Minute™ Lipid Raft Isolation Kit for Mammalian Cells/Tissues

Catalog Number: LR-039

Description

Lipid rafts are small plasma membrane domains containing high level of cholesterol and sphingolipids. Lipid rafts 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 other proteins are excluded. Lipid rafts are also found to be associated with Na^+/K^+ ATPase on plasma membrane. Lipid rafts are traditionally isolated by sucrose-gradient or OptiPrep gradient using ultracentrifugation that requires large amount of starting material. The protocol is tedious and time consuming. This kit was developed using our proprietary spin-column-based technologies, offering a rapid and easy way to isolate lipid rafts. Total membrane fraction of the sample 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 table top microcentrifuge. Highly enriched lipid rafts can be obtained from cultured cells/tissues in less than 90 min without using density gradient and ultracentrifugation.

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	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 RT.

Important Information:

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

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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 buffer A and B prior to use.
3. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Protocol

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 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 30-40 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.
4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds. Centrifuge at 1000 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 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 cytosolic fraction).
6. Resuspend the pellet in 500 µl cold buffer B by repeat pipetting up and down for 20-30 times and vortex vigorously for 10 seconds. Immediately 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. Centrifuge the tube 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 in the tube becomes clouded). Incubate the tube on ice for 2 min. Centrifuge at 10,000 X g for 10 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 and completely. Alternatively, a 2 ml

syringe equipped with a 21gauge needle can also be used. The lipid rafts will adhere to the wall of the microfuge tube after removal of the aqueous phase.

9. Resuspend the lipid rafts in 200 µl cold buffer A by pipetting up and down for 10-20 times. Centrifuge at 16,000 X g for 5 min. Remove supernatant completely. The pellet is isolated lipid rafts that can be resuspended in 50-200 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 30-100 ug/sample depending upon the cell/tissue types.

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.