

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.
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