PREPARATION OF TUMOR LYSATE FROM FROZEN TISSUE (BY PRECELLYS HOMOGENIZER)

1. **REAGENTS AND MATERIAL**: Frozen tumor tissue set on dry ice, Scalper, Weighing dish, Tweezers, Lysis buffer with protease inhibitors set on ice, tubes from Precellys Ceramic Beads Kit (1.4 mm, Cat # 10011152, from Cayman Chemical, www.caymanchem.com), 1.5ml microcentrifuge tubes 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 tumor tissue (approximately the size of a grain of rice) and place in 2ml tubes with Ceramic Beads (for Precellys homogenizer). (We can work with small volume by Precellys homogenizer. We estimate protein yield at 60ug from 1mg of tissue.)

3. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is calculated as 40mg of tumor /ml.

4. For using Precellys, place the tubes on the rack, put the white lid on, and choose from program 1 or 2, click valid to start. Program 1 sets at 30 second per cycle for 2 cycles and Program 2 sets at 45 seconds per cycle for 2 cycles.

5. Centrifuge at 4^o C for 15 minutes at maximum speed (13,000~14,000 rpm).

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

7. Determine protein concentration by BCA or Bradford reaction and adjust protein concentration to 1.5 ug/ul. (Use lysis buffer to dilute)

8. 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…)