**PhosTag / Western Blot / Broad-Range Sample Preparation**

**Materials:**

* Lysing matrix tubes
* 4M urea Extraction Buffer
* Tissue homogenizer
* Centrifuge
* Filter tubes
* Tabletop test tube I Wencubator set to 90C

**Methods:**

**\*\*Samples should remain on ice throughout the preparation\*\***

1. Weigh out ~10 mg of frozen human ventricular tissue and place in labelled lysing matrix tubes.
2. Pipet room temperature [extraction buffer](#Extraction_Buffer) (vortex before use) into lysing matrix tubes in a 1:20 ratio of sample to buffer
3. Homogenize with beads (40 seconds @ 6 m/s)
4. Spin down for 1 minute to reduce bubbles in mini centrifuge.
5. Dump sample with beads into labeled filter tube.
6. Spin down lysing matrix tubes for 1 minute to reduce bubbles in mini centrifuge.
7. Pipette remaining sample into filter tube
8. Centrifuge filter tubes for 12 minutes at 15g’s
9. Remove filter portion and discard.
10. Keep filtrate in tube and incubate at 90 degrees C for 5 minutes in order to solubilize the proteins. Vortex after.
11. **If required - Perform lowry protein assay (per kit instructions) to calculate protein concentrations on extracted supinate.**
12. Sample is ready for PhostagTM-SDS-PAGE. Store at -80.

**4M urea Extraction buffer (100mL):**

1. Add 40 mL of DI water to a beaker and place on hotplate (Ramsey) with a stir-rod of 250 RPM and a temp 30 degrees C
2. Add in;
   1. 0.788 g Tris (for 50 mM in 100mL solution)
   2. 2g of SDS (to get 2% (w/v) in 100mL solution)
   3. 6 ml of glycerol (to get 6% (v/v) in 100mL solution)
   4. 24.024 of Urea (to get 4M in 100mL solution)
   5. 1 ml of beta-mercapto-ethanol (to get 1% (v/v) in 100mL solution)
3. Add HCl to bring solution to a pH of 6.8 by added 5-100uL at a time. (tris buffer zone is 8ish, so it will be hard to get through this but after the pH is below 8, GO SLOW! – roughly should take 400-500 µL of HCL) – use KOH if you undershoot.
4. Lastly, take the final solution and fill to 100mL and aliquot out into two 50 mL tubes labelled and dated.