

Whole Cell Extracts From Cells

RIPA buffer (0.1% SDS)

1ml NP40

500 ul 20% SDS (for 0.1%)

5ml 20x PBS (also called Solution A)

93.5ml H₂O

100ml total-mix and sterile filter. Store at +4.

Add protease inhibitors **fresh on the day of harvest:**

To each ml of RIPA, add:

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

40 ul 25x Complete (Roche)

RIPA+ = RIPA plus inhibitors

1. Rinse cells with PBS at RT
2. From this point on every thing should be ice cold
3. Add 70 ul RIPA+ per well of a 6-well plate
4. Scrape and transfer to microfuge tube
5. Repeat #3-4 and transfer to same tube
6. Pass through 21g needle to sheer DNA (2-3x slowly to avoid foaming)
7. Incubate on ice 30 min
8. Microfuge 20 min in cold room at 12,000rpm
9. Transfer top phase to a clean tube-make several aliquots
10. Aliquot and store at -80

To make 25x Complete:

Tablets stored at +4

Dissolve tablet in 2ml H₂O and store at -20 for up to 20 days