

More than 0.5 g of tissue or 5.0×10^7 cells.



Homogenize tissue samples with a tight Potter–Elvehjem homogenizer (1000 rpm). This step can be omitted for culture cells.



Nitrogen cavitation (400-800 psi, 15 min, 4 °C).



Centrifuge the homogenate (10,000g, 10 min, 4 °C).



Centrifuge the supernatant (100,000g, 40 min, 4 °C).



The pellet and supernatant are crude membrane and cytosolic fractions, respectively. Dissolve the pellet and centrifuge it with 38w/v% sucrose solution (100,000g with swing rotor, 40 min, 4 °C).



Collect turbid layer, and centrifuge it (100,000g, 40 min, 4 °C).



Dissolve the pellet (plasma membrane fraction), and store at -80 °C.