Quick protein extract for determining expression in transfected cell lines

A. For MDCK cells use cells in one near confluent well of a 6 well dish.

B. For Rat2 cells use cells from one near confluent 100mm dish.

1. Remove the medium.
2. Add 1ml PBS and scrape off the cells, transfer to a microfuge tube.
3. Spin down gently: if you have an adjustable speed microfuge set the speed between 1 and 2 and spin for 5mins, in a non-adjustable speed microfuge pulse for 2 x 2secs.
4. Remove as much liquid as possible then resuspend the cell pellet in 25æl cold NP40 lysis buffer, place on ice for 5min.
5. Spin down harder: full speed for 10secs.
6. Remove supernatent to a fresh tube containing 25æl 2 X Tricine gel loading Buffer (TLB).
7. Either run directly on a Tricine gel and western blot for your expressed protein or store at -20oC until you have enough samples ready to run gels.

NP40 Lysis Buffer

50mM Tris pH 8.0
150mM NaCl
1% NP40
4X protease inhibitors (PIN)

2 X TLB: see under Tricine gels p14.

200 X PIN: see under Transcription/Translation p18.