Minute™ Plasma Membrane Protein Isolation and Cell Fractionation Kit

Catalog number: SM-005

Description

Minute™ plasma membrane (PM) protein isolation kit is a novel and patented native PM protein isolation kit. The principle of isolation is: Cells/Tissues are first sensitized by buffer A then pass though a filter that allows cells to pass through with a zigzag path. The cell membranes are ruptured during the process and leave nuclei intact therefore the final PM prep is basically free of nuclear membrane and nuclear protein contamination. PM is separated from a mixture of un-ruptured cells (very small amount), nuclei, cytosol and organelles by subsequent differential centrifugation and density centrifugation without using ultracentrifugation. Since cell membranes are ruptured during the zigzag path when high speed centrifugal force is applied there is no need to use a homogenizer that are required by other kits. When a homogenizer is used in PM isolation, inconsistency is a major problem because variation in the duration of homogenization results in a different protein profile every time therefore resulting in a significant variation in final PM purity (inter-experiment variation). As a comparison, we use the same amount of starting cell, defined centrifugal force and pre-defined duration in every experiment and the result is much more consistent. The procedure can be completed in less than 45 min.

Applications

The kit is designed to rapidly isolate native membrane proteins from cultured cells or tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, membrane protein structure analysis, 2-D gels, enzyme activity assays and other applications. This kit provides the most rapid method currently available for preparation of native membrane proteins.

Buffer Formulations: Proprietary

Kit components (50 preps)

1. 25 ml buffer A
2. 10 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5 4 plastic rods
6. Tissue dissociation beads

Storage: Store Buffer A and Buffer B at -20^{o}C upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge

Important Information:

1. Read the entire procedures carefully. Thaw buffer A and buffer B completely, invert the bottles a few times and place them on ice. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated mirocentrifuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. The use of protease inhibitor cocktails is optional.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Membrane Protein Isolation Procedures

A. Isolation of Total Membrane Proteins

1. Place the filter cartridges in collection tubs, and incubate on ice.

2. **For cultured cells**, collect 1-50 X 10^6 cells by low speed centrifugation (500-600 X g 5 min). Go to step 3a. **For tissue samples**, go to step 3b.

   Note: For isolation of plasma membrane proteins from cultured cells (see below) it's recommended to use 20-50 X 10^6 cells. The rpm in this protocol is based on A Eppendoff 5415C table top microcentrifuge.

3a. Wash cells once with cold PBS. Remove supernatant completely and resuspend the pellet in buffer A (200 µl for a starting cell number less than 5 million and 500 µl for a starting cell number greater than 5 million). Incubate the cell suspension on ice for 5-10 min. **Vortex the tube vigorously for 10-30 seconds**. Immediately transfer the cell suspension to the filter cartridge. Go to step 4.

3b **For tissue samples** place a piece of fresh tissue (10-30 mg) or frozen tissue (20-30 mg) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue with a plastic rod for one min by pushing the tissue against the surface of the filter repeatedly with twisting force (Note: if you are working with skeletal or cardiac muscles, it is recommended to add 100-120 mg tissue dissociation beads to the filter prior to grinding). Add 300 µl buffer A to the same filter cartridge, mix by pipette up and down a few times and incubate the tube on ice with **cap open** for 5 min. Go to step 4.

   Note: The presence of a small amount of un-homogenized tissue will not affect the quality of the sample. The plastic rod is reusable. For cleaning, wipe it with 75% alcohol or rinse it with distilled water.

4. Cap the filter cartridge and centrifuge at 14,000 rpm (16,000 X g) for 30 seconds **(it is recommended to use a table top centrifuge that can reach maximum speed in less than 10 seconds**).

   Optional: For cultured cells it is recommended to resuspend the pellet in collection tube from step 4, transfer the cell suspension to the same filter and spin at 14,000 rpm for 30 seconds. Re-passing the cells through the filter can increase the yield by 20-30%.

5. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds.

   Following procedures separate total cellular components into four fractions: nuclei, cytosol, organelles and plasma membrane.

6. Centrifuge at 3000 rpm (700 X g) for one min (the pellet contains intact nuclei). Transfer the supernatant to a fresh 1.5 ml microcentrifuge tube and centrifuged at 4°C for 10-30 min at 16,000 X g (longer centrifugation time will increase yield). Remove the supernatant (this is the cytosol fraction) and save the pellet (this is the total membrane protein fraction including organelles and plasma membranes). Store the pellet at -70°C or dissolve it in detergent-containing buffers of your choice. The yield is typically 10-500 µg/sample. You may stop here if isolation of plasma membrane proteins is not needed. Continue to step 7 for plasma membrane protein isolation. Don't freeze total membrane protein fraction if further isolation of plasma membrane proteins is desired.

B. Isolation of Plasma Membrane Proteins

7. Resuspend the total membrane protein fraction from step 6 in 200 µl buffer B by repeatedly pipetting up and down or vortexing. Centrifuge at 10,000 rpm (7,800 X g) for 5 min at 4°C (Note: if final plasma membrane prep is contaminated by organelle membranes, increase centrifugation time up to 20 min can improve the purity). The pellet contains organelle membrane proteins.

8. Carefully transfer the supernatant to a fresh 2.0 ml microcentrifuge tube and add 1.6 ml cold PBS. Mix by inverting the tube a few times. Centrifuge at 14,000 rpm (16,000 X g) for 15-30 min (longer centrifugation will improve yield). Discard the supernatant and save the pellet (isolated plasma membrane proteins). Typically, 10-300 µg plasma membrane proteins can be obtained. Pellet of plasma membrane proteins can be dissolved in 20-200 µl detergent containing buffers of your choice depending upon specific downstream applications. Reagents in following table are recommended for solubilization of the pellet. For isoelectric focusing (First dimension of 2D gel) we recommend to use: 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use).

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.

About Evaluation of Isolated PM Proteins

Many researchers use Western blotting to access the purity of isolated membrane proteins. Some commonly used "cytosolic markers" are not exclusively cytosolic. For example, actin (1), GAPDH (2) and tubulin (3) are mainly cytosolic but they are also associated with plasma membranes. It's not surprising to detect week signals of these marker proteins in PM preps in certain cell and tissue types. For more info please refer to following publications:

(1). Gruenstein E., et al. (1975). Actin associated with membranes from 3T3 mouse fibroblast and Hela cells. Journal of cell Biology. 64:223-234.
(2). Terrasse R., et al. (2012). Human and pneumococcal cell surface glyceraldehydes -3-phosphate dehydrogenase (GAPDH) proteins are both ligands of human C1q protein. J. Biol. Chem. 287:42620-42633.
(3). Wolff J. (2009). Plasma membrane tubulin. Biochemica et Biophysica Acta. (BBA)-Biomembranes 1788:1415-1433.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting cell numbers Increase incubation time to 10 min (step3)
Low protein activity	Keep lysate cold/add protease inhibitors
Retention of cell lysate in protein filter cartridge after 30 seconds of centrifugation	Reduce amount of starting material or increase centrifugation time to 2 min
Contamination of PM by cytosolic proteins	Wash PM pellet with 0.5 ml cold PBS containing 0.3 M NaCl, Ph. 9.5