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OrgFrontier™ Plasma Membrane Isolation Kit

(Catalog # K414-10; 10 preparations; Store at -20°C)

I. Introduction:

Plasma membrane is one of the most studied cellular membranes. Prokaryotic and eukaryotic organisms possess plasma membranes which, in summary, enclose and protect the cytosol, and cellular organelles while communicating to their surroundings due to their semi-permeable characteristics. Plasma membranes are selectively permeable lipid bilayers: they allow nutrients and other metabolites to be transported into the cytosol and allow for export of cellular waste at the same time. Oxygen, CO_2 and other gaseous or lipophilic small molecules freely diffuse across the plasma membrane; however, ions, amino acids and sugars follow strict passive and active cellular transportation mechanisms. The plasma membrane lipid bilayer is primarily composed of four phospholipids: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin. Phosphatidylinositol, a fifth phospholipid, is also localized in the inner side of the membrane. Additionally, many proteins are embedded or found adhered to both sides of the plasma membrane. For example, glycoproteins are usually found attached to the outer side of the membrane. BioVision's OrgFrontier™ Plasma Membrane Isolation Kit provides a proprietary set of reagents and buffers designed to enable optimized extraction and isolation of intact plasma membrane from mammalian tissues and cells. The protocol requires an ultracentrifuge to be performed and can be completed in a few hours.

II. Applications:

- Isolation of plasma membrane fractions from tissues and cultured cells.
- Plasma membrane studies and plasma membrane protein profiling.
- Enrichment of membrane-associated proteins for western blot and ELISA.

III. Sample Type:

- Cultured cells and tissues

IV. Kit Contents:

Components	K414-10	Cap Code	Part Number
Homogenization Buffer	50 ml	NM	K414-10-1
EZBlock™ Protease Inhibitor Cocktail	1 ml	Orange	K414-10-2
Gradient Dilution Buffer	100 ml	NM	K414-10-3
OptiPrep™ Density Gradient Medium	85 ml	WM	K414-10-4

V. User Supplied Reagents and Equipment:

- Centrifuge (refrigerated) and Tubes
- Ultracentrifuge (capable of 200,000 x *g*) and Compatible (>17 ml) Ultracentrifuge Tubes
- Dounce Homogenizer
- Microsonicator
- Scalpel/Scissors
- 1X PBS
- Dithiothreitol (optional)

VI. Storage Conditions and Reagent Preparation:

Store kit components at -20°C, except for the OptiPrep™ Density Gradient Media, which should be stored at 4°C upon receipt. Protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Homogenization Buffer and Gradient Dilution Buffer**: Thaw completely and chill bottles on ice prior to use. Store at -20°C or 4°C.
- **Protease Inhibitor Cocktail**: Thaw at room temperature prior to use.
- **OptiPrep™ Density Gradient Medium**: Thaw completely and store at 4°C. Mix thoroughly before use.
- **Working Buffer Solution (WB)**: Add 10 µl of protease inhibitor cocktail per every 5 ml Homogenization Buffer prior to use. Keep on ice and discard unused solution after 8 hours.
- **Gradient Working Solution (GWS):** Prepare 10 ml GWS (equivalent to one isolation) by mixing OptiPrep™ with Gradient Dilution Buffer at a 5:1 ratio (8.33 ml of OptiPrep™ and 1.67 ml Gradient Dilution Buffer). Keep GWS on ice at all times.
- **2.5% Gradient Solution:** Prepare 3 ml of 2.5% Gradient Solution (equivalent to one isolation) by mixing GWS with Gradient Dilution Buffer at a 1:19 ratio (150 µl GWS and 2.85 ml Gradient Dilution Buffer). Keep 2.5% Gradient Solution on ice at all times.
- **25% Gradient Solution:** Prepare 10 ml of 25% Gradient Solution (equivalent to one isolation) by mixing GWS with Gradient Dilution Buffer at a 1:1 ratio (5 ml GWS and 5 ml Gradient Dilution Buffer). Keep 25% Gradient Solution on ice at all times.

VII. Plasma Membrane Isolation Protocol:

A. Sample Preparation:

Cultured Cells

1. Collect 0.2-10 x 10^8 cells by centrifugation (700 x *g*, for 5 min at 4°C). For adherent cells, aspirate growth medium and wash cells once with 1X PBS, remove cells using a cell scraper and pellet by centrifugation (700 x *g*, for 5 min at 4°C).
2. Wash both types of cells once with 5 ml of ice cold 1X PBS and centrifuge samples at 1000 x *g*, for 5 min at 4°C.
3. Re-suspend the cell pellet in 1 ml of ice-cold Working Buffer Solution in a pre-chilled Dounce Homogenizer (BioVision Cat # 1998) and homogenize cells on ice for 20-25 strokes.*

Mammalian Tissues

1. Mince Tissues, ~0.2-0.5 g, into small pieces using a very clean scissors or scalpel.
2. Wash samples with 1X PBS.
3. Transfer samples to an ice-cold Dounce homogenizer.
4. Add 1 to 1.5 ml of the Working Buffer Solution and homogenize tissues using a tight fitting homogenizer until tissues have been thoroughly lysed (20-40 times*).

