

# General Protocol of Immuno-Precipitation Assay

## Stack Lab

### CELL LYSATE

1. Rinse the cells 2 times with PBS (1 confluent P100 dish)
2. Add 1 ml of Lysis Buffer\* (pre-chilled), shake the dish 10 minutes at 4°C.
3. Scrape cells off the dish, collect to eppendorf tube.
4. Rotate the tube at 4°C, 1 hour
5. Pass the lysate through 26 ½ gauge syringe 2 times.
6. Spin the samples at 14,000 rpm, 15 minutes, 4°C
7. Transfer the supernatant to a new tube --- that's "Total cell lysate"

### PRE-CLEAR (optional step depending on experiment)

8. Add 30 ul beads (protein G or A/G) to the samples, rotate for 2 hours at 4°C
9. Spin 15 sec at 10,000 rpm. **COLLECT supernatant.** Measure the total protein if needed.

### Immunoprecipitate

10. Add 5ug of primary Ab to approximately 500 ug of total protein.
11. Bring volume to 1 ml with lysis buffer.
12. Rotate at 4°C overnight
13. Add 35 ul beads to each tube.
14. Rotate at 4°C for 2 hours.
15. Spin down beads for 2mins at 6000-7000 rpm.
16. Wash the beads 5 times with 1 ml of lysis buffer.
17. Add 50 ul the 2X sample buffer with BME to beads. (samples can be frozen down at this stage).
18. Boil and run western.

### \*Lysis Buffers

All lysis buffer need add inhibitors. We use Protease Inhibitors Cocktail tablets (Roche # 11897100) 1 tablet in 10 ml (Can be aliquoted and frozen).

### **mRiPA Buffer**

1% Triton X-100 (5 ml of 20% SDS)  
50 mM Tris PH 7.5  
150 mM NaCl  
5 mM EDTA  
0.1% SDS  
20 mM NaF (Sodium Fluoride)

10 mM  $\text{Na}_2\text{P}_2\text{O}_7$  (Sodium Pyrophosphate)

**Alternative Lysis Buffer**

1% Brij 97

25 mM Hepes PH 7.5

150 mM NaCl

5 mM  $\text{MgCl}_2$