LABELING MUSCLE ACTIN WITH CARBOXYFLUORESCEIN SUCCINIMIDYL ESTER

Day 0 and 1

Materials

1. 0.5 mM ATP, 0.2 mM $CaCl_2$, 1 mM PIPES, pH 6.8 at 4^oC, 250 ml for day 0.

2. 0.5 mM ATP, 0.2 mM $CaCl_2$, 70 mM KCl, 150 mM PIPES, pH 7.0, 1 ml.

3. 16 mM ATP, 0.2 mM $CaCl_2$, 5 mM DTT, 5 mM lysine, 10 mM glutamic acid, 20 mM Tris-HCl, pH 8.0, 10 ml.

4. 3 mM $CaCl_2$, 1.2 M KI, 50 mM Tris-HCl, pH 8.0, 5 ml.

5. 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM DTT, 2 mM Tris-HCl, pH 8.0 at 4^oC, 1 liter.

6. CFSE (Molecular Probes, C-1311; not the diacetate), prepare a stock solution of 50 mg/ml in DMSO.

7. Small SS34 tubes and adaptors, 50Ti tubes, small vial.

8. G-25-150 column, ~30x1.5 cm.

Procedure (perform under reduced light, 4^oC unless otherwise noted)

1. Resuspend 10 mg lyophilized actin in 2 ml buffer 1. Be careful not to make bubbles.

2. Add DTT to 0.5 mM.

3. Dialyze against 250 ml buffer 1 for 8 hrs or overnight.

4. Equilibrate G-25 column with buffer 5.

5. Collect actin from dialysis tubing and transfer to a small vial with a stir bar. Measure volume. Add KCl to 70 mM and $CaCl_2$ to 1 mM. Incubate at room temperature for 30 min.

6. While vortexing, add 166 µl (8.31 mg) CFSE stock solution to 1 ml of buffer 2.

7. Mix dye solution immediately with actin solution, by gentle pipeting or stirring. Incubate at room temperature for 1 hr.

8. Centrifuge in a 50Ti rotor at 40,000 rpm, 4^{o}C for 1 hr.

9. Resuspend pellet in 1.0 ml of buffer 3. Measure the total volume.

10. Add slowly an equal volume of buffer 4 while stirring. Stir gently on ice for 30 min.

11. Centrifuge in a SS34 rotor at 18,000 rpm, 4^{o}C for 20 min.

12. Run supernatant through the G-25 column, collect 10 drop fractions.

13. Collect fluorescent fractions in the void volume, measure volume in a volumetric conical tube.

14. Polymerize actin by adding KCl to 100 mM and $MgCl_2$ to 2 mM. Incubate for 30-60 min at room temperature.

15. Centrifuge in a 50Ti rotor for 1 hr at 40,000 rpm, 15^{o}C.

16. Soak pellet(s) in 0.6 ml buffer 5 for 1-2 hr, resuspend by gentle pipeting.

17. Dialyze against buffer 5 overnight.

Day 2 on

Materials

1. 50Ti tubes.

2. Buffer 5 as for day 1, 200 ml.

Procedure

1. Centrifuge in a 50Ti (1 hr, 40,000 rpm) or 42.2Ti (30 min, 25,000 rpm) at 4^{o}C.

2. Polymerize actin by adding KCl to 100 mM and $MgCl_2$ to 2 mM. Incubate for 30-60 min at room temperature.

3. Centrifuge in a 50Ti rotor for 1 hr at 40,000 rpm, 15^{o}C, or 42.2Ti rotor for 1/2 hr at 25,000 rpm.

4. Soak pellet(s) in 0.4 ml buffer 5 for 1-2 hr, resuspend by gentle pipeting.

5. Dialyze against buffer 5 overnight.

6. Centrifuge in a 50Ti (1 hr, 40,000 rpm) or 42.2Ti (30 min, 25,000 rpm) at 4^{o}C.

7. Measure concentration and dye/protein molar ratio. Dilute 1:40 with the dialysis buffer and read the OD at 495 nm.

$$D/P = \{OD_{495} \times 41 / 60{,}000\} / \{(mg/ml) / 43{,}000\}$$

8. Dilute to 3-5 mg/ml with the dialysis buffer. Calculate total mg of actin. Drop freeze in liquid N_2 after dissolving 2 mg sucrose per mg actin.

9. Dialyze against 0.05 mM $MgCl_2$, 0.2 mM ATP, 2 mM Tris-acetate, pH 6.95 overnight before microinjection.
