Tissue Protein Extraction Buffer (1X Hepes based Buffer, pH 7.5) For Immunoprecipitation and Western blot

Protocol (All solutions should be kept on ice. The protocol below is scalable).

1. Pulverize frozen tissue and remove approximately 90 μl of tissue. Place tissue in a 1.5 ml round bottom microcentrifuge tube.
2. Add general phosphatase and protease inhibitor cocktails to approximately 500 μl of ice-cold Tissue Protein Extraction Buffer.
3. Add the 500 μl of Tissue Protein Extraction Buffer with inhibitors to the pulverized tissue.
4. Homogenize tissue with a mini pestle-homogenizer using 15 strokes, 3 seconds/stroke on ice.
5. Centrifuge 12000g for 15 min at 4^{o}C.
6. Remove supernatant (without lipid layer) and transfer into another 1.5 ml tube.
7. Centrifuge again at 12000g for 15 min at 4^{o}C.
8. Transfer supernatant to another tube. This supernatant fraction contains the extracted proteins.
9. The Bradford assay can be used to measure the extracted protein concentration.

Safety. Contains irritants. Avoid ingestion and contact.
Storage. Aliquot, -20^{o}C