

## Whole Cell Extracts From Tissue

### RIPA buffer

60 ul NP40

150 ul 20% SDS (for 0.5%)

6 ul PMSF 17 mg/ml (100 mM) MeOH

240 ul 25x Complete (Roche)

QS to 6mls with PBS

**(PMSF AND COMPLETE MUST BE ADDED FRESH TO THE RIPA BEFORE THE PROCEDURE. RIPA CAN BE MADE AHEAD OF TIME AND STORED AT +4 FOR AN EXTENDED PERIOD)**

- Keep cold
- add 300 ul RIPA to sample (in epi tube) ~ 50 mg-100 mg
- sonicate:
  - power 4
  - 50% Duty
  - Timer on
  - Keep in beaker with ice while sonicating
  - ~ 15 bursts
- ice 30 min
- spin 20 min
- transfer top phase
- aliquot and store at -80

### To make 25x Complete:

Tablets stored at +4

Dissolve tablet in 2ml H<sub>2</sub>O and store at -20 for up to 20 days