Minute™ Plant Lipid Raft Isolation Kit

Catalog number: PL-051

Description

There are substantial evidences that support the existence of detergent-resistant membrane (DRM) subdomains in both animal and plant membrane systems, which is also referred to as lipid rafts. Lipid rafts are shown to have increased sphingolipid to protein ratio and higher cholesterol concentration. The lipid rafts are believed to play an important role in signal transduction and protein trafficking in eukaryotic cells. Traditional method for lipid raft isolation involves cold non-ionic detergent extraction followed by sucrose gradient ultracentrifugation. The protocol requires large amount of starting material (hundred grams amount) and specialized equipment in addition to lengthy protocol time. We have developed a rapid method for isolation of lipid rafts from plant tissues using only milligram amount of starting material and the protocol can be completed in about 1h without using ultracentrifugation.

Kit Components (20T):

Buffer A	10 ml
Buffer B	10 ml
Buffer C	1.2 ml
Buffer D	10 ml
Plastic rods	2
Protein Extraction powder	2g
Filter Cartridge/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 A, B, and C on ice prior to use.
3. Solution required but not provided: Cold ddH_2O.

Protocol

1. Place 200-250 mg fresh young plant leaf/seedling in a filter in a 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 80-100 times to reduce the volume (this step takes about 1-2 min). Add 50-80 mg protein extraction bead to the filter.
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 ddH_2O 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 8,000 X g for 10 min. This step is to remove chloroplasts, nuclei and other larger plastids. After centrifugation, discard the filter and transfer all supernatant to a fresh 1.5 ml microfuge tube.

4. Centrifuge the tube at 16,000 X g for 30 min. Remove and discard supernatant. Add 1 ml cold ddH_2O to the tube without disturbing the pellet and remove it immediately.
5. Resuspend the pellet in 450 µl buffer B by pipetting. Add 50 µl buffer C and mix well. Incubate at 4°C for 30 min. Vortex vigorously for 20 seconds and centrifuge at 10,000 X g for 5 min. Transfer the supernatant to a fresh microfuge tube.
6. Add 500 µl buffer D to the tube, vortex briefly to mix and incubate on ice for 10 min. Spin the tube at 10,000 X g for 5 min. After centrifugation the lipid rafts are floating on top of the tube.
7. Insert a fine pipette tip (such as the SDS-PAGE sample loading tip) attaches to a transfer pipette to the bottom of the tube and remove aqueous phase slowly. Alternatively, a 2 ml syringe equipped with a 21gauge needle can also be used. The white-light green colored lipid rafts will adhere to the wall of the microfuge tube after the aqueous phase is removed (save this phase if non-lipid raft membrane proteins are of the interest).
8. Centrifuge at 16,000 X g for 5 min to bring down lipid rafts to the bottom of the tube. Remove residual reagent completely. Carefully add 200 µl cold ddH_2O to the tube without disturb the pellet and immediately remove the water completely. The pellet is isolated lipid rafts that can be resuspended in 100-200 µl buffers listed in the table below or in other buffers of your choice depending upon the downstream applications. The final protein yield is in the range of 50-100 ug/sample depending upon the sample types.

Tech note:

1. The isolated lipid rafts may need to be dialyzed to reduce salt concentration for applications such as 2D gel analysis.
2. The non-lipid raft protein in the supernatant can be precipitated by our protein precipitation kit (Cat#: WA-006).
3. The isolated lipid rafts are mainly derived from plasma membrane and other endomembrane systems.
4. Lipids can be extracted from isolated lipid rafts by traditional organic extraction method.

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.