

LABELING ALPHA-ACTININ WITH IATR

Materials

1. Small vial and stir bar.
2. Bio-Beads SM-2 in 0.7x15 cm column.
3. 2 mM Tris-HCl, pH 8.5, 4°C, 250 ml.
4. Centricon-30 concentrator.
5. 5 mM Tris-acetate, 0.1 mM DTT, pH 6.95, 4°C, 250 ml.
6. 200 mM K-borate, pH 9.0, 10 ml.
7. DTT, 100 mM stock.
8. IATR (tetramethylrhodamine iodoacetamide; Molecular Probes).

Procedure (perform under reduced light)

1. Get 2 mg gizzard alpha-actinin, stored in 2 mM PIPES, 0.02% azide, pH 7.0 in liquid nitrogen. Thaw quickly in warm water and chill in ice.
2. Resuspend about 1 mg IATR in 150 ul acetone; pipet and grind against the side of the test tube until dye forms fine slurry. It is very important at this point that the dye be dissolved as much as possible.
3. Add IATR/acetone slurry dropwise to buffer 6 in a small vial with good stirring. The volume (ml) of borate buffer = mg IATR x 9 / (mg/ml) alpha-actinin.
4. Clarify IATR solution at 25,000 rpm, 4°C, for 10 min in a 42.2Ti rotor.
5. Mix equal volumes of clarified dye and alpha-actinin in a test tube; remove only top part of dye solution from the centrifuge tube with a Pipetman p-200. Pump about 3 times with pipetman to mix, cover with parafilm and foil.
6. Incubate on ice for 2 hr in the dark. Equilibrate SM-2 column with buffer 3.
7. Add DTT to 10 mM. Clarify at 25,000 rpm, 4°C for 20 min in a 42.2Ti rotor.
8. Apply supernatant to SM-2 column. Pool fractions that appear pink in room light.

9. Concentrate with Centricon-30 (concentrate in a SS34 rotor at 6,500 rpm, 4°C for 20 min. Then collect at 2,000 rpm for 3 min).

10. Dialyze overnight against buffer 5 in the cold room.

11. Clarify at 25,000 rpm, 4°C, for 20 min in a 42.2Ti rotor.

12. Determine the concentration of alpha-actinin using Lowry assay. Determine dye/protein molar ratio by diluting conjugate 1:40 (10 ul in 400 ul of injection buffer) and reading OD₅₅₅.

$$D/P = \{OD_{555} \times 41 / 60,000\} / \{(mg/ml) / 100,000\}$$

13. Dilute to 5 mg/ml for microinjection if necessary. Extra conjugated alpha-actinin can be stored as aliquots in liquid N₂.
