Making Caveolae by the Alkaline Detergent-Free Procedure
(modified from Song et al. (1996) J. Biol. Chem. 271:9690-9697)

Solutions needed:

Carbonate buffer
150 mM sodium carbonate, pH 11
1 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. Use one or two confluent D150 dishes per gradient. One plate is adequate for seeing protein or lipid distribution.

2. Wash the plate once with PBS. Scrape into 1 ml of carbonate buffer. Lyse by passing the material 10 times through a 23g needle.

3. Sonicate 3 times for 15 sec each using a microtip with the power set to maximum for the microtip. Do sonications on ice. Rest one minute between sonications. Remove the tip from the membrane solution between sonications and let the tip sit in the ice water. This will ensure that there is no heat build up on the tip. Be sure to keep the membranes as cold as possible.

4. Mix the sonicate with an equal volume of 80% sucrose in MBS. Place this at the bottom of a 12-ml ultracentrifuge tube. Carefully layer 6 ml of 35% sucrose on top of the lysate. Then layer 4 ml of 5% sucrose on top.

5. Spin for 3 hr at 175,000 x g (39,000 rpm) in a SW41 swinging bucket rotor. Centrifugation should be carried out at 4˚ C.

6. Remove tubes from rotor. A band of membrane can normally be seen floating at the 5%/35% interface. Fractionate by taking sequential 1.2 ml fractions carefully off the top of the gradient. Caveolae are normally found in fraction 4 and possibly 5.