- RIPA buffer (RIPA buffer enables the extraction of cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification. RIPA Buffer does not contain protease or phosphatase inhibitors. However, if desired, protease and phosphatase inhibitors can be added to the RIPA buffer just before use to prevent proteolysis and maintain phosphorylation of proteins.)
- Protease inhibitor cocktail (proprietary mixture)

RIPA buffer

5 ml 1M tris-cl PH 7.4
30 ml 5 M NaCl
5 ml 20% NP-40 or (I used TRITON-X since NP-40 is not commercially available anymore)
5 ml 10 % sodium deoxycholate
0.5 ml 20% SDS
50 mL ddH2O

Procedure

1. Prepare RIPA Lysis buffer
    1. Add 10 µL PMSF solution, 10 µL sodium orthovanadate solution and 10 µL protease inhibitor cocktail solution to 1ml of 1X RIPA buffer to prepare complete RIPA Lysis buffer
2. Pour off media from tissue culture dish into waste container
3. Wash cells twice with PBS pouring excess off into waste beaker
4. Carefully soak up any extra PBS with a Kimwipe
5. Add 500 ul of RIPA lysis buffer to the culture dish
6. Use cell scraper to scrape cells from the bottom of the dish
7. Pass cell lysate through pipette 20 times to form homogeneous lysate
8. Transfer lysate to 1.5 ml microcentrifuge tube
9. Allow samples to stand for 5 mins at 4C (cold room)
10. Centrifuge the resulting mixture at 14,000g for 15 mins at 4C to separate cell debris from protein
11. Transfer supernatant to a new tube and store at -20C