**PhosTag / Western Blot / Broad range Sample preparation:**

1. Combine Frozen human ventricular tissue samples (10 mg) with 200 µL [extraction buffer](#Extraction_Buffer)
2. Homogenize with beads (40 seconds @ 6 m/s)
3. Spin down for 1 minute to reduce bubbles in mini centrifuge
4. Dump into filter tube.
5. Spin down for 1 minute to reduce bubbles in mini centrifuge
6. Pipette into filter tube
7. Spin 1 more minutes and dump into filter tube
8. Centrifuge 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.