**LEE LAB RESEARCH LABORATORY**

**PROTEIN LYSATE AND WESTERN BLOT PROTOCOL**

**Protein Lysate Preparation Protocol (Culture)**

1. Wash cells using cold PBS followed by ice-cold 1x TNE lysis buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA adjusted to pH at 7.4) containing 1% SDS, 0.5% NP-40, 0.5% DOC and protease/phosphatase inhibitors.
2. Use cell scraper to remove all detached cells and keep on ice for 5 minutes.
3. Transfer lysates to 1.7 mL collection tubes and sonicate for 2 pulses (at 20% amplitude, 10 sec each), heat for 10 minutes on hot plate (100°C) and centrifuge at 16,000 x g for 10 minutes at 4°C.
4. Transfer resulting supernatants to new tubes (final total lysate).
5. Perform BCA protein assay following manufacturers instruction and prepare 1xLaemlli samples of standardized concentration for SDS-PAGE.

**Protein Lysate Preparation Protocol (Tissue)**

1. If tissue stored at -80C, place in -20C for at least 4 hours or O/N prior to

homogenization

1. Weigh tissue out in mg
2. Add in 10 volumes of 1X TNE (e.g. 10 uL of TNE per 1 mg of tissue)
3. Either by mechanical (Dounce) or homogenizer machine, homogenize tissue gently and

on ice

1. This is TNE crude lysate (no detergents)
2. Add equal volume of 1xTNE +1% SDS, 0.5% NP-40, and 0.5% Doc (Complete TNE)
3. Sonicate at 40C (3 pulses: 10s On/2s Off at 20% amplitude)
4. Boil (10minutes)

**\*STOPPING Point: Sample should be in “Complete TNE”. Make sure to sonicate and boil prior storage\***

1. Centrifuge (16,000xg for 10 minutes, 40C)
2. Collect supernatant for BCA protein assay

**Immunoblotting (Western) Protocol**

1. Run protein lysates using desired CriterionTM TGXTM gels
2. Transfer onto nitrocellulose membranes.
3. Detect protein of interest using appropriate primary antibodies at 4C overnight, followed by horseradish peroxidase **(**HRP)-conjugated secondary antibodies for 1h at room temperature.
4. To visualize, use chemiluminescent substrates and image using ImageQuant LAS 4010 detection system.
5. Perform densitometry on Western blot images using ImageQuant TL 8.1 software