PI-0104

Plasma Membrane Protein Isolation Kit

(Sufficient for 50 Isolations, Store at -20°C)

Introduction:

The Plasma Membrane proteins are a group of specialized proteins involved in transport, cell signaling and cell adhesion, recognition and communication. The low abundance of these proteins makes their study challenging. AkrivisBio's Plasma Membrane Isolation Kit provides optimized reagents in a two phase extraction system for the effective isolation of the plasma membrane from the other cellular membranes, based on differential affinity towards polyethylene glycol and dextran, wherein the plasma membranes selectively enter the upper PEG rich phase while the other membranes are found in the lower dextran rich phase. This protocol results in a yield of about 20% of the plasma membrane with a purity over 90%. Subsequent solubilization of the membrane proteins prepared using the kit allows for analysis in a variety of ways, such as Western blotting, 2-D gels, and enzyme analyses, etc. The kit is good for up to 50 preps and the entire procedure take less than 1 hour.

Kit Components:

Swelling/Homogenization Buffer	100 ml	NM	PI-0104A
Upper Phase Solution	20 ml	NM	PI-0104B
Lower Phase Solution	20 ml	WM	PI-0104C
Protease Inhibitor Cocktail	lyoph	Red	PI-0104D

Storage and Handling:

Store kit at -20°C. Keep all kit components on ice at all times while in use. Bring to room temperature before use.
Reconstitute Protease Inhibitor Cocktail: Add 250 µl of DMSO to the vial and mix until completely dissolved.
Before use, prepare sufficient Swelling/Homogenization Buffer for the number of samples to be prepared, add 2 µl Protease Inhibitor Cocktail per 1 ml buffer. Some precipitation may occur after adding the Protease Inhibitor Cocktail. It can be ignored.
The following protocol is designed for extraction of ~ 10^8 cells.

Cell Membrane Isolation Protocol:

1. Collect cells ~1 g wet weight (~2 x 10^8) by centrifugation (at 1500 X g for 6 minutes at 4°C).
2. Resuspend in 1 ml of Swelling/Homogenization Buffer and place on ice for 10-30 minutes.
3. Homogenize using a Polytron (40 sec, 10000 rpm) or Dounce homogenizer (~50 strokes) on ice.
4. Check for cell breakage by light microscopy.

- Pipette 2-3 µl of the suspension onto a cover slip and observe under a microscope. A shiny ring around the nuclei indicates that cells are intact. Proceed if 90% are lacking the shiny ring, otherwise, repeat homogenization step.

5. Transfer homogenate to a 1.5 ml microcentrifuge tube. Centrifuge at 175 X g for 10 minutes at 4°C. Collect supernatant and discard the pellet.
6. Transfer supernatant to a fresh tube and centrifuge at 25,000 X g for 30 min at 4°C to prepare a plasma membrane enriched fraction.
7. Discard supernatant. The pellet is the total cellular membrane (containing both plasma membrane and cellular organelle membrane).

Note: Stop here if you only need total cellular membrane proteins. If you need to isolate plasma membranes, continue below.

Purification of Plasma Membranes:

1. Resuspend the total cellular membrane pellet in 200 µl of Upper Phase Solution. Add 200 µl of the Lower Phase Solution. Vortex to get a homogenous cloudy suspension. Place on ice for 5 minutes.
2. Prepare a counterbalance tube containing 400 µl of water and mark the cap.
3. Centrifuge both tubes in a microcentrifuge at 1000 X g for 5 minutes. The phases will separate but it can be difficult to visualize.
4. Carefully transfer the upper phase to a new tube, mark it as Upper and place on ice.
5. Extract the tube containing lower phase again by adding 100 µl of Upper Phase Solution. Vortex and centrifuge at 1000 X g for 5 minutes.
6. Carefully transfer the upper phase to the tube marked Upper.
7. Extract the combined upper phase by adding 100 µl of Lower Phase Solution. Vortex until homogeneous and centrifuge at 1000 X g for 5 minutes.
8. Carefully transfer the upper phase to a fresh tube. Dilute upper phase with 5 volumes of water. Chill on ice for 5 minutes.
9. Centrifuge at 16,000 X g for 10 minutes at 4°C. Remove the supernatant. The pellet is the plasma membrane fraction.
10. Store the plasma membranes at –80°C. The membrane fraction can be solubilized in 0.2-1% Tergitol 15-S-9 or other appropriate detergent in the buffer of your choice.

    The yield is approximately 200 µg of plasma membrane protein from 10^8 cells**.**

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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