Tissue Homogenization and RNA + Protein Extraction using TRizol® reagent
Protocol Specified on the basis of Human Heart Preparation and Lung Tissue Preparation.
Duke Proteomics Core Facility
http://www.genome.duke.edu/cores/proteomics/sample-preparation/

This protocol should generate approximately 0.7 to 1.5 mg protein for analysis, depending composition of the tissue. This precipitation protocol is intended as a substitute for more traditional protocols utilizing SDS-based or urea-based tissue homogenization, which does not afford the harvest of RNA during the protocol. Sample should be kept at 4C (on ice) during this protocol unless otherwise stated.

1. Weigh tissue section immediately upon removal from -80C by taring a 15 mL round-bottom polypropylene falcon tube and adding tissue. Add 1 mL ice-cold TRizol® per 0.1 g tissue weight to falcon tube.
2. Homogenize tissue section vigorously using 5 second bursts at max speed. Homogenate should be even consistency with no visible chunks.
3. Transfer desired quantity of homogenate for processing to 2 mL Lo-Bind eppendorf tubes in 1 to 1.5 mL aliquots.Store remaining homogenate at -80C. According to manufacturer, this homogenate is good for at least 1 year at -80C.
4. Incubate TRizol tissue homogenate on ice/at 4C for 30 minutes with mixing if possible. If not, vortex occasionally.
5. Incubate TRizol tissue homogenate for 5 mins at 30C. Cool sample on ice. Spin at 12,000 rcf for 5 minutes at 4C; unlysed tissue debris and cells will form a pellet at the bottom of the tube and clear TRizol lysate should form supernatant.
6. Pipet 0.8 mL clear TRizol lysate to 2.0 mL LoBind eppendorf tube for protein extraction.
7. Decant any remaining clear TRizol-tissue homogenate and store at -80C. ***Steps 8-9 for generating an additional “sonication-soluble” fraction***. If not isolating sonication-soluble fraction, skip to step 10.
8. Decant all clear trizol homogenate from the top of the pellet. Add 0.5mL of fresh, cold TRizol reagent to the top of the pellet.
9. Sonicate the pellet/0.5mL TRizol at Power = 3 with probe sonicator. Sonicate with two 10-second bursts, cooling on ice between. Repeat steps 4-6 above for isolation of clear lysate from sonication-soluble fraction. At step 6, you will only be able to isolate 0.4mL of clear TRizol lysate. Proceed from step 10, with reagent volumes cut in half for each step in the protocol.
10. Add 0.2mL chloroform to 2 mL epi tube containing 0.8 mL TRizol lysate, and vortex to mix. Centrifuge at 12,000rcf x 5 min to perform phase separation. Remove upper aqueous layer (contains RNA, process if necessary). 0.5 mL of RNA/aqueous layer should be able to be removed without disturbing the protein/phenol/chloroform layer.
   Approximately 200 uL will be available for sonication-soluble fraction.
11. To the remaining phenol/chloroform layer, add 1.2 mL coldMeOH, and vortex. Store sample for 10 mins at 30C, then cool the sample on ice and subsequently sediment the protein by centrifuging at 12,000 rcf for 10 mins. (per Kline et. al. JPR2008 v7 p5055)
12. Remove supernatant and wash pellet with one 1.0 mL aliquot of cold MeOH. Spin at 12,000 rcf between washes if needed.
13. Add 500 uL cold MeOH, then sonicate the protein pellet using burst sonication (5 seconds at 30 percent power) to generate a fine protein powder. Use the same volume, 500 uL, for sonication-soluble fraction for this step. Use approximately 4 bursts, and store in ice between bursts. This step is critical for protein re-solubilization.
14. Allow the protein pellet to settle or use low-speed centrifugation to settle protein (2000 rcf x 1 minute). Remove supernatant MeOH. Do not allow protein pellet to dry, but allow all MeOH from above the pellet to evaporate.

15. Add 200 uL 0.25% Rapigest in 50mM ammonium Bicarbonate and resolubilize pellet with assistance from vortex and heat to 60C. If needed, probe sonication can be used. Utilize same volume (200 uL), for resolubilization of sonication-soluble TRizol lysate.

16. Protein concentration should be between 2.5 and 7.5 mg/mL; perform 1:10 dilution of a small aliquot (2 uL:18uL) in 50 mM Ammonium Bicarbonate, and use a Bio-Rad mini-Bradford Assay to quantify protein (BSA as standard).

17. Normalize protein content and follow standard in-solution digestion protocol.