Protein Extraction using phenol and methanolic ammonium acetate precipitation

1. Put fresh tissue in 1.5 ml tube on ice with water.
2. Add 150 ul of extraction media (0.1 M Tris-HCl pH 8.8, 10 mM EDTA, 0.9 M sucrose).
3. Sonicate tissue less than ONE minute on ice and cool down.
   ➔ **Adjust the dial 5 for optimization of the output power (watt).**
   ➔ **Output watt will be 14 watt. Do not exceed over 20 watt.**
4. Repeat step 3 trice.
5. Add 75 ul of extraction media (0.1 M Tris-HCl pH 8.8, 10 mM EDTA, **1.2% β-mercaptoethanol**, 0.9 M sucrose)
6. Add **225 ul of Tris pH8.8 buffered phenol** and mixing well (vortexing).
7. Centrifuge 10 min at 5000 g, 4 C.
8. Transfer the phenol phase (**should be top phase; there is protein**) into **new 2 ml tube** and back-extract aqueous phase with 100 ul + 100 ul of extraction media and phenol by vortexing. Centrifuge and combine with first extraction.
   ➔ **If your sample includes high salt, the phase will be reversed.**
9. Precipitate phenol extracted proteins by adding 5 volumes of **COLD** 0.1 M ammonium acetate in 100% methanol (stored at –20 °C).
10. Vortex and incubate at –20 C for at least 1 h or **overnight**. Collect the precipitate by centrifugation (20 min, 20,000 g, 4 °C).
    ➔ I preferred this procedure overnight.
11. Wash the pellet **twice** with 0.1 M ammonium acetate in methanol\
    ➔ I preferred this procedure overnight.
12. Wash the pellet **twice** with ice-cold 80% acetone (stored at –20 °C)
    ➔ I preferred this procedure overnight.
13. Resuspend final pellet with 100 ul of protein buffer by pipetting and vortexing at 25 °C. Incubate sample for 1 h at room temperature with agitation. **Do not heat sample (<30** °C) **under any circumstances as this will lead to carbamylation of proteins.**

14. Do acetone precipitation.
15. Please do EZQ protein assay

Notes:

- Preparation of samples must be performed with labware that has never been in contact with nonfat milk, BSA, or any other protein blocking agent to prevent carryover contamination.
- Always use **non-latex gloves** when handling samples, keratin and latex proteins are potential sources of contamination.
- Never re-use any solutions, abundant proteins will partially leach out and contaminate subsequent samples.

- Trisma-base
- pH 8.8 Phenol
- HCl
- EDTA
- β-mercaptoethanol
- sucrose
- 2ml tube (eppendorf)
- ammonium acetate
- methanol
- acetone
- nitrile gloves