Minute™ Plasma Membrane-Derived Lipid Raft Isolation Kit

Catalog Number: LR-042

Description

Lipid rafts are small membrane domains containing high level of cholesterol and sphingolipids. Lipid rafts have been found in plasma membrane (PM) and internal organellar membranes such as mitochondria-associated membrane (MAMs) and endoplasmic reticulum. Lipid rafts are implicated in numerous cellular processes such as signal transduction, membrane trafficking and protein sorting. Lipid-modified proteins and some transmembrane proteins are concentrated in the rafts while other proteins are excluded. Lipid rafts are also found to be associated with Na^+/K^+ ATPase on PM. Traditional methods for lipid raft isolation involve isolation of detergent resistant membrane subdomain from total membranous structures, which does not distinguish plasma membrane-derived and/or organelle-derived lipid rafts. Using the patented spin-column-based technologies, we have developed this kit specifically for isolation of plasma membrane-derived lipid rafts. Larger plasma membrane vesicles are first isolated and treated with a non-ionic detergent containing buffer followed by isolation of detergent resistant fraction by flotation centrifugation using a table top microcentrifuge. Highly enriched plasma membrane derived lipid rafts can be obtained in about 1 hour without using traditional homogenizer and ultracentrifugation.

For total lipid raft isolation, please refer to Minute™ Total Lipid Raft Isolation Kit under Cat # LR-039.

Kit Components (20 prep)

1.	Buffer A	15 ml
2.	Buffer B	10 ml
3.	Buffer C	10 ml
4.	Plastic rods	2
5.	Filter Cartridge with collection tubes	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000 X g. **The centrifuge should be able to reach maximum speed within 10 seconds.**

Shipping and Storage: Ship at ambient temperature and store at 4°C.

Important Information:

1. **All centrifugation steps should be performed at 4-8°C in a cold room or in a refrigerated microfuge.**

2. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. If protein degradation is a concern, add protease inhibitor cocktails to aliquot of buffer A and B prior to use.
3. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce).

Protocol

Note: Warm buffer C to room temperature and mix well prior to use.

1. Place the filter cartridges in a collection tube and incubate on ice. Pre-chill buffer A and B on ice prior to use. **Don't pre-chill buffer C!**

2. **A. For cultured cells**, collect 30-40 X 10^6 cells by low speed centrifugation (500-600 X g for 5 min). Wash cells once with 1 ml cold PBS. Remove supernatant completely and resuspend the pellet in 500 µl buffer A. Incubate the cell suspension on ice for 5 min. **Vortex the tube vigorously for 10-30 seconds**. Immediately transfer the cell suspension to the filter cartridge. Go to step 3.

   B. For soft tissue samples, place 40-50 mg tissue (fresh or frozen) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue with a plastic rod by pushing the tissue against the surface of the filter repeatedly with twisting force for 2-3 min. After grinding, add 300 µl buffer A to the same filter cartridge. Go to step 3. **For muscle tissues,** Place tissue on surface of a clean glass or plastic plate. Mince the tissue with a sharp blade into tissue slurry or past. Transfer the tissue past to the filter cartridge and grind as above. **The plastic rod is reusable. Clean it with 70% alcohol or water.**

3. Cap the filter cartridge, invert a few times and centrifuge at 16,000 X g for 30 seconds (for cultured cells, the pass through can be repassed through the same filter to increase the final yield).
4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds.
   Centrifuge at 1900 X g for 5 min (the pellet contains nuclei, large cell debris and some un-ruptured cells).
5. Transfer all supernatant to a fresh 1.5 ml microfuge tube and centrifuged at 3000 X g for 15 min. Carefully remove and discard the supernatant. The pellet is isolated plasma membrane fraction (larger PM vesicles).
6. Resuspend the pellet in 0.4 ml cold buffer B by repeat pipetting up and down for 20-30 times and incubate the tube on ice for 30 min. Vortex briefly every 10 min and immediately return the tube onto ice to keep it cold all the time.
7. Add 0.4 ml buffer C to the tube and mix well by vortexing briefly (the solution in the tube becomes clouded). Centrifuge at 10,000 X g for 5 min. After centrifugation the lipid raft is floating on top of the tube.
8. 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-grey colored lipid rafts will adhere to the wall of the microfuge tube after removal of the aqueous phase.

9. Centrifuge the tube at 16,000 X g for 2 min to bring down lipid rafts to the bottom of the tube. Remove residual reagent completely. The pellet is isolated PM-derived lipid rafts that can be resuspended in 100-300 µl buffers listed below or in other buffers of your choice depending upon the downstream applications. The final protein yield is in the range of 30-200 ug/sample depending upon the cell/tissue types. The isolated lipid rafts can be dissolved in different reagent for different applications.

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.