TriReagent Protein Isolation

1. See 1-4 of TriReagent RNA Isolation protocol. You will be using the pink bottom layer. Cut values in half if using 500 µL of TriReagent.
2. Thaw samples on ice. Turn on the damn centrifuge so it cools down!
3. Discard any remaining aqueous (colorless) phase.
4. Add 0.3 mL of 100% ethanol to the tube. Invert a few times and incubate at room temp for 2-3 min.
5. Centrifuge at 2000 rcf for 5 min at 4oC to sediment DNA, then transfer pink supernatant to new tube. Discard the tube with the DNA pellet.
6. Precipitate protein by adding 1 ml of isopropanol, giving the tube a quick shake, and incubating for 10 min at room temp.
7. Centrifuge at 12000 rcf for 10 min at 4oC. Remove supernatant and discard as Trizol waste (toxic).
8. Wash pellet in 2mL of 0.3M guanidine hydrochloride 95% ethanol solution, let stand 20 min at room temp, then centrifuge at 7500 rcf for 5 min at 4oC. Repeat this step 2 more times. (In this wash solution, the pellet should be stable for 1 yr @ -20oC.)
9. Vortex 3 sec in 1 mL 100% ethanol, let sit for 20 min at room temp.
10. Centrifuge at 7500 rcf for 5 min at 4oC, remove supernatant and discard, let air dry 5 min. Dissolve pellet in Lysis buffer (amount depends on pellet size and desired concentration). To dissolve pellet, heat @ 65oC for 5 min, occasionally vortexing.

**Lysis Buffer (200 mL)**

1.169g EDTA

1.636g NaCl

10g SDS

2.4228 Tris

DI water to reach 200mL