**Protocol for Tissue Preparation for Reverse Phase Protein Array Analysis**

**Reagents/supplies required, but not supplied by Proteomics Core**

Sample Tubes RB (Qiagen, Cat# 990381)

Dry ice and/or liquid nitrogen

**Tissue Preparation Procedure:**

1. Label all tubes prior to adding tissue samples. Label tubes using the following format:

Example:

The name on the label should match the RPPA Sample Submission Form.

1. Obtain a small tissue sample, usually 5-15 mg, unless it is fat, normal breast, or lung tissues, not to exceed 50 mg. . Try to provide a consistent size for all the samples.   
   Please test the size needed to figure out the optimal tissue size/weight, based on your tissue type. Usually for most tissues like tumor and solid tissues i.e. Liver, Heart, Brain etc., 15 mg tissue is more than sufficient. Some tissues, like lung or normal breast tissues, will require a larger size.   
   Four tubes of testing tissue pieces of similar samples and sizes are usually required for us to test the protocol first.
2. All the tissue samples will need to be in the Sample Tubes RB(Qiagen, Cat#990381) to be processed by the Core.
3. Transfer samples to labeled Sample Tubes RB (perform on dry ice or in liquid nitrogen).
   1. While keeping the Sample Tubes RB on dry ice, transfer pellets from original tube to labeled Sample Tubes RB.   
      If you have a large piece, please refrain from thawing the tissues, as this could lead to protein degradation. Instead, break the tissues in liquid nitrogen using a pre-cooled, clean pestle and mortar.
   2. Work quickly but steadily using a pipette tip to transfer the tissue. For very small tissue samples, be careful not to lose the pellet during the transfer.
   3. Be sure to keep all tubes on dry ice so that tissue samples don’t begin to thaw prematurely. Premature thawing of tissue samples may cause degradation of proteins and affect results.
   4. Transfer 10 samples at a time. Once tissue samples have been transferred to a 2 ml Eppendorf tube, store samples in -80°C.
4. Place all labeled Sample Tubes RB in a freezer box and label as follows:

PI Name/ Investigator Name Type of samples

Contact number

Date of delivery

1. Store tissue samples at -80°C and deliver to Core in dry ice (if not extracting your own protein).
2. Email RPPA Sample Submission Form and make an appointment with the Core prior to delivering samples.
   1. Include a brief description as to how tissue was obtained and processed.
   2. Be sure to note if you have added any type of stabilizing reagent to the tissue. (Ex: RNA*later*)