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Minute™ Plasma Membrane-Derived Lipid Raft Isolation Kit

Catalog Number: LR-042

Description

Lipid rafts are small membrane domains containing a high level of cholesterol and sphingolipids. Lipid rafts have been found in the 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 the 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 tabletop microcentrifuge. Highly enriched plasma membrane-derived lipid rafts can be obtained in about 1 hour without using a 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)

	Component	Amount
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°C in a cold room or a refrigerated microfuge.**
2. For protein phosphorylation study, **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 buffer A and B prior to use.
3. It is recommended to use BCA Protein Assay Kit for the determination of protein concentration (Pierce, Cat #:23227).

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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 them on ice. Pre-chill buffer A and B on ice before 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 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 the 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 the 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 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 may be repassed through the same filter to increase the final yield).

4. Discard the filter and resuspend the pellet by vigorous 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 centrifuge at 3000 X g for 15 min. Carefully remove the supernatant. The pellet is the isolated plasma membrane fraction (larger PM vesicles).

6. Resuspend the pellet in 0.4 ml cold buffer B by repeated pipetting up and down for 20-30 times and incubate on ice for 30 min. Vortex briefly every 10 min and immediately return onto the ice.

7. Add 0.4 ml buffer C and mix well by vortexing briefly (the solution becomes cloudy). Centrifuge at 10,000 X g for 5 min. and the lipid raft will float on the top.

8. Insert a fine pipette tip (such as the SDS-PAGE sample loading tip) attached to a transfer pipette to the bottom of the tube and remove the aqueous phase slowly. Alternatively, a 2 ml syringe with a 21gauge needle can 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 at 16,000 X g for 2 min to bring down lipid rafts to the bottom of the tube. Remove residual reagent completely. Carefully add 1 ml cold ddH2O to the tube without disturbing the pellet then remove the water completely (see tech note below). The pellet is isolated PM-derived lipid rafts that can be resuspended in 100-300 µl buffers listed below or in buffers of your choice. 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 reagents for different applications.

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Tech Note:

1. The reason to rinse the pellet with water in step 9 is to remove residual buffer components that may interfere with downstream applications such as SDS-PAGE and Western blotting. In most cases, this treatment is sufficient for reducing the interference.
2. If still not satisfactory, the pellet can be resuspended in 100-200 µl WA-009 (see table below) and pass through a desalting column (such as Zeba desalting column of ThermoFisher) to further remove the interfering components.
3. Another alternative is to dialyzing resuspended lipid raft against cold water using a dialyzer (such as 10 K Slide-A-Lyzer Mini Dialysis Device).
4. The aqueous phase in step 8 contains non-lipid raft proteins that can't be readily analyzed by Western blotting (WB) due to the presence of interfering components. However, non-lipid proteins can be precipitated from the aqueous phase by MinuteTM High-Efficiency Protein Precipitation Kit (Cat# WA-006) for WB analysis. If WA-006 is used, pay attention to the following technical details: Transfer 0.6-0.8 ml of the aqueous phase to a 1.5 ml microfuge tube and follow the standard protocol of WA-006. Perform all incubations on ice and remove supernatants by pipetting instead of decanting. After the washing buffer is added to the tube that contains precipitated proteins, the washing buffer appears slightly white-grey in color (this is normal). Make sure the precipitated protein pellet stays in the bottom after washing.
5. It is important to have equal loading in SDS-PAGE and Western blotting for the determination of the enrichment of lipid raft as compared to the total cell lysate.

Recommended reagents:

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.
Minute™ High-Efficiency Protein Precipitation Kit (30 ml)	WA-006	Precipitation of non-lipid raft proteins (see tech notes).

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