Detergent-Free Caveolae Prep
(from: Liu, Casey and Pike, BBRC 245: 684-690 (1998)

Solutions Needed:

Tris lysis buffer:
25 mM Tris, pH 7.4,
250 mM sucrose,
2 mM EDTA

MES-buffered saline (MBS):
25 mM MES, pH 6.5;
150 mM NaCl,
2 mM EDTA

5%, 35% and 80% sucrose in MBS

Phosphate buffered saline

Procedure:

Chill ultracentrifuge to 4° C with vacuum on

1. Wash cells in ice cold PBS. Be sure to remove as much of the wash as possible so it does not add a lot of volume to your lysate.

2. Add 1 ml Tris lysis buffer to cells on ice. Scrape cells into buffer. Homogenize 20 strokes in Dounce homogenizer. Pass lysate through 23 g needle ten times and put in 17 x 100 tube on ice.

3. Sonicate 3 times for 20 seconds each using the maximum power setting for a microtip. Do sonications on ice . Rest sample for 2 minutes on ice between sonications to keep sample from getting warm.

4. Mix 0.9 ml of lysate with 1.1 ml of 80% sucrose in MES-buffered saline. Mix using 23 g needle and syringe. Be sure the two components are completely mixed.

5. Put mixture in the bottom of a 13 ml centrifuge tube for the SW41 rotor. Add 6 ml 35% sucrose in MBS and then 4 ml 5% sucrose in MBS. (Note that this is different from Triton X-100 prep which uses 30% sucrose.)

6. Balance tubes by weighing them and adding or subtracting 5% sucrose as necessary

7. Centrifuge in SW41 rotor for 3 hr at 39,000 rpm (175,000 x g).

8. Remove gradients and fractionate into 10 fractions of 1.2 ml each. The final fraction is often a little larger or a little smaller than the rest. Keep to 10 fractions.

9. Resuspend pellet in 1.2 ml PBS by homogenization.

- If doing lipids, cells should be labeled with ^{3}H-inositol 48 hr in advance. Remove 800 μl of each fraction and extract for lipids.
- For protein gels, use 50 μl aliquots of each fraction + 50 μl of sample buffer.
- For proteins, use 50 μl aliquots for fractions 1 through 7 and 10 μl aliquots for fractions 8 through pellet.