

Protein Isolation from Cell Culture Plates

For each tissue culture flask, use 1 ml of lysate buffer

Lysate Buffer:

RIPA Buffer	1.0 ml
Sodium orthovanadate, 100mM	10 ul
PMSF, 10 mg/ml in isopropanol	10 ul
Aprotinin, (Sigma A4529)	10 ul
Leupeptin (Sigma L2023)	10 ul
Pepstatin A 1 mg/ml	10 ul

RIPA Buffer:

	<u>100 ml</u>
1x dH2O (or PBS)	90 ml
1 % Nonidet P-40 (Amresco) or Igepat CA-630 (Sigma)	1 ml
0.5% sodium deoxycholate (deoxycholic acid, sodium salt, Sigma D6750)	0.5 g
0.1% SDS	0.1 g
Mix and Bring to 100 ml with dH2O	

Protocol:

1. Place RIPA Buffer in an ice bucket and prepare a cold lysate solution by adding aliquoted amounts of inhibitor stock solutions.
2. Wash tissue culture plate two times with cold PBS without calcium
3. Tilt plate at an angle of the ice bucket to allow excess PBS to drain to the bottom of the plate/flask
4. Aspirate excess PBS (procedure will not work well in presence of PBS)
5. Add 600 ul lysate buffer to plate
6. Scrape cells using a rubber tissue culture cell scraper and collect in 1.5 ml tube
7. Tilt plate and rise using 300 ul more lysate buffer across the top of the plate/flask
8. Collect cells in same tube. Final volume should be approximately 1 ml.
9. Use a 22 gauge needle and syringe to shear the cells. Pump up and down 10-20 times.
10. Leave on ice for 30 minutes.
11. Sonicate if possible.
12. Centrifuge on low speed at 4 c for 30 minutes
13. Aliquot the supernatant (don't take the pellet). Make 2x10 ul aliquots for protein assay, rest in 100 ul aliquots.
14. Label and store at -70C.