Smart Prep of Caveolae

(Smart, E.J., Ying, Y.-S., Mineo, C. and Anderson, R.G.W. (1995) A Detergent-Free Method for Purifying Caveolae Membrane from Tissue Culture Cells. PNAS 92: 10104-10108)

Buffers Needed:

Buffer A: 0.25 M sucrose
1 mM EDTA
20 mM Tris, pH 7.8

Buffer B: 0.25 M sucrose
6 mM EDTA
120 mM Tris, pH 7.8

Buffer C: 50% Optiprep in buffer A prepared by diluting OptiPrep 1 to 5 with buffer B.
(N.B.- Optiprep is supplied as a 60% solution)

All procedures are carried out at 4˚ C.

Plasma Membrane Preparation:

1. Wash cells twice with 5 ml buffer A
2. Scrape into 5 ml buffer A
3. Pellet in tabletop centrifuge for 5 min at 1000 x g (2200 rpm in tabletop)
4. Suspend cells in 1 ml buffer A. Homogenize 20 strokes in Dounce. Pass through 21 g (or higher) needle 20 times
5. Transfer to 15 ml conical tube and centrifuge for 10 min at 1000 x g (2200 rpm, velocity ~6)
6. Remove supernatant and save
7. Resuspend the pellet in 1 ml buffer A and homogenize as above. Centrifuge 10 min as above.
8. Remove supernatant and combine with first supernatant. This is the post-nuclear supernatant (PNS).
9. Apply 2 ml PNS to top of 8 ml of 30% Percoll in buffer A.
10. Centrifuge 30 min. at 84,000 x g (33K in Ti70 rotor)

11. Collect the membrane fraction that bands at approximately the middle of the tube. This is the plasma membrane fraction.

If analysis of the Percoll gradients indicates a large degree of contamination of the plasma membrane with other internal membranes, the position of the plasma membrane can be moved farther into the gradient by increasing the pH of the Percoll gradient to 9.6.

Caveolae/Lipid Rafts

1. Adjust volume of plasma membranes to 2 ml

2. Sonicate 6 x 15 sec with one min rest in between times. Do this on ice and keep the probe cold in ice water between sonications to prevent excess heating of the sample.

3. Mix sonicate with 1.84 ml buffer C + 0.164 ml buffer A. Place in bottom of a 12 ml centrifuge tube.

4. Pour a 20% to 10% Optiprep gradient in a total of 8 ml. Dilute buffer C with buffer A to make the 10% and 20% Optiprep solutions.

5. Centrifuge at 52,000 x g for 90 min at 4˚ C in ultracentrifuge using SW41 rotor (21,000 rpm)

6. Collect the top 5 ml and mix with 4 ml buffer C.

7. Overlay with 2 ml of 5% Optiprep made by diluting buffer C with buffer A.

8. Centrifuge at 52,000 x g for 90 min at 4˚ C. as above. You will see a distinct opaque band 4-5 mm above the interface that represents the caveolar fraction.