Carbonate Extraction of Nuclear Envelopes

(Rout Lab, 1999)

Purpose: to extract proteins from the nuclear envelope using carbonate

1. Mix 25 µl of a nuclear envelope fraction with 225 µl BT/Mg/DMSO in TLA-45 tubes. Less NE can be used if necessary and brought up in a total volume of 250 µl with BT/Mg/DMSO.

2. Spin tubes in TLA-45 rotor for 40K, 40 min., 4˚C.

3. Aspirate supernatant and resuspend remaining pellet with 250 µl of 0.1M Na-Carbonate extraction buffer (this is usually a 1:10 dilution of a 1M Na-Carbonate buffer stock with water). Vortexing and pipetting up and down will be sufficient to resuspend pellet. Do NOT sonicate.

4. Incubate tubes on ice for 1 hour.

5. Spin tubes in TLA-45 rotor for 40K, 40 min., 4˚C.

6. Transfer supernatant to a fresh tube labelled “S”. Wash pellet with 250 µl Na-Carbonate extraction buffer.

7. Spin tubes in TLA-45 rotor for 40K, 40 min., 4˚C.

8. Transfer supernatant to “S” tube. Add 500 µl water to “S” tube and 750 µl water to tube containing pellet.

9. Add 100 µl 0.3% NaDOC and 100 µl 72%TCA to all tubes to precipitate proteins.
Mix by inversion and incubate on ice for 1 hour.

10. Spin tubes in microcentrifuge at 14K for 20 min., 4˚C.

11. Aspirate supernatant and resuspend pellet in 0.5 mL 90% acetone. Incubate in freezer for 3 hours to overnight. (Overnight is preferred)

12. Spin tubes in microcentrifuge at 14K for 20 min., 4˚C.

13. Aspirate supernatant and resuspend pellet in 5 µl sol. A and 5 µl sol. B. Heat at 65˚C for 10 minutes and run on protein gel.