

Labeling Tubulin with Tetramethylrhodamine Succinimidyl Ester

Materials

1. Drop frozen 2x cycled tubulin in a polymerization-promoting buffer. Stored at -80°C
2. GTP, 100 mM stock, titrate to pH ~ 7 . Need 2 ml.
3. Waterbath, set at 37°C
4. SS34 rotor, 5 ml and 15 ml tubes and adaptors. Bring the rotor, adaptors, and tubes to $\sim 37^{\circ}\text{C}$ by soaking in warm water. If possible, keep a separate centrifuge and SS34 rotor at 4°C .
5. PEM buffer: 0.1 M PIPES, 1.0 mM EGTA, 0.5 mM MgCl_2 , pH 6.9, prepare 50 ml. Keep ~ 20 ml at 37°C and the rest on ice.
6. DMSO.
7. TRSE, Molecular Probes C-1171. Prepare fresh 10 mg/ml stock in DMSO. Need 2.5 mg per 50 mg of tubulin.
8. Potassium glutamate, 1 M. Prepare 1 M glutamic acid and titrate with KOH to pH 6.9.
9. Injection Buffer: 50 mM potassium glutamate, 0.5 mM MgCl_2 , pH 6.5-6.7. Prepare 10 ml. Keep ~ 5 ml at 37°C and the rest on ice.
10. 1 M PIPES, pH 6.9. Need 5-10 ml.

Procedure

1. Weigh out drop frozen pellets of 2x cycled tubulin to obtain ~ 50 mg of tubulin. Thaw in a water bath.
2. Add an equal volume of 1 M PIPES (volume in mls equals tubulin weight in grams). Mix well. Add GTP to 1 mM and DMSO to 10% and mix well. Incubate at 37°C for 10 min. The solution should turn turbid and viscous.
3. Centrifuge in a SS34 rotor at 18,000 rpm, 37°C for 20 min. Keep the rotor warm in an incubator afterwards.
4. Discard the supernatant. Resuspend pellets in 3 ml of ice cold PEM buffer by gentle pipeting. Measure the total volume.

5. Incubate at 0°C for 10 min.
 6. Add GTP to 1 mM, and DMSO very slowly while stirring to 10%. Incubate at 37°C for 10 min. Prepare the TRSE stock solution.
 7. Transfer the tubulin solution to a vial with a stir bar. While stirring vigorously, add very slowly 250 microliters of the TRSE stock.
 8. Incubate at 37°C for 5 min, then add potassium glutamate to 5 mM to stop the reaction.
 9. Centrifuge in a SS34 rotor at 18,000 rpm, 37°C for 20 min. Chill down the rotor afterwards.
 10. Rinse pellet quickly with 2x2 ml warm PEM buffer. Resuspend pellet in 2 ml of cold PEM buffer (solution appears opaque).
 11. Incubate at 0°C for 10 min.
 12. Centrifuge in a SS34 rotor at 18,000 rpm, 4°C for 20 min. Warm up the rotor afterwards.
 13. Collect supernatant and measure its volume. Bring GTP to 1 mM and DMSO to 10%.
 14. Incubate at 37°C for 10 min.
 15. Centrifuge in a SS34 rotor at 18,000 rpm, 37°C for 20 min. Chill down the rotor afterwards.
 16. Rinse the pellet quickly with 2x2 ml warm PEM buffer. Resuspend pellet in 1 ml of cold PEM buffer by gentle pipeting.
 17. Cycle tubulin as in steps 11 through 15. Use 5 ml centrifuge tubes for smaller volumes.
 18. Rinse pellet twice, quickly, with warm injection buffer. Resuspend pellet in ~500-800 microliters of ice cold injection buffer. Incubate at 0°C for 10 min.
 19. Centrifuge in a SS34 rotor at 18,000 rpm, 4°C for 20 min
 20. Drop freeze the labeled tubulin.
 21. Final tubulin concentration is determined by the Lowry assay. Take a 10-20 ul aliquot and dilute with 400 ul buffer. Read OD at 556 nm and calculate dye concentration based on the molar extinction coefficient of 50,000. D/P should be around 0.75 per tubulin monomer.
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