PREPARATION OF CELL LYSATE FOR REVERSE PHASE PROTEIN ARRAY (6-WELL FORMAT)

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, containing freshly added protease and phosphatase inhibitors from Roche Applied Science Cat. # 05056489001 and 04906837001, respectively

   4XSDS 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. Seed cells in 6-well plate 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 $\times 10^6$ cells/3ml is recommended in each well of 6-well plates.

3. Treat cells according to experimental design.

4. Wash the cells twice with PBS. Add lysis buffer to the plates (100-150ul for each well of 6-well plate).

5. Incubate the plates on ice (leveled) for 20 minutes with occasional shaking every 5 minutes.

6. Scrape cells off the plates and collect the cell lysate into microcentrifuge tubes. Centrifuge the cell lysate in microcentrifuge at 14,000 rpm (maximum speed) for 10 minutes at 4 °C.

7. Carefully collect supernatant. Discard the pellet.

8. Determine cellular protein concentration by BCA or Bradford reaction. Adjust protein concentration to 1.5 ug/ul. (Use lysis buffer to dilute)

9. Mix the cell lysate with (4XSDS + B-Me) sample buffer without bromophenol blue (3 parts of cell lysate plus one part of 4XSDS sample buffer). Boil the samples for 5 minutes, and store in –80 °C until sample submission.

*****Note: Please provide at least 80 uL of the cell lysate for each replicate separately in a 1.5 ml standard flip-cap microcentrifuge tube. Please label tubes in numerical number according to your sample list and make each replicate name on the sample list different (i.e. SampleA-1, SampleA-2, SampleB-1, SampleB-2…)*****