

LABELING MUSCLE ACTIN WITH IATR

Day 0 and 1

Materials

1. 0.5 mM ATP, 0.2 mM CaCl₂, 2 mM Tris-HCl, pH 8.0 at 4°C, 250 ml for day 0 and 1000 ml for day 1.
2. 100 mM KCl, 100 mM boric acid, pH 8.4 at 4°C, 10 ml.
3. IATR (tetramethylrhodamine iodoacetamide; Molecular Probes).
4. 50Ti tubes, small vial.

Procedure (perform under reduced light, 4°C 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 MgCl₂ to induce polymerization. Let sit on ice for 20 min.
5. Get ≥ 1 mg IATR in a test tube. Add 100-200 μ l acetone and make a fine slurry by grinding particles against the tube with a pipet.
6. Add IATR slurry very slowly by dipping a small amount at the tip of a Pasteur pipet into 3 ml of buffer 2 in a small vial under constant stirring.
7. Clarify in a 50Ti rotor at 40,000 rpm for 10 min, 4°C.
8. Collect clarified dye solution. Dilute 5 μ l into 1 ml distilled water and measure OD at 555 nm. The OD should be in the range of 0.05 to 0.10.
9. (optional) Calculate the volume (ml) of IATR required for reaction as $0.15 / \text{OD}$ or use an volume equal to that of actin. Mix gently with actin with a Pasteur pipet.
10. Let sit on ice for 2 hr.
11. Stop the reaction by adding DTT to 10 mM.

12. Dialyze against 1 liter buffer 1 overnight.

Day 2

Materials

1. G-25-150 column, ~30x1.5 cm.
2. Buffer 1 as for day 1, 4°C, 1000 ml.
3. 50Ti tubes, volumetric conical tube.

Procedure

1. Equilibrate G-25 column with Buffer 1.
2. Clarify dialyzed actin in a 50Ti rotor for 1 hr at 40,000 rpm, 4°C.
3. Run supernatant through the G-25 column, collect 10 drop fractions.
4. Collect fluorescent fractions in the void volume, measure volume in a volumetric conical tube.
5. Polymerize actin by adding KCl to 100 mM and MgCl₂ to 2 mM. Let sit for 30-60 min at room temperature.
6. Centrifuge in a 50Ti rotor for 2 hr at 40,000 rpm, 15°C.
7. Soak pellet(s) in 0.4 ml Buffer 1 for 1-2 hr, resuspend by gentle pipeting. Sometimes there is a dark center on the surface of the pipet, which can come off as an aggregate when handled gently. Discard the aggregate by careful pipeting.
8. 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°C.
2. Measure concentration and dye/protein molar ratio. Dilute 1:40 with the dialysis buffer and read the OD at 555 nm.

$$D/P = \{OD_{555} \times 41 / 60,000\} / \{(mg/ml) / 43,000\}, \text{ should be } 0.5-1.1.$$

3. Dilute to 3-5 mg/ml with the dialysis buffer. Calculate total mg of actin. Store as aliquots in liquid N₂ after dissolving 2 mg sucrose per mg actin.

4. Dialyze against 0.05 mM MgCl₂, 0.2 mM ATP, 2 mM Tris-acetate, pH 6.95 overnight before microinjection.
