For larger scale preparations and mT immunoprecipitation

A. MDCK cells use one 100mm dish of cells

B. Rat2 cells use 2 to 3 100mm dishes.

1. Remove medium, wash and remove cells as above.
2. Add 200ul NP40 Lysis buffer + PIN, place on ice 5mins,
3. Spin down full speed, 10secs and remove supernatent to fresh tube.
4. Measure protein concentration.

5A. For MDCK cells run 10ugs on an SDS-Tricine gel, then western blot. Flash freeze the rest and store at -80oC.

5B. For Rat2 cells add 5ul anti-mT antibody (mix of 5 monoclonals), incubate on ice 1hour, add 100ul 10% protein G Sepharose in Lysis buffer, place on rotator at +4oC for 1hour. Spin down 10secs, wash 3 X 500ul Lysis buffer, add 20ul 1XTLB and run on a SDS-Tricine gel. Use the rabbit anti mT serum for the blot.