PREPARATION OF TUMOR LYSATE FROM FROZEN TISSUE (BY ELECTRIC OR HAND HOMOGENIZER)

1. **REAGENTS AND MATERIAL**: Frozen tumor tissue set on dry ice, Scalper, Weighing dish, Tweezers, Lysis buffer with protease inhibitors set on ice, 5ml tubes (round bottom) labeled with sample number and set on ice.

   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 volume.**

2. Remove the tumor tissue from cryovials and set in weighing dish at room temperature for a short while (Do not wait for complete thaw). Cut a small piece of the tumor (approximately the size of a grain of rice) and weigh by analytical balance. Try to put the remaining tumor tissue back on dry ice as soon as possible.

3. Put the small piece of tumor tissue into a 5ml tube on ice. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is calculated as 40mg of tumor/ml.

4. Homogenize the tumor tissue by electric or hand homogenizer for 8 seconds. The tumor tissue should be set on ice while homogenizing to prevent heat. Wash the homogenizer probe twice with ice-cold water in between samples and dry the probe with Kimwipe.

5. Optional: Set the samples on ice for 10 minutes.

6. Transfer the samples to microcentrifuge tubes and centrifuge at 4^0C, 14,000rpm for 10 minutes.

7. Collect supernatant (tumor lysates) and transfer to another set of microcentrifuge tubes.

8. Determine protein concentration by BCA or Bradford reaction and 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…)