

Detergent-free sucrose gradient membrane fractionation Stack Lab

*** The whole process should be done on ice!

- 1 Wash the cells twice with ice-cold PBS
- 2 Scrape cells off the plate with 1.2 ml of scraping buffer**. Pass thru 25 gauge needle 10 times to shear the DNA and transfer the supernatant to an eppendorf micro-centrifuge tube.
- 3 Centrifuge the lysates at full speed (14,000 ~ 16,000 rpm) at 4°C for 10 minutes.
- 4 Transfer the supernatants to a 15 ml conical tube. Sonicate at full power for 30 seconds. (Sample will become foamy)
- 5 Pre-chill centrifuge to 4°C. Centrifuge samples at 3000 rpm for 2 minutes.
- 6 Prepare a 5%, 35% and 45% sucrose gradient:
 - a. Make a 2X stock of each: 10%, 70% and 90% sucrose dissolved in 25 mM MES (pH6.5) and 150 mM NaCl (MES/NaCl buffer).[Note: The 90% buffer may require heating at 60°C to form a clear solution.]
 - b. **For the 5% and 35% final concentrations**, mix the corresponding 2X sucrose solution 1:1 with MES/NaCl buffer containing 2X protease inhibitor cocktail and 10 mM EDTA (for 5% - mix with 10% sucrose, 35% - mix with 70% sucrose).
 - c. **For the 45% final sucrose concentration**, mix the sonicated cell lysate with 90% sucrose solution 1:1.
- 7 Load the gradient into an ultra-clear centrifuge tube (Beckman, 344057) by using a 2.5 ml Hamilton syringe from bottom in the sequence of 1 ml of 5%, 2 ml of 35% and 2 ml of 45%.
- 8 Ultracentrifuge the sample at 55,000 rpm and 4°C for 20 hours with low acceleration and no brake. (Beckman L-90K, SW55Ti rotor)
- 9 After centrifugation, a cloudy layer could be seen about 1/3 from the top. Collect from the top 10 fractions (500ul each)
- 10 Analyze fractions on an 8% SDS-page gel (1.5 mm) for MT1-MMP, or 10 ~ 12% gel if using caveolin-1 as positive control (20 kDa, BD pAb at 1:5000).

** Scraping buffer: 500 Na₂CO₃, PH11, freshly supplemented with 2x proteases inhibitors and 10 mM EDTA