Whole worm glycerol assay

Reagents needed:

1. Dry Ice
2. Mortar and pestles
3. 1N Perchloric acid (6%)
4. 5N KOH (+ 100 mM KPO4 to buffer)
5. BCA protein assay kit (Pierce)
6. Glycerol assay kit (R-biopharm)

Protocol

1. Wash worms off plates w/ M9 (make sure osmolarity matches that of plate)
2. Wash twice
3. Resuspend worm pellet in 10 ml M9 and allow worms to evacuate bacteria for 15 minutes.
4. Split into 3 X 1.5ml eppendorf tubes (to get accurate protein measurments, you need at least 200ul worms/tube – if there's not enough worms, only do 1 or 2 tubes)
5. Spin in microfuge at max speed for 30 seconds and remove as much supernatant as possible.
6. Freeze worm pellet in LN2; store at -80 degrees indefinitely
7. **STOPPING POINT**

8. Pop worms out of tube into a pre-cooled mortar and pestle on dry ice (this takes some practice!)
9. Grind worms to a fine powder on dry ice (~5-10 minutes of grinding)
10. Rim container w/ **1ml** of perchloric acid, letting it freeze around the top of the vessel.
11. Place frozen container with worms and PCA into a pan of room temperature water and let it thaw (takes about 15 minutes)
12. Once thawed, tilt mortar towards you at a 45° angle and remove solution + white precipitate (proteins) to a 15 ml polypro tube → **BE PATIENT!!!!!!!! Allowing ALL of the solution to collect in the bottom of the mortar is essential for accurate assays.**
13. Rinse the container with 1 ml of PCA and remove to the 15ml tube used in step 12. Repeat step once more (total amount of added PCA is 3.0 ml).
14. **NOTE: The goal of step 12 and 13 is to get as much of the white protein precipitate as possible into the 15 ml tube. BE PATIENT AND KEEP THE MORTAR AT AN ANGLE. AFTER STEP 13, THERE SHOULD BE VERY LITTLE PROTEIN LEFT IN THE MORTAR.**
15. Allow extraction to proceed on ice for at least 60 minutes (overnight is OK)
16. **STOPPING POINT**

17. Spin solution at 3200RPM, 4 degrees for 15 minutes to pellet protein
18. Remove EXACTLY 3ml of supernatant. Measure the remaining volume on the protein pellet → **IF YOU RECOVERED < 3.0 ML, YOU WERE NOT PATIENT ENOUGH WHILE RECOVERING THE PCA IN STEPS 14 AND 15.**
19. Solublize the protein pellet in 2ml of 0.2N NaOH (this can sometimes take a while; use ample vortexing to resupend the pellet)
20. Use this solution for the BCA protein assay (see 'BCA Assay' protocol)
21. Neutralize the 3 ml PCA extract with 5N PO4 buffered KOH. Goal is to attain a pH of 7.5-8.0.
22. Start by adding 500ul KOH solution and rechecking pH. If the solution is still acidic, add 5ul of KOH and recheck the pH. DO NOT ADD MORE THAN 5ul AT A TIME or you will overshoot the target pH!!!.
23. Once the goal pH is reached, allow the solution to sit on ice for 30 minutes and then recheck the pH. Adjust as necessary.
24. Split the neutralized solution into 3X1.5 ml eppendorf tubes, spin at 4 degrees, max speed for 10 minutes.
25. Use this supernatant for the glycerol assay (For low glycerol concentrations, use 1-2ml/ assay. For high glycerol concentrations, use 100ul/assay)
26. Follow R-biopharm kit protocol for glycerol assay (I measure pre-reaction after 7 minutes and postreaction after 15 minutes)
27. For BCA assay, run the room temp protocol.