



OCEAN RIDGE BIOSCIENCES

10475 Riverside Dr. Suite 1
Palm Beach Gardens, FL 33410
Tel: 561-427-7845
Cell: 561-427-5548
Fax: 561-740-8710

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Preparing Tissue Lysates for Cytokine Profiling on Luminex System

1. Quickly dissect region of interest on ice. Tissue chunks can be up to 10 mg with approx 2 mm thickness.
2. Immediately put the tissue chunk in to 15 volumes of ice-cold Procarta Lysis Buffer (Panomics/ Affymetrix Part # PC6002) containing protease inhibitors. (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 H2O since detergents in the lysis buffer can interfere with DC Protein Assay Kit.

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 ug/ ul.