Plasma Membrane Protein Extraction Kit

(Catalog #K268-50; 50 preparations; Store at –20°C)

I. Introduction:

The Membrane Protein Extraction Kit provides optimized buffers and reagents for effective extraction of membrane proteins from mammalian tissues and cells. Unlike other available procedures that can only extract the total cellular membrane proteins (combinations of plasma and organelle membrane proteins), BioVision's kit was designed to not only extract the total cellular membrane proteins, but also purify the plasma membrane proteins specifically. The procedure offers consistent yield and high purity (over 90 %). Membrane proteins prepared using the kit can be utilized in a variety of applications, such as Western blotting, 2-D gels, and enzyme analyses, etc. The entire procedure takes less than 1 hour.

II. Kit Contents:

Component	K268-50 50 assays	Color Code	Part Number
Homogenization Buffer	100 ml	NM	K268-50-1
Upper Phase Solution	20 ml	NM	K268-50-2
Lower Phase Solution	20 ml	WM	K268-50-3
Protease Inhibitor Cocktail	1 vial	Red	K268-50-4

III. General Consideration and Reagent Preparation:

- Read the entire protocol before beginning the procedure. Be sure to keep all buffers and reagents on ice at all times during the experiment.
- Reconstitute Protease Inhibitor Cocktail by adding 250 µl of DMSO, mix well.
- Before use, prepare sufficient Homogenization Buffer for the number of samples to be prepared, add 1/500 volume of the reconstituted Protease Inhibitor Cocktail (e.g., Add 2 µl Protease Inhibitor Cocktail per 1 ml buffer) to make the Homogenization Buffer Mix. (Note: Some precipitation may occur after adding the Protease Inhibitor Cocktail. You may continue using the buffer or simply remove the precipitates by centrifugation).
- The following protocol is described for extraction of ~ 5-10 x 10^8 cells. If more cells are used, scale up the volume proportionally.

IV. Membrane Protein Extraction Protocol:

B. Extraction of Total Cellular Membrane Proteins:

1. Collect cells ~1 g wet weight (0.2-10 x 10^8) by centrifugation (700 x g, 5 minutes at 4°C). For adherent cells, scrape cells in PBS and then spin down (700 x g, 5 minutes) to pellet cells.
2. Wash cells once with 3 ml of ice cold PBS.
3. Resuspend cells in 2 ml of the Homogenization Buffer Mix in an ice-cold Dounce Homogenizer (Cat.# 1998-1). Homogenize cells on ice for 30-50 times.
4. For tissue samples, homogenize tissues in 2 volume of the 1X Homogenization Buffer, until completed lysed (30-50 times).

   Note: Efficient homogenization depends on the cell 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 percent of the nuclei do not have the shiny ring, proceed to the next step. Otherwise, perform 10 - 30 additional passes.
5. Transfer the homogenate to a 1.5 ml microcentrifuge tube. Centrifuge at 700 x g for 10 minutes at 4°C. Collect supernatant and discard the pellet.
6. Transfer the supernatant to a new vial and centrifuge at 10,000 x g for 30 min at 4°C.
7. Collect supernatant (This is the Cytosol Fraction). The pellet is the total cellular membrane protein (containing proteins from both plasma membrane and cellular organelle membrane).

   Note: You may stop here if you only need the total cellular membrane proteins. If you would like to further isolate the plasma membrane proteins specifically, continue with the following steps.

C. Purification of Plasma Membrane Proteins:

1. Resuspend the total membrane proteins pellet in 200 µl of the Upper Phase Solution. Add 200 µl of the Lower Phase Solution. Mix well and incubate on ice for 5 minutes (Mark the tube as **A**).
2. Prepare a fresh phase tube without sample. Adding 200 µl of Upper Phase Solution and 200 µl of Lower Phase Solution (Mark the tube as **B**).
3. Centrifuge both tubes A & B in a microcentrifuge at 1000 x g for 5 minutes.
4. Carefully transfer the upper phase from tube A to a new tube (tube **C**), keep on ice.
5. To maximize the yield, extract the tube A lower phase again by adding 100 µl of the Upper Phase Solution from tube B. Mix well and centrifuge at 1000 x g for 5 minutes.
6. Carefully collect the upper phase. Combine with the upper phase from Step 4 (tube C). Extract the combined upper phase by adding 100 µl of the Lower Phase Solution from tube B, Mix well and centrifuge at 1000 x g for 5 minutes.
7. Carefully collect the upper phase. Dilute the upper phase in 5 volumes of water. Keep on ice for 5 minutes.
8. Spin at 15,000 x g in a microcentrifuge tube for 10 minutes at 4°C. Remove the supernatant. The pellet is the plasma membrane protein.
9. Store the plasma membrane proteins at –70°C for further studies. The membrane fraction can be dissolved in 0.5 % Triton X-100 in PBS or other buffers before use.

Generally 30-100 µg plasma membrane proteins can be obtained.

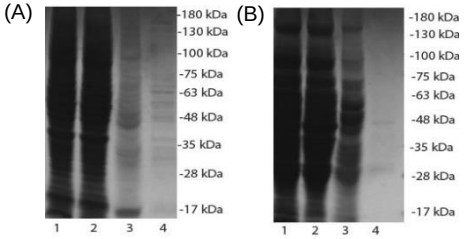

Figure: Membrane protein extraction: ~4x10^7 HeLa cells (A) were lysed in 500 µl homogenization buffer and ~0.5 g rat liver (B) was homogenized in 1 ml homogenization buffer. 10 µl was loaded onto 4-20% gradient gel and stained with Coomassie Blue. 1-Whole cell lysate, 2-Cytosolic portion, 3-Total membrane protein, 4-Plasma membrane protein.

FOR RESEARCH USE ONLY! Not to be used in humans.