

LABELING GIZZARD MYOSIN WITH IATR (MHC AND 17KD MLC)

Day 1-2

Materials

1. 0.5 M KCl, 10 mM HEPES, pH 7.5, 4°C, 250 ml.
2. 0.5 M KCl, 50 mM HEPES, pH 8.0, 4°C, 250 ml.
3. Bio-Beads SM-2 in a 0.7x15 column.
4. 20 mM KCl, 20 mM PIPES, pH 7.0, 4°C, 250 ml.
5. IATR (tetramethylrhodamine iodoacetamide; Molecular Probes).

Procedure

1. Thaw appropriate volume of frozen gizzard myosin to obtain 3-5 mg.
2. Dialyze against buffer 1 overnight to remove residual ammonium sulfate.
3. Clarify myosin solution at 20,000 rpm, 4°C, for 30 min in a 50Ti rotor.
4. Dilute 20 ul myosin with 400 ul dialysis buffer. Determine concentration of myosin by reading OD₂₈₀ (UV).

$$\text{concentration (mg/ml)} = \text{OD} * 21 / 0.508$$

5. Measure volume of myosin. Determine total amount of myosin.
6. Dissolve 0.5-1 mg IATR in 100 ul of DMSO; pipet until dye forms a fine slurry. It is very important at this point that the dye be dissolved as much as possible.
7. Add the slurry dropwise to buffer 2 while stirring, to obtain a concentration of 0.1 mg/ml.
8. Clarify dye at 35,000 rpm, 4°C, for 15 min in a 50Ti rotor.
9. Carefully collect supernatant. Dilute 100 to 1000 fold and read OD at 555 nm. The volume required for reaction (in ml) is calculated as:

$(1.4/OD_{555}) \times \text{mg of myosin}$

10. Add dye to myosin, mix gently by pipeting. Incubate on ice for 2 hr in the dark. Equilibrate column with buffer 2.
11. Apply to Bio-Beads column. Pool fractions that appear pink in room light.
12. Dialyze overnight against buffer 4 to precipitate myosin.

Day 3

Materials

1. 2 M KCl, 10 mM PIPES, pH 7.0, 4°C, 250 ml.
2. 0.45 M KCl, 2 mM Tris-acetate, pH 7.0, 4°C, 100 ml (injection buffer).

Procedure

1. Pellet myosin at 15,000 rpm, 4°C, for 10 min in a SS34 rotor.
2. Soak and resuspend pellet in ~200 ul of buffer 1. Handle gently.
3. Clarify at 25,000 rpm, 4°C, for 20 min in a 42.2Ti rotor.
4. Determine myosin concentration using Lowry assay. Determine dye concentration by diluting conjugate 1/40 and reading OD at 555 nm. Calculate the dye/protein molar ratio.

$$D/P = \{OD_{555} \times 41 / 60,000\} / \{(\text{mg/ml}) / 470,000\}$$

5. For microinjection, dialyze 2 hr against injection Buffer.
 6. Ideal myosin concentration for injection is 7-10 mg/ml. Typical labeling ratio = 4.0-5.0.
 7. Gizzard myosin good for ~2 day. Continue to dialyze against the injection buffer until just before use, then clarify.
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