Labeling Gizzard Myosin with Tetramethylrhodamine Iodoacetamide (Primarily 17kDa Light Chain)

Day 1

Materials

1. 50 mM KCl, 0.2 mM EGTA, 2 mM ATP, 2 mM MgCl2, 10 mM HEPES, pH 7.0. Need 500 ml.

Procedure

1. Thaw appropriate volume of frozen gizzard myosin to obtain 10-20 mg.

2. Centrifuge in a SS34 rotor at 9,000 rpm, 4°C for 20 min.

3. Resuspend pellet in a small volume of buffer 1 to obtain 15-20 mg/ml concentration. Dialyze against 500 ml of buffer 1 for >=2 hours.

4. Measure volume. Dilute with dialysis buffer to obtain a myosin solution of 12-15 mg/ml.

5. Get an aliquot of IATR, dissolve in DMF or DMSO at 7 mg/ml. The amount needed for reaction is 0.12 mg IATR per 10 mg of myosin. Add the calculated volume very slowly to myosin. Mix gently.

6. React on ice for 6 hours.

Day 2 on

Materials

1. 50 mM KCl, 0.2 mM EGTA, 1 mM DTT, 10 mM HEPES, pH 7.0. Need 2 liters.

2. Buffer 1 with 10 mM MgCl2, 10 ml.

3. Buffer 1 with 2 mM MgCl2, 2 mM ATP, 10 ml.

4. 50 mM KCl, 0.2 mM MgCl2, 0.2 mM ATP, 0.1 mM DTT, 20 mM Tris-acetate, pH 7.0. Need 250 ml.

Procedure
1. Add DTT to 10 mM. Centrifuge in a 50Ti rotor at 30,000 rpm, 4°C for 30 min.

2. Dialyze supernatant against buffer 1 at 4°C for >=3 hours.

3. Measure the volume and bring MgCl2 to 10 mM. The solution should turn turbid. Allow solution to sit on ice for 30 min.

4. Centrifuge in a SS34 rotor at 9,000 rpm, 4°C for 15 min.

5. Resuspend pellets in 3-5 ml of buffer 2, centrifuge as in step 4.

6. Resuspend pellets in a small volume of buffer 3.

7. Dialyze overnight against buffer 4.

8. Centrifuge in a 50Ti rotor at 35,000 rpm, 4°C, for 60 min. The supernatant is good for microinjection for 2-3 days. Most of the fluorophores are located at 17 kD MLC.