

Triton X-100 Caveolae Prep

Solutions needed:

MES-buffered saline (MBS):

25 mM MES, pH 6.5,
150 mM NaCl,
2 mM EDTA

MES-buffered saline + 1% Triton X-100

MBS + 5% sucrose ; MBS + 30% sucrose; and, MBS + 80% sucrose

Instructions:

Chill ultracentrifuge to 4° C with vacuum on

1. Wash cells in ice cold PBS. Be sure to remove as much of wash as possible so it does not add a lot of volume to your lysate.
 2. Add 1 ml MES-buffered saline (MBS) containing 1% Triton X-100 to cells on ice. Scrape cells into buffer. Pass lysate through 23 g needle five times and put in 1.5 ml Eppendorf tube on ice.
 3. Incubate on ice for 10 min with periodic inversion of the tube.
 4. Mix 1 ml of lysate with an equal volume of 80% sucrose in MES-buffered saline. Mix using 23 g needle and syringe. Be sure the two components are completely mixed so that the lighter phase will not float up into the higher parts of the gradient.
 5. Put the lysate in 40% sucrose in the bottom of a 13 ml centrifuge tube for the SW41 rotor. Add 6 ml 30% sucrose in MBS and then 4 ml 5% sucrose in MBS. (Note that this is different from detergent-free caveolae prep which uses 35% sucrose.)
 6. Balance tubes by weighing them and adding or subtracting 5% sucrose as necessary
 7. Centrifuge in the Beckman Ultracentrifuge using the 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 in volume. Keep to 10 fractions.
 9. Resuspend pellet in 1.2 ml PBS by homogenization. This requires a lot of homogenization because the pellet is so sticky.
- If doing lipids, cells should be labeled with ³H-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.