Cell Lysate Preparation for RPPA from Cell Pellet

A. Preparation of Suspension Cell Pellets

1. Collect cells by spinning down by centrifugation (minimum of 1 million cells per sample is required)
2. Wash cell pellet twice with PBS (resuspend and centrifuge)
3. Aspirate PBS as much as possible without disturbing cell pellet

B. Lysis of Cell Pellets

1. REAGENTS
   - Lysis Buffer: 1% Triton X-100, 50mM HEPES pH 7.4, 150mM NaCl, 1.5mM $MgCl_2$,1mM EGTA, 100mM NaF, 10mM Na pyrophosphate, 1mM Na_3VO_4, 10% glycerol, freshly added protease and phosphatase inhibitors from Roche Applied Science cat. nos. 05056489001 and 04906837001, respectively. Complete lysis buffer can be stored in -20°C. Before use, thaw on ice.
   - 4×SDS Sample Buffer: 40% Glycerol, 8% SDS, 0.25M Tris-HCL, pH 6.8. Before use, add Beta-mercaptoethanol (B-Me) at 1/10 of the volume.
2. Thaw dry cell pellets on ice. Add 80μl of cold lysis buffer to the pellets and mix by pipetting up and down. Set the sample mixture on ice for 30 minutes, vortexing every 5 to 10 minutes.
   - **Note: Volume of lysis buffer to be added depends on the number of cells and the size of the pellet. Minimum of 80μl containing 1-2 million cells per sample is required for sufficient protein concentration for RPPA.**
3. Centrifuge the cell lysate in a microcentrifuge at 14,000 rpm (maximum speed) for 10 minutes at 4°C.
4. Carefully collect the supernatant. Discard the pellet.
5. Determine the cellular protein concentration by BCA or Bradford reaction. Adjust protein concentration to 1.5μg/μl. (Use lysis buffer to dilute.)
6. Mix the cell lysate with 4×SDS sample buffer + B-Me without bromophenol blue (3 parts cell lysate plus one part 4×SDS sample buffer). Boil the samples for 5 minutes and store in -80°C until sample submission.

Please provide at least 80μl of each sample separately in a 1.5 ml standard flip-cap microcentrifuge tube. Label tubes numerically in order according to your sample list. Do not place stickers on the sides of the tubes as we will place our own labels there.