

## **Preparation of Tissue Lysates Samples for Cytokine Analysis** (with shipping instructions)

Please read the manual accompanying the Lysis Buffer carefully. Volume of Procarta Lysis Buffer should be scaled to obtain at least 3 micrograms of protein per microliter of lysate.

1. Quickly dissect region of interest on ice. Tissue chunks can be up to 10 mg with approximately 2 mm thickness.
2. Immediately put the tissue chunk in to 15 volumes of ice cold Procarta Lysis Buffer Panomics (Affymetrix CAT#: EPX-99999) supplemented with 1mM PMSF (final concentration) and 1X protease inhibitor cocktail (Sigma-Aldrich CAT #: P8340), pipette up and down several times and then incubate on ice for 5 minutes. Please list concentrations of buffer components on sample submission sheet. (150 ul lysis buffer for up to 10 mg tissue chunk)
3. The tissue is then homogenized in a dounce homogenizer until there are no more tissue clumps (approx. 30-40 strokes). Other types of homogenizers should work as well.
4. Centrifuge at 14,000 rpm (bench top centrifuge) for 10 minutes at 4° C.
5. Transfer the supernatant to a new microfuge tube and store at –80C until ready for assay.
6. (Optional) Perform a DC Protein assay using a small aliquot of the lysate. The sample should be diluted in dd H<sub>2</sub>O since detergents in the lysis buffer can interfere with DC Protein Assay Kit.2b.

### **Sample Requirements for Profiling of Tissue Lysates on Luminex System**

- For optimal results please provide 225 ul of cleared lysate at a protein concentration of 5-10 ug/ ul.
- The minimum amount of sample that would provide useful results is 75 ul of lysate at a concentration of >3 micrograms of protein per microliter of tissue lysate.

### **Shipping**

Select a box for shipment that has an external dimension of at least 25 cm in each dimension. The box should have a sturdy cardboard exterior and inner Styrofoam box with wall thickness of at least 2.5 cm. Fill the storage box with 1.5 kg of dry ice. Cool a 133 mm x 133 mm x 48 mm (2 ml tubes) or 73 mm (5 ml tubes) vial storage box in the shipping container.<sup>3</sup> Transfer the frozen sample tubes to the vial storage box. Fill in the free areas of the vial storage box with dry ice and secure the vial storage box top over the samples using wire or string. Fill the Styrofoam shipping container with additional dry ice, and packing peanuts (if necessary) in order to minimize free space in the package. The total dry ice content should be at least 2.5 kg. Use FedEx overnight delivery to ship the package to ORB at the address below. Affix the dry ice sticker to the exterior of the package and record the same dry ice weight on the sticker as was used during set-up of the shipment (may require metric to English unit conversion).

Attention:  
David Willoughby  
Ocean Ridge Biosciences, LLC  
394 SW 12<sup>th</sup> Avenue  
Deerfield Beach, Florida 33442  
Phone: 754-600-5128  
Emergency Phone: 561-427-5548