Protocol: TCMP

Preparation of cell lysates

Reagents:

1. Lysis buffer: 8M Urea, <=1% SDS, 200mM EPPS pH 8.5, protease inhibitor + as needed, phosphatase /Lysine deacetylase inhibitors.
    a. cOmplete EDTA-free Protease Inhibitor Cocktail
    b. Phosphatase Inhibitor Mini Tablets
2. Tissue/Cell sample
3. Tissue grinder/ Sonicator/ bead-beater
4. Ice bucket

Procedure

1. Make sure to wash cells at least 3X to remove serum proteins from the media. For tissue chunks, try to wash away as much blood as possible.

2. Add Lysis buffer (LB) to sample.

    - LB containing urea should always be made fresh. The target protein concentration in the lysate is 0.5-8 mg/mL.

    - Urea takes time to dissolve. Do not heat Urea containing buffer.

3. Mechanically grind tissue in LB; sonicate cell pellets in LB; use Bead beater for yeast or bacterial cells in LB. Try to avoid overheating the lysates and use ice bucket as needed, keeping in mind Urea will precipitate if it is too cold.

4. Measure Protein concentrations using the bicinchoninic acid (BCA) assay before sending them and make sure the protein amounts are sufficient for your desired analysis. We will reconfirm the assay at Thermo Center. Please keep in mind that different version of the assay may be incompatible with some substances in the LB.

5. Don't forget to fill out the sample submission form, including any additional helpful information about the nature of your samples in the "Special Instructions" field.

6. Ship properly labelled sample on dry ice, early in the week to avoid any issues with shipping delays.