

Isolation of Plasma Membranes from Mammalian Liver Homogenate using the Thermo Scientific Fiberlite F50L-8x39 Carbon Fiber Fixed Angle Rotor

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KEY WORDS

- Plasma Membrane Perturbation
- Differential Centrifugation
- Density Gradient Alteration
- Thermo Scientific Fiberlite F50L-8x39 Rotor
- Thermo Scientific Sorvall WX Ultracentrifuge



Figure 1: Thermo Scientific Sorvall WX Ultracentrifuge and Fiberlite carbon fiber rotor

Introduction

Differential centrifugation is commonly used to isolate subcellular components from tissue homogenate. This method can quickly eliminate many unwanted components from a large amount of starting material. Once collected, the components of interest may be analyzed or purified further by density gradient centrifugation using discontinuous gradients.

Discontinuous step gradients are often used for the further separation of plasma membranes. The nuclei will pellet while the plasma membranes and mitochondria band together at one of the density steps. Dense sucrose layers are used for the gradients because membranes which band at a density of 1.18 g/mL are impermeable to sucrose, but mitochondria which would otherwise band at the same density may be permeable due to the high g-forces used in centrifugation. The density alteration

in the mitochondria resulting from the uptake of sucrose permits the separation to take place.

The Thermo Scientific Fiberlite F50L-8x39 carbon fiber rotor can

be used in conjunction with the Thermo Scientific Sorvall WX 80 ultracentrifuge to isolate plasma membranes from tissue homogenate using these suggested protocols.



Figure 2: Thermo Scientific Fiberlite F50L-8x39 rotor

Plasma membranes

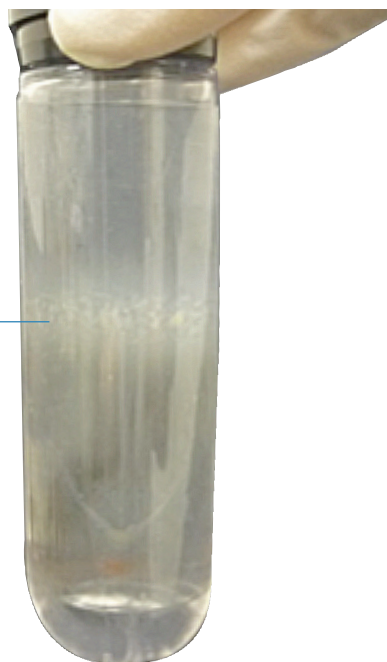


Figure 3: Plasma membranes separated in the center of the centrifuge tube

Protocol for Separation of Plasma Membranes

1. Pre-cool all rotors to be used at 4°C
2. Prepare 15% (w/v), 20% (w/v), 30% (w/v), 40% (w/v), and 50% (w/v) sucrose solutions in TE (100 mM Tris, 10 mM EDTA, pH 7.5)
3. Homogenize 2.0 g of mammalian liver tissue in 50 mL of the 15% sucrose solution
4. Pellet the mitochondria at 10,000 rpm for 10 minutes at 4°C
5. Transfer the membrane containing supernatant into a new centrifuge tube and centrifuge at 100,000 xg for 1 hour at 4°C
6. Discard the supernatant, being careful to not disturb the pellet. Resuspend the membranes containing pellet in 25 mL of the 40% sucrose solution
7. Prepare clean centrifuge tubes with a 4-step sucrose gradient using a 10 mL disposable syringe with a long, wide, bore metal canula. The third step should contain the membrane sample in the 40% sucrose solution
8. Place 6 mL of the 20% sucrose solution into a 39 mL centrifuge tube. Carefully under-layer with

6 mL of the 30% sucrose solution. Now under-layer with 6 mL of the membrane sample resuspended in the 40% sucrose solution. Finally, under-layer with 6 mL of the 50% sucrose solution

9. Place the four-step sucrose gradient samples into a Fiberlite® F50L-8x39 rotor and centrifuge for 2.5 hours at 4°C

Conclusion

Of the numerous procedures reported in the literature for the isolation of plasma membranes, nearly all employed differential centrifugation to first separate the membranes from most other cellular components. After the sample tissue is homogenized and screened, the filtrate is centrifuged at low speed to remove the nuclei and other large debris, leaving the plasma membrane fragments and mitochondria in the supernatant.

In another method for plasma membrane purification, Barden *et al.* (1983) reported an isolation called density perturbation employing a change in the membrane density. Plasma membranes contain various protein receptors each binding a specific ligand, which in turn

coupled to a high-density particle. The increase in membrane density, which results from the binding of the particular ligand-high density particle complex to the receptor, facilitates the separation of the membranes from other cellular components by discontinuous gradient centrifugation. To increase the purity of the plasma membranes the sample was placed in the third step of the discontinuous gradient for the separation.¹

References

1. Evans, W.H. (1969), Subfractionation of Rat Liver Plasma Membranes. *FEBS Lett* 3 (4), 237-241
2. Barden, A., Lmieux, G., Pallotta, D., (1983) Purification and characterization of plasma Membranes from *Physarum polycephalum amoebae*, *Biochim. Biophys. Acta* 730, 25-31

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