Technical Note:
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Isolation of Plasma Membrane from Endothelial Cells Using the Thermo Scientific Sorvall MTX Micro-Ultracentrifuge and S55-A2 Rotor

KEY WORDS

- Plasma Membrane Isolation
- Percoll Gradient
- Bovine Aortic Endothelial Cells
- S55-A2 Rotor
- Fiberlite F50L-8x39 Rotor
- Sorvall WX Ultracentrifuge
- Sorvall MTX 150 Micro-Ultracentrifuge
- Sorvall MX Micro-Ultracentrifuge

Introduction

Obtaining a pure plasma membrane (PM) preparation is very important in the field of membrane research. Often this fraction is contaminated with other organelle membranes that can contribute to erroneous results.

There are many reports in the literature where researchers have used sucrose density gradients for purification of PM. One disadvantage of this method is the long centrifugation process. A more rapid method of PM isolation using a Percoll® gradient has been reported.[1] However, before doing experiments with the PM fraction, it is desirable to remove the Percoll. This brief describes a method for the isolation of PM from endothelial cells and the use of the Thermo Scientific Sorvall MTX Micro-Ultracentrifuge to remove the Percoll. The greatest advantage of this protocol and equipment is that very small microliter volumes can be centrifuged using the Thermo Scientific S55-A2 rotor.

Procedures

Plasma Membrane (PM) Isolation

Plasma membrane was isolated from bovine aortic endothelial cells (EC) following the method of Smart *et al* (1995) with some modifications. EC were grown to confluence in 150 mm dishes. Cells were made quiescent by keeping in serum free medium for 24 hours.

1. After required treatments, wash cells twice with cold PBS and harvest by scraping in 3 mL of buffer A (250 mM sucrose, 20 mM tricine, 1 mM EDTA, pH 7.8).
2. Perform centrifugation using the Thermo Scientific general purpose centrifuge with the following parameters: 1,000 x g for 5 minutes at 4 °C.
3. Suspend the cell pellet in 1 mL of buffer A and hand homogenize with 20 strokes.
4. Transfer the homogenate to a 1.5 mL tube and perform centrifugation with the following parameters: 1,000 x g for 10 minutes at 4 °C.
5. Collect the postnuclear supernatant (PNS) and store on ice.
6. Using the pellet, repeat Steps 3-5. Pool the two PNS supernatants and keep on ice.
7. Measure the concentration of protein by Biuret method using BSA as a standard.
8. Layer PNS containing 4 mg of protein on the top of 23 mL of 30% Percoll in buffer A in a 39 mL polycarbonate (PC) bottle assembly (PN 010-1371, actual volume = 26.3 mL).
9. Perform centrifugation in a Thermo Scientific Fiberlite F50L-8x39 fixed-angle rotor and the Thermo Scientific Sorvall WX Ultracentrifuge using the following parameters: 84,000 x g for 30 minutes at 4 °C.
10. Collect the PM fraction and store in 1.5 mL high performance microtubes (PN 314352H01).

   Note: The PM fraction will be visible as a band/ring at a distance of approximately 5.7 cm from the bottom of the tube.

Removal of Percoll

The PM fraction contains Percoll and it is desirable to remove[2,3] prior to performing experiments with the PM.

1. Using high performance microtubes, perform centrifugation of the PM fractions in a Thermo Scientific S55-A2 rotor and Sorvall® MTX 150 Micro-Ultacentrifuge using the following parameters: 105,000 x g (39,800 rpm) for 90 minutes at 4 °C; a Thermo Scientific Sorvall MX Micro-Ultracentrifuge may alternatively be used.
2. Carefully remove the PM and place in a new high performance microtube. If necessary, dilute with buffer A. *Note: The Percoll will be formed in a tightly-packed pellet at the bottom of the tube and the PM fraction will be floating above the pellet.*
3. Remove any residual Percoll by centrifugation using the following parameters: 105,000 x g for 90 minutes at 4 °C.

