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CONJUGATION OF PROTEIN TO FITC

Reference and nomograph for F:P ratio: Goldman, M. Fluorescent Antibody Methods, Academic Press, 1968, pp. 97-138 (nomograph on p. 125).

Reagents

1. Buffers - Carbonate-Bicarbonate Buffer 0.5M

Solution A: Dissolve 26.5 grams sodium carbonate (M_r of 105.99) in distilled water. Final volume 500 ml volumetrically.

Solution B: Dissolve 21.0 grams sodium bicarbonate (M_r of 84.01) in distilled water. Final volume 500 ml volumetrically.

Mix one part Solution A and eight parts Solution B for buffer used in protein conjugation. The pH of the mixture should be between 9.0 and 9.25.

2. Affinity-purified Ig or purified MAb are conjugated to FITC at pH 9.0 to 9.5. Ig should be dialyzed against 0.1M Cl, 0.1M Na $NaHCO_3$ before use, and extensively away from compounds with amino or sulfhydryl groups which are FITC-reactive.

For tissue fluorescence, isolate IgG, conjugate, separate from free FITC, and then pass conjugate through DEAE and elute at 3 different salt concentrations in order to obtain fractions with different F:P ratios; alternatively instead of repurifying through DEAE, absorb the conjugate with tissue powder.

For membrane fluorescence, DEAE fractionation is unnecessary.

3. FITC (fluorescein isothiocyanate) - store in desiccator in cold room. Keep dry. Allow to warm before opening.

Conjugation

1. Place solutions in small beaker or erlenmeyer at room temperature.

2. Ig should be about 5-10 mg per ml (You can use, with less coupling efficiency, as little as 1 mg or as much as 50 mg per ml).

3. Add 0.18 ml of buffer per every ml IgG; check pH which should be 9.0 to 9.5.

4. FITC should be used at a concentration of 0.05 mg/mg of protein. Weigh FITC, place in 5 or 10 ml beaker; add two-three drops of buffer with Pasteur pipette and add to protein solution. Rinse Pasteur in protein solution several times and wash beaker with it to be sure all FITC particles have been dissolved and carried to protein solution.

5. Mix well. Check pH for first five minutes, and if necessary, adjust with carbonate solution.

6. Place in refrigerator for 18-24 hrs.

7. To remove free FITC, pass through a G-25 Sephadex column (the FITC is retarded relative to FITC-protein both by its smaller size and by adsorption to the Sephadex).

8. Centrifuge at 20,000 rpm x 10 min in order to eliminate any precipitated protein.

9. Calculate F:P ratios. Dilute solution and read in spectrophotometer at 493 mμ and 276 mμ. Calculate ratios on nomograph. Express molar ratios. To convert ratio of μg of FITC per mg of IgG to molar ratios, multiply by 0.41; to do the reverse (molar to weight ratio), multiply by 2.4.

10. Store in small aliquots in -80°C freezer. Microfuge each time before use.