Minute™ Total Lipid Raft Isolation Kit for Mammalian Cells/Tissues

Catalog Number: LR-039

Description

Lipid rafts are small membrane domains containing high cholesterol and sphingolipids. Lipid rafts are found in the plasma membrane (PM) and internal organellar membranes such as mitochondria-associated membrane (MAMs) and endoplasmic reticulum. They have been implicated in numerous cellular processes including but not limited to signal transduction, membrane trafficking, and protein sorting. Lipid-modified proteins and some transmembrane proteins are concentrated in the rafts, while others are excluded. Lipid rafts are also found to be associated with Na^+/K^+ ATPase on the plasma membrane. Lipid rafts are isolated traditionally by sucrose-gradient or OptiPrep gradient using ultracentrifugation, which requires a large amount of starting material and is tedious and time-consuming. This kit was developed using our proprietary spin-column-based technologies, offering a quick and easy way to isolate lipid rafts. Total membrane fraction (including PM and organelle membranes) is first isolated and treated with a non-ionic detergent-containing buffer, followed by isolation of detergent-resistant fraction by flotation centrifugation using just a tabletop microcentrifuge. Highly enriched total lipid rafts can be obtained from cultured cells/tissues in less than 90 min without using density gradient and ultracentrifugation.

For isolation of plasma membrane-derived lipid rafts, please refer to Minute™ Plasma Membrane-Derived Lipid Raft Isolation Kit under Cat #: LR-042

Kit Components (20T) :

1.	Buffer A	15 ml
2.	Buffer B	10 ml
3.	Buffer C	10 ml
4.	Plastic rods	2
5.	Filter Cartridge	20
6.	Collection Tube	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000 Xg. **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).

Protocol

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

1. Place the filter cartridges in collection tubes 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 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 30-40 mg fresh or frozen tissue in a filter cartridge (For muscle tissue, mince with a sharp blade into slurry or past on surface of a clean glass or plastic plate before transfer to the filter cartridge). Add 200 µl buffer A and grind the tissue with the 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.
   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.

4. Discard the filter and resuspend the pellet by vigorous vortexing for 10 seconds. Centrifuge at 1,000 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 4°C for 30 min at 16,000 X g. The pellet is the total membrane fraction. Carefully transfer all supernatant to a fresh 1.5 ml tube and save if desired (this is the cytosolic fraction).

6. Resuspend the pellet in 500 µl cold buffer B by repeat pipetting up and down for 20-30 times followed by vigorous vortex for 10 seconds. Immediately incubate on ice for 30 min. Vortex briefly every 10 min and immediately return the tube onto the ice to keep it cold all the time.

7. Centrifuge at 16,000 X g for 10 min. Transfer all supernatant to a fresh 1.5 ml microfuge tube. Add 0.5 ml buffer C to the tube and mix well by vortexing briefly (the solution becomes cloudy). Incubate on ice for 2 min. Centrifuge at 10,000 X g for 10 min. The lipid rafts will float on the top after centrifugation.

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 and completely. Alternatively, a 2 ml syringe with a 21gauge needle can also be used. The lipid rafts will adhere to the wall of the microfuge tube after the removal of the aqueous phase.

9. Centrifuge at 16,000 X g for 5 min. Remove residual buffer completely. Carefully add 1 ml cold ddH_2O without disturbing the pellet, remove the water completely (see tech note below). The pellet contains isolated lipid rafts that can be resuspended in 50-200 µl buffers listed below or in buffers of your choice. The final protein yield is in the range of 30-100 µg/sample depending upon the cell/tissue types.

Tech Notes:

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 Minute™ 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).