Minute™ Plasma Membrane Protein Isolation Kit

Catalog number: SM-005

Description

Invent Biotechnologies Minute™ plasma membrane protein isolation kit is composed of optimized buffers and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly isolate native total membrane proteins (organelle membrane proteins) and native plasma membrane proteins from cultured mammalian cells or tissues. This kit can sequentially separate cellular components into four fractions: nuclei, cytosol, organelles and plasma membrane. Due to the use of protein extraction filter cartridges, the membrane protein isolation is simple, easy and user friendly with high yield. Unlike many commercial membrane preparation kits that require large amount of starting cells (5 millions and up). This kit offers wide range of starting cells (1-50 millions/sample). The buffers are detergent and EDTA free. A Dounce homogenizer or a tissue blender is not needed. 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^{o}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

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 for 30 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 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 rpm (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 for 5 min at 4°C. 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 16,000 rpm 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 such as 0.5% Triton X-100 in PBS

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