

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. While worms evacuate, record the weight of each 1.5 ml to be used in step 5.
5. Split into 3 X 1.5ml eppendorf tubes (need at least 200ul worms/tube – if there's not enough worms, only do 1 or 2 tubes)
6. Spin in microfuge at max speed for 30 seconds and remove as much supernatant as possible.
7. Weigh each tube again and subtract from weight in step 4 to determine the worm "wet weight"
8. Freeze worm pellet in LN₂; store at -80 degrees indefinitely
9. **STOPPING POINT**
10. Pop worms out of tube into a pre-cooled mortar and pestle on dry ice (this takes some practice!)
11. Grind worms to a fine powder on dry ice (at least 10 minutes of grinding)
12. Rim container w/ 1ml of perchloric acid, letting it freeze around the top of the vessel.
13. Place frozen container with worms and PCA into a pan of room temperature water and let it thaw (takes about 15 minutes)
14. 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.**
15. Rinse the container with 1 ml of PCA and remove to the 15ml tube used in step 14. Repeat step once more (total amount of added PCA is 3.0 ml).
16. **NOTE: The goal of step 15 and 16 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 15, THERE SHOULD BE VERY LITTLE PROTEIN LEFT IN THE MORTAR.**

17. Allow extraction to proceed on ice for at least 60 minutes (overnight is OK)

18. STOPPING POINT

19. Spin solution at 3200RPM, 4 degrees for 15 minutes

20. 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.

21. Solublize the protein pellet in 0.5ml of .2N NaOH (this can sometimes take a while; use ample vortexing to resuspend the pellet)

22. Use 10ul of this solution for the BCA protein assay (**dilutions may be required depending on the pellet size**)

23. Neutralize the 3 ml PCA extract with 5N PO₄ buffered KOH. Goal is to attain a pH of ~8.0.

24. 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!!!**

25. Once the goal pH is reached, allow the solution to sit on ice for 30 minutes and then recheck the pH.

26. Split the neutralized solution into 3X1.5 ml eppendorf tubes, spin at 4 degrees, max speed for 10 minutes.

27. Use this supernatant for the glycerol assay (For low glycerol concentrations, use 1-2ml/ assay. For high glycerol concentrations, use 100ul/assay)

28. Follow R-biopharm kit protocol for glycerol assay (I measure pre-reaction after 7 minutes and postreaction after 15 minutes)

29. For BCA assay, run the room temp protocol.