P100 membrane prep fom tissue culture cells

This is a method for isolating the membrane fraction of cells, for determining expression of presumed membrane proteins in transfected or infected cell lines.

1. Scrape off cells from 2 to 5 confluent 10cm dishes, transfer the cells to a 15ml conical tube. Spin down at #2 in the bench top centrifuge, 5 mins.
2. Wash cells 2 X with 5ml PBS: resuspend, vortex and spin as before.
3. Resuspend the cell pellet in 5ml cold Hypotonic buffer, put on ice for 5mins.
4. Transfer the cell suspension to a cold Dounce homogenizer and lyse the cells with 30 strokes of the B pestle(up and down = 1 stroke). Keep on ice! Or use the Potter homogenizer in the cold room.
5. Transfer to a 15ml conical tube and spin at 2500rpm, +4oC in the Sorvall RT6000B centrifuge, for 10mins. This pellets the nuclei.
6. Transfer 5ml supernatent to a 5ml Optiseal tube for the TL100.4 rotor (ask DWA for help if you have not used this rotor before). Spin in the TL100 centrifuge, 65,000rpm, +4oC, 1hr to pellet the membranes.
7. Remove the supernatent, If you want to save some by freezing in liquid nitrogen then store at -80oC.
8. Resuspend the pellet in 100ul NP40 Lysis buffer + PIN (this buffer dissolves the membranes and releases the proteins into solution), measure the protein concentration, aliquot 20 to 50ugs to run on SDS-PAGE, add gel loading buffer and store at -20o until ready to run gel. Flash freeze the rest of the sample in liquid nitrogen and store at -80oC.
9. For Rat2/mT extracts resuspend the pellet in 200ul NP40 Lysis buffer + PIN and immunoprecipitate the mT as described in step 5B on TC - 13.
10. Alternatively, if you need the membranes intact, resuspend the pellet in 100ul Dog Buffer C. Freeze and store as above. The membranes must first be solubilized in some detergent (SDS, NP40 etc) before the protein concentration can be measured.

Buffer recipes

Hyptotonic Buffer

10mM HEPES pH 8.0
15mM KCl
2mM MgCl2
0.1mM EDTA

add DTT to 1mM and protease inhibitors to 4X (from the 200X lab stocks, Translation p) immediately before use.

NP40 Lysis Buffer

25mM Tris pH 8.0
150mM NaCl
1% NP40

add DTT to 1mM and protease inhibitors to 4X (from the 200X lab stocks, Translation p) immediately before use.

Dog Buffer C

50mM Triethanolamine (TEA) pH 8.0
250mM sucrose