Minute™ Plasma Membrane Isolation Kit for Mammalian Red Blood Cells

Catalog Number: RP-057 (20 preps)

Introduction

Red blood cells (RBCs) are essential components of the circulatory system, responsible for transporting oxygen and carbon dioxide throughout the body. Understanding their surface composition, particularly the diverse array of proteins is crucial for research in blood biology and disease. This protocol offers a rapid and efficient method for isolating native plasma membranes (PM) vesicles from RBCs with associated membrane proteins. The method utilizes differential centrifugation and a specialized protein extraction powder for gentle RBC lysis while enriching for PM fractions. Importantly, this approach preserves the native protein conformation and avoids the use of detergents, facilitating for downstream studies of membrane-associated proteins. The entire protocol can be completed in approximately 1 hour, yielding highly enriched PM with minimal contamination from intracellular components like spectrin and hemoglobin.

Kit components

1.	Buffer A	30 ml
2.	Buffer B	30 ml
3.	Protein extraction powder	5.0g
4.	pestles for microfuge tube	2
5.	1.5 ml microfuge tube	20

Storage: Store the kit at 4°C.

Additional Materials Required

Table-Top Microcentrifuge. 1 X PBS

Important Product Information

Prior to plasma membrane isolation, addition of protease inhibitor cocktail to aliquot of buffer A is recommended. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. Purified RBCs or white blood cell and platelet-depleted samples are recommended as starting material although anti-coagulated whole blood can also be used.

Protocol

1. Harvest RBCs by centrifugation at 2000 X g for 3 min in a microfuge tube provided. The wet pellet size should be 50-150 µl. We recommend to use 100 µl wet pellet as staring material and don't use more than 150 µl.

2. Wash RBCs pellet with 1 ml cold PBS by pipetting up and down and centrifuge at 2,000 X g for 3 min. Remove supernatant completely.
3. Add 50 µl buffer A to the pellet and resuspend by stirring, followed by addition of 100-120 mg protein extraction powder to the bottom of the tube. Grind the pellet with the pestle provided for about 3 min with back-and-forth twisting force (200-300 times). This step is for lysing RBCs.
4. Add 0.4 ml buffer A to the tube and continue to grind for about 1 min. Add another 0.4 ml buffer A to the tube (total 0.8 ml added). Cap the tube and vortex vigorously for 20 seconds. Incubate on ice for 10 min to allow protein extraction powder to sink to the bottom (note: The pestle is reusable, for cleaning simply rinse with water and dry it with paper towel).
5. Centrifuge the tube at 100 X g (about 1000 rpm) for 3 min at 4ºC. This is to remove protein extraction powder. Transfer the supernatant to a fresh pre-chilled microfuge tube without disturbing the white pellet.
6. Centrifuge the tube at 16,000 X g for 20-30 min at 4ºC to pellet crude plasma membranes. Remove supernatant without disturbing the pink pellet and save it if desired (this is cytosolic fraction).
7. Add 0.5 ml buffer A and 0.5 ml buffer B to the tube. Resuspend the pellet by pipetting for 20-30 times. Cap the tube and vortex vigorously for 20 seconds. Centrifuge the tube at 16,000 X g for 10 min. Remove and discard supernatant completely.
8. Resuspend the pellet in 1 ml buffer B by pipetting, incubate on ice for 5-10 min and centrifuge at 16,000 X g for 10 min. This is to wash the crude plasma membranes. The pellet can also be washed with 0.5 ml buffer B twice that may yield cleaner prep.
9. After washing, the pellet contains isolated PM that is water-insoluble. It can be dissolved in 50-150µl detergent-containing buffers (see table below for recommendations) depending upon downstream applications. For 100 µl starting material 40-60 ug of membrane protein can be expected.

Tech Notes:

1. The amount of protein extraction powder used should be adjusted proportionally to the starting red blood cell (RBC) pellet volume.
2. We recommend using 100 mg protein extraction powder for wet pellet volumes between 50-100 µl and 150 mg for volumes exceeding 100 µl. This ensures optimal protein extraction efficiency across different sample size.
3. Buffer A is a hypotonic buffer for lysis of RBCs and buffer B is a washing buffer for dissociation of proteins that are non-specifically associated with plasma membrane. This helps to enrich the isolated plasma membrane fraction by removing unwanted contaminants.
4. This kit only works for mammalian RBCs and is not applicable to non-mammalian RBCs.
5. Isolated plasma membranes can be directly utilized in Western blotting for protein analysis. However, for applications like ELISA and immunoprecipitation, a further washing step with PBS is recommended. Residual Buffer B in the final pellet (step 8) may interfere with these assays. A single wash with 0.5 ml PBS can effectively remove residual buffer B and improve assay performance.

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.