P100 - "Membrane Prep." (Alexandra and Gil's version)

1. Grow cells to 80% confluency.
2. If necessary, trypsinize cells.
3. Wash cells 3x with PBS - use IEC clinical centrifuge in tissue culture, speed is depending on type of cells you are processing. We use 3000 rpm for MRC5, 293 N3S and Rat2 cells.
4. For processing 10 dishes of 293 N3S, MRC5 or Rat2 cells add 10 ml of cold hypotonic buffer and incubate 10 min on ice.
5. Homogenize using approximately 30 strokes with pre-cooled Potter homogenizer - if possible in the cold room with the drill machine (middle speed)
6. Spin prep. in hermle in coldroom at 2400 rpm (1000 x g)for 5 min - spins out nuclei.
7. Supernatant contains membranes. Keep supernatant and aliquot it in 1ml Beckman TL-100 clear polyalomer ultracentrifuge tubes. Spin at 65000 Rpm for 1hr @ 40C in the TL100.2 rotor/ Beckman TL100 ultracentrifuge.

   Note: Rotor should be cooled to 40C before hand. Alternatively the supernatent can be dispensed into 2 5ml Optiseal tubes for the TL100.4 rotor.
8. Pellet (P100) contains the membranes and the supernatant (S100) cytosolic contents. Resuspend the membrane pellet in Dog buffer C - resuspend in as little as possible. Note: I normally use 25ul-50ul of Dog buffer C per tube. If necessary keep also supernatant. Freeze both in liquid nitrogen and store at -800C.

Measure protein content with bradford assay, the membranes must first be solubilized in some kind of detergent before protein concentration can be measured..

Hypotonic Buffer	Dog Buffer C
10mM HEPES pH 7.8	250mM Sucrose pH 7.5
15mM KCl	50mM TEA pH 8.0
2mM MgCl2	
0.1mM EDTA	
1mM DTT (add fresh)	
1mM PMSF (fresh)	
1X PIN (fresh)	