RPPA Sample Preparation from Frozen Tissue
by Precellys Homogenizer

A. Reagents and Materials:

- Frozen tumor tissue set on dry ice
- Scalpel
- 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
- Weighing dish
- Tweezers

- **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. no. 05056489001 and 04906837001, respectively. Completed 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.

B. Procedure:

1. 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 volumes by Precellys homogenizer. We estimate protein yield at 60μg from 1mg of tissue.
2. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is calculated as 40mg of tumor/ml.
3. For using Precellys, place the tubes on the rack, secure the white lid, and choose from program 1 or 2; click valid to start. Program 1 is set at 30 second per cycle for 2 cycles and Program 2 is set at 45 seconds per cycle for 2 cycles.
4. Centrifuge at 4°C for 15 minutes at maximum speed (13,000-14,000 rpm).
5. Collect supernatant (tumor lysates) and transfer to another set of microcentrifuge tubes.
6. Determine the protein concentration by BCA or Bradford reaction and adjust protein concentration to 1.5 μg/μl. (Use lysis buffer to dilute)
7. 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 80 μl 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.