Labeling Vinculin with TRITC

Materials

1. 200 mM K-borate, pH 9.0, 10 ml.

2. 100 mM lysine, 100 mM K-borate, pH 9.0, 1 ml aliquots stored at -20^{o}C.

3. 2 mM Tris-HCl, pH 8.5, 4^{o}C, 250 ml.

4. 100 mM DTT stock.

5. Centricon-30 concentrator.

6. 5 mM Tris-acetate, 0.1 mM DTT, pH 6.95, 4^{o}C, 250 ml.

7. TRITC, 10% on celite (Research Organics).

8. Bio-Beads SM-2 in 0.7x15 column.

Procedure (perform under reduced light)

1. Get 1-2 mg vinculin, stored in 2 mM Pipes, 0.02% Azide, pH 7.6, in liquid nitrogen. Thaw quickly in warm water and chill in ice.

2. Mix vinculin solution 1:1 with 200 mM K-borate pH 9.0. Add 1.2 mg TRITC on celite per mg vinculin.

3. Stir on ice for 2 hr in the dark.

4. Pellet celite by centrifuging in a 42.2Ti rotor at 4^{o}C, 25,000 rpm for 10 min.

5. Collect supernatant and add equal volume of 100 mM lysine. Incubate for 2 hr on ice in the dark.

6. Equilibrate SM-2 column with buffer 3.

7. Apply the solution to the SM-2 column. Elute slowly. Pool fractions that appear pink in room light.

8. Measure volume, add DTT to 10 mM.

9. Concentrate to about 100 ul (for 2 mg vinculin) using a Centricon-30 (concentrate in a SS34 rotor at 6,500 rpm, 4^{o}C for about 20 min. Then collect at 2,000 rpm for 3 min).

10. Dialyze overnight against buffer 6 in the cold room.

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

12. Determine the concentration of vinculin using Lowry assay. Determine dye/protein molar ratio by diluting conjugate 1:41 (10 ul in 400 ul of injection buffer) and reading OD555.

D/P = {OD555 x 41 / 60,000} / {(mg/ml) / 130,000}

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