separate plant tissue homogenate into four fractions: Nuclei, cytosol, organelles and plasma membrane.

5. Centrifuge at 3000 rpm (700 X g) for one min (**the pellet contains nuclei and larger debris**).

6. Transfer the supernatant to a fresh 1.5 ml microcentrifuge tube and centrifuged at 4ºC for 30 min at 16,000 X g (longer centrifugation time will increase yield). Remove the supernatant (**this is the cytosolic fraction**) and save the pellet (this is the total membrane fraction including organelle membrane and plasma membrane. You may stop here and freeze the pellet at -80ºC if further isolation of plasma membrane proteins is not needed. For plasma membrane protein isolation continue to step 7. Don't freeze total membrane protein fraction if further isolation of plasma membrane proteins is desired.

7. Resuspend the total membrane protein fraction from step 6 in 200 µl buffer B by repeatedly pipetting up and down or vortexing. Centrifuge at 10,000 rpm (7,800 X g) for 5-10 min at 4ºC (Note: if final plasma membrane prep is contaminated by organelle membranes, increase centrifugation time up to 20 min can improve the purity). The pellet contains organelle membrane proteins.

8. After centrifugation in step 7, carefully transfer the supernatant to a fresh 2.0 ml microcentrifuge tube and add 1.6 ml cold PBS. Cap the tube and mix by inverting the tube a few times. Centrifuge at 14,000 rpm (16,000 X g) for 30 min (longer centrifugation will improve yield). Discard the supernatant and save the pellet (isolated plasma membrane fraction). Typically,10-100µg plasma membrane proteins can be obtained/sample. Several pellets of isolated PM can be combined to increase total amount. The PM proteins can be dissolved in 20-200 µl detergent containing buffers of your choice depending upon specific downstream applications. Reagents in following table are recommended for solubilization of the pellet. For isoelectric focusing (First dimension of 2D gel) we recommend to use: 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use).

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.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting material Increase incubation time to 10 min (step3)
Low protein activity	Keep lysate cold/add protease inhibitors
Retention of cell lysate in protein filter cartridge after 30 seconds of centrifugation	Reduce amount of starting material or increase centrifugation time to 2 min
Contamination of PM by cytosolic proteins	Wash PM pellet with 0.5 ml cold PBS containing 0.3 M NaCl, Ph. 9.5