***Note:** Efficient homogenization depends on the cell or tissue type. To check the efficiency of the homogenization, pipette 2-3 µl of the homogenized suspension onto a cover slip and observe under a microscope. A "shiny ring" around the nuclei indicates that cells are still intact. If 70 - 80% of the nuclei do not have the shiny ring, proceed to the next step. Otherwise, perform 10-30 additional strokes.

B. Plasma Membrane Isolation (Perform All Steps on Ice or at 4°C):

1. Transfer the homogenate to a clean, pre-chilled 1.5 ml microcentrifuge tube.
2. Sonicate samples using two 10-second pulses, with 30 seconds between pulses, using a microsonicator. Keep samples on ice and keep probe away from the sample-air interface to minimize foaming.
3. Centrifuge at 700 x *g* for 10 min at 4°C. Carefully remove and discard the fatty residue from the top of the Supernatant. Collect the remaining supernatant and transfer it to a new pre-chilled tube. Store the supernatant on ice.
4. (Optional) Using the pellet from step 3, add 0.5 ml of Working Buffer and repeat steps 2 and 3.
5. Pool the supernatant fractions. ***This is the post-nuclear fraction or PNS.*** Discard the pellet.
6. Sonicate the PNS using two 10 second pulses, 30 seconds between pulses, using a microsonicator. Keep samples on ice and keep probe away from the sample-air interface to minimize foaming.

C. Ultracentrifugation and Fraction Collection:

7. Mix 1 ml of the PNS with 4 ml of the GWS. Place it on the bottom of a fresh, clean, ice-cold ultracentrifuge tube.
8. Layer 10 ml of the 25% Gradient Solution carefully on top of the PNS mix. *Do not mix or perturb the layers*.
9. Layer 2 ml of the 2.5% Gradient Solution carefully on top of the 25% solution. *Do not mix or perturb the layers*.
10. Ultracentrifuge the tube(s) at 200,000 x *g* for 90 minutes at 4°C.
11. At the end of the ultracentrifugation, the plasma membrane fraction(s) will be in ***the visible band at the interface of the 2.5%/25% gradient solutions.***
12. Carefully remove the clear portion of the 2.5% gradient layer and then collect the band below it in a separate, ice-cold tube. This is the isolated 'plasma membrane fraction.'

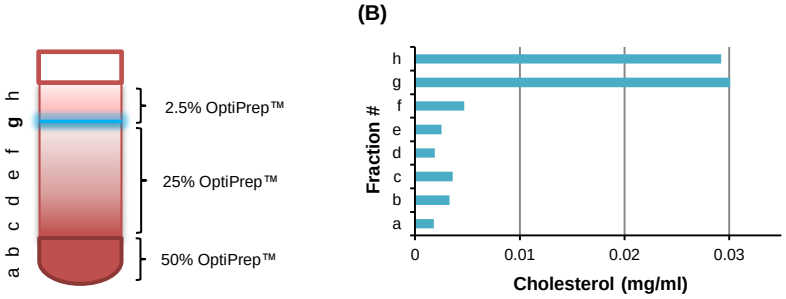

Figure: (A) Representation of an ultracentrifuge tube with OptiPrep™ density gradient from steps 9 through 11. Each fraction (labeled a-h) corresponds to ~2 ml volume. Following ultracentrifugation, the isolated plasma membrane fraction (labeled as fraction **g**) is localized at the interface between the 2.5% and 25% density layers (denoted by the bold shaded line). **(B)** Cholesterol content by fraction, assayed using BioVision's Total Cholesterol & Cholesteryl Ester Assay Kit (BioVision Cat # K603). Cholesterol is highly enriched in the plasma membrane (approximately 22% of the total lipid content of the plasma membrane), but is found in much lower concentration (2%-6% of total lipids) in other organelle membranes.

VIII. RELATED PRODUCTS:

Nuclear/Cytosol Fractionation Kit (K266)
Lactate Dehydrogenase Activity Assay Kit (K726)
BCA Protein Assay Kit (K818)
Lysosome Purification Kit (K235)
Plasma Membrane Protein Extraction Kit (K268)
Total Cholesterol & Cholesteryl Ester Assay Kit (K603)
OrgFrontier™ Chloroplast Isolation Kit (K468)
Microsome Isolation Kit (K249)

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