

Isolation of Plasma Membrane from Endothelial Cells Using the Thermo Scientific S55-A2 Rotor and Sorvall Discovery M150 SE Microultracentrifuge

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KEY WORDS

- Plasma Membrane Isolation
- Percoll Gradient
- Microultracentrifuge
- S55-A2 Rotor

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 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. Smart *et al.*¹ have reported a more rapid method of PM isolation using a Percoll gradient. However, before doing experiments with the PM fraction, it is desirable to remove Percoll. This brief describes a method for the isolation of plasma membrane from endothelial cells and the use of the Thermo Scientific Sorvall Discovery M150 SE microultracentrifuge to remove the Percoll by ultracentrifugation. The greatest advantage of this protocol and equipment is that very small microliter volumes can be centrifuged using the Thermo Scientific S55-A2 rotor.

Procedure

Plasma membrane was isolated from bovine aortic endothelial cells (EC) following the method of Smart *et al.* 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. After required treatments, cells were washed twice with cold PBS and harvested by scraping in 3 mL of buffer A

(250 mM sucrose, 20 mM tricine, 1 mM EDTA, pH 7.8).

The cell pellet was obtained by centrifuging at 1,000 x g for 5 minutes. The cells were suspended in 1 mL of buffer A and hand homogenized with 20 strokes. The homogenate was transferred to a 1.5 mL tube and centrifuged at 1,000 x g for 10 minutes in a tabletop centrifuge at 4 °C. The postnuclear supernatant (PNS) was collected and stored on ice. The process of homogenization and centrifugation was repeated with the pellet supernatant collected and pooled with PNS.

Concentration of protein was measured by Biuret method using BSA as standard. PNS containing 4 mg protein was layered on the top of 23 mL of 30% Percoll in buffer A in a 25 x 89 mm tube and centrifuged in a fixed-angle rotor at 84,000 x g for 30 minutes at 4 °C in an ultracentrifuge. The plasma membrane fraction visible as a band/ring at a distance of 5.7 cm from the bottom of the tube was collected and stored in microcentrifuge tubes. This PM fraction contained Percoll and it was desirable to remove^{2,3} it before doing experiments with the plasma membrane.

The 1.5 mL microcentrifuge tubes with PM samples and Percoll were placed in a Thermo Scientific S55-A2 rotor and centrifuged in a Sorvall® Discovery™ M150 SE microultracentrifuge at 105,000 x g (39,800 rpm) for 1 hour and 30 minutes. Percoll formed a tightly-packed pellet at the bottom of the tube. PM fraction was floating above the pellet. PM was carefully



Thermo Scientific Sorvall Discovery M150 SE Microultracentrifuge

removed, diluted with buffer A, and centrifuged again at 105,000 x g for 1 hour and 30 minutes to remove any residual Percoll. Finally, PM was removed carefully and suspended in buffer A.

Results

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

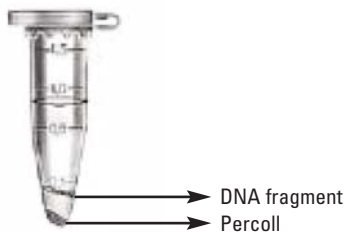


Figure 1. Schematic representation of microcentrifuge tube after centrifugation at 105,000 x g for 30 mins. in a Thermo Scientific Sorvall Discovery M150 SE Microultracentrifuge.

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 confirm that the purified PM fraction was free of ER contaminants.

To evaluate the reproducibility of the microultracentrifugation method, PM preparations from control and 4 different treatments were analyzed using western blots (Figure 3). As shown in the figure, equal intensities of the Na-K ATPase bands suggested the presence of equal amounts of PM in all the preparation.

Conclusion

In conclusion, the ultracentrifugation protocols described in this article were successful at purifying plasma membrane from bovine aortic endothelial cells. In particular, the microultracentrifugation method described to separate

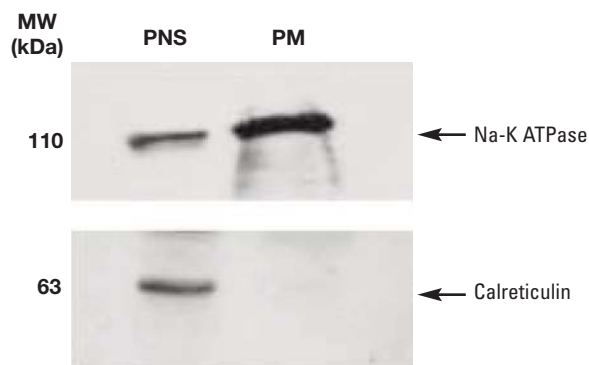


Figure 2. Western blot analysis of plasma membrane preparation. Western blot analysis was done to check (A) the enrichment of the marker enzyme Na-K ATPase in the PM fraction compared to PNS by using antibody against Na-K ATPase, and (B) the presence of ER contaminant in PM fraction by using antibody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

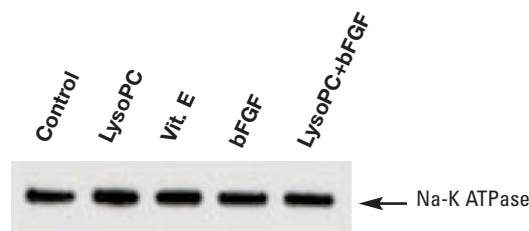


Figure 3. Western blot analysis to check the reproducibility of the isolation method. PM was isolated from EC cultures with or without treatment of 10 μ M lysoPC, 50 μ M vitamin E, 10 ng/mL basic FGF and 10 μ M lysoPC and basic FGF. Protein concentration was determined and equal amount of protein was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

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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