Cell Lysate Preparation for RPPA from 6-Well Plate

A. REAGENTS

1. 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.
2. 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.

B. PROCEDURE

1. Seed cells in 6-well plates for overnight or 24-hour incubation. Cell number per well is dependent on the cell size, cell growth rate and experimental design (e.g., duration of treatment). Generally, 0.3-0.5×10^6 cells/3ml is recommended in each well of 6-well plates.
2. Treat cells according to experimental design.
3. Wash the cells twice with PBS. Add lysis buffer to the plates (100-150μl for each well of 6-well plate).
4. Incubate the plates on ice (leveled) for 20 minutes with occasional shaking every 5 minutes.
5. Scrape cells off the plates and collect the cell lysate into 1.5ml microcentrifuge tubes. Centrifuge the cell lysate in microcentrifuge at 14,000 rpm (maximum speed) for 10 minutes at 4°C.
6. Carefully collect the supernatant. Discard the pellet.
7. Determine cellular protein concentration by BCA or Bradford reaction. Adjust protein concentration to 1.5μg/μl. (Use lysis buffer to dilute.)
8. Mix the cell lysate with (4×SDS + B-Me) sample buffer 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.