Thermo Scientific Sorvall MTX 150 Micro-Ultracentrifuge

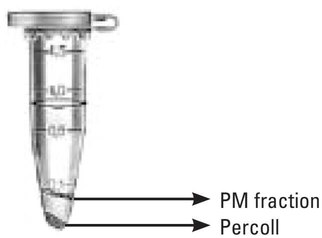

Figure 1. Schematic representation of a 1.5 mL high performance microtube after centrifugation at 105,000 x g for 90 minutes in a Sorvall MTX Micro-Ultracentrifuge.

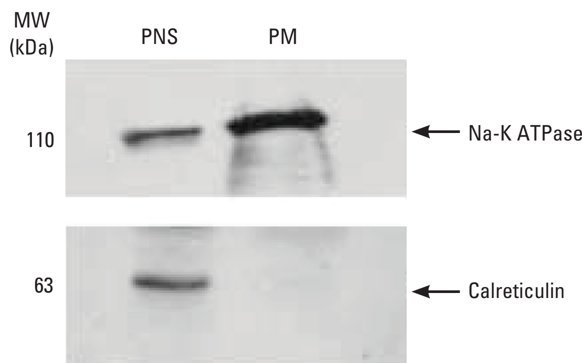

Figure 2. Western blot analysis of plasma membrane preparation. Western blot analysis was done to check (A) the enrichment of the marker enzyme Na-K ATPase in the PM fraction compared to PNS by using antibody against Na-K ATPase, and (B) the presence of ER contaminant in PM fraction by using antibody against ER marker calreticulin.

4. Carefully remove the PM and suspended in buffer A.

Results

Figure 1 schematically illustrates the separation and purification of PM from Percoll after centrifugation in a micro-ultracentrifuge. The PM fraction separated as a distinct layer suspended above the Percoll pellet. For subsequent experiments, it is important to have a pure PM preparation free of organelle contaminations, particularly endoplasmic reticulum (ER). To evaluate the purity of the isolated PM and ensure it was free of ER contamination, fractions were probed by western blots using antibodies against proteins specific for the PM and ER respectively (Figure 2).

As shown in Figure 2, the antibody against Na-K ATPase recognized a 110 kDa polypeptide in both the crude PNS and the purified PM fraction. High enrichment of the Na-K ATPase in the PM fraction could be observed compared to the PNS fraction. In contrast, the calreticulin-specific antibody recognized a 63 kDa polypeptide only in the crude PNS fraction but not in the purified PM preparation. These western blot results confirmed that the purified PM fraction was free of ER contaminants.

To evaluate the reproducibility of the micro-ultracentrifugation method, PM preparations from control and four different treatments were analyzed using western blots. As shown in the Figure 3, equal intensities of the Na-K ATPase bands suggested the presence of equal amounts of PM in all the preparation.

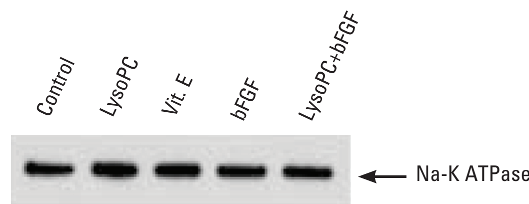

Figure 3. Western blot analysis to check the reproducibility of the isolation method. PM was isolated from EC cultures with or without treatment of 10 µM lysoPC, 50 µM vitamin E, 10 ng/mL basic FGF and 10 µM lysoPC and basic FGF. Protein concentration was determined and equal amount of protein was loaded to each lane of a 12% SDS polyacrylamide gel. Proteins were separated and probed with an antibody against PM marker Na-K ATPase.

Conclusion

The ultracentrifugation protocols described in this brief were successful at purifying PM from bovine aortic endothelial cells. In particular, the micro-ultracentrifugation method described to separate Percoll and isolate pure PM fraction is a rapid and reproducible protocol for isolation of PM-enriched fraction from cultured cells.

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