RPPA Sample Preparation from Frozen Tissue
by Electric or Hand Homogenizer

A. Reagents and Materials:

- Frozen tumor tissue set on dry ice
- Scalpel
- Lysis buffer with protease inhibitors set on ice
- 5ml tubes (round bottom) labeled with sample number and set on ice
- 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 weigh by analytical balance. Try to put the remaining tumor tissue back on dry ice as soon as possible.
2. 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.
3. 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.
4. Optional: Set the samples on ice for 10 minutes.
5. Transfer the samples to microcentrifuge tubes and centrifuge at 4°C, 14,000rpm for 10 minutes.
6. Collect supernatant (tumor lysates) and transfer to another set of microcentrifuge tubes.
7. Determine the protein concentration by BCA or Bradford reaction and adjust protein concentration to 1.5 μg/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 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.