Labeling Muscle Actin with 5-Iodoacetamidofluorescein

Day 0 and 1

Materials

1. 0.5 mM ATP, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 8.0 at 4^oC, 250 ml for day 0.

2. 100 mM KCl, 100 mM boric acid, pH 8.4 at 4^oC, 10 ml.

3. 0.5 mM ATP, 2 mM $MgCl_2$, 100 mM KCl, 2 mM DTT, 2 mM PIPES, pH 7.0, 1000 ml.

4. IAF (Molecular probes)

5. 50Ti tubes, small vial.

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 (100 mM stock) to 5 mM.

3. Dialyze against 250 ml buffer 1 overnight.

4. Collect actin from dialysis tubing, add 100 mM KCl and 2 mM $MgCl_2$ to induce polymerization. Let sit on ice for 20 min.

5. Get 2.4 mg IAF in a test tube. Add 100-200 µl acetone and make a fine slurry by grinding particles against the tube with a pipet. Adjust the volume in step 6 if the amount actually weighed is higher.

6. Add IAF slurry very slowly by dipping a small amount at the tip of a Pasteur pipet into 2 ml of buffer 2 in a small vial under constant stirring.

7. Add 2 ml of the dye solution to actin and mix gently with a Pasteur pipte.

8. Let sit on ice for 2 hr.

9. Stop the reaction by adding DTT to 10 mM.

10. Dialyze against 1 liter buffer 3 overnight.

Day 2

Materials

1. Buffer 1 as for day 1, 4^{o}C, 2000 ml.

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

3. 50Ti tubes, volumetric conical tube.

4. Colloidin bag for dialysis.

Procedure

1. Equilibrate G-25 column with Buffer 1.

2. Pellet actin in a 50Ti rotor at 40,000 rpm, 4^{o}C, for 2 hr.

3. Rinse pellet briefly with buffer 1, soak and resuspend in 2 ml.

4. Dialyze against 500 ml buffer 1 at 4^{o}C for 4-5 hr in a colloidin bag.

5. Clarify dialyzed actin in a 50Ti rotor for 1 hr at 40,000 rpm, 4^{o}C.

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

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

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

9. Centrifuge in a 50Ti rotor for 2 hr at 40,000 rpm, 15^{o}C.

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

11. Dialyze against buffer 1 overnight.

Day 3 on

Materials

1. 50Ti tubes.

Procedure

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

2. 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} x 41 / 60,000} / {(mg/ml) / 43,000}, should be 0.5-0.9.

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

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