

## E2-41 Preparation of fluorescent labelled protein for immunoassays

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Immunoassay is a very common bio-analytical technique which can be used to quantify trace amounts of biologically important substances. The aim of this study is to develop a simple, convenient technique to attach a fluorochrome to a protein.

To avoid the direct reaction between the fluorochrome and the protein, a solution of fluorochrome (FITC-fluorescein isothiocyanate) is kept in a semipermeable sac dipped in a protein solution (BSA) and stirred at a constant rate. The rate of migration of the FITC is monitored using fluorescence spectroscopy. The molecular weight cut off of the membrane is approximately 12,000 - 14,000 and migration is only permitted to FITC from the sac to the solution. Two factors have been studied to optimize the conditions for labeling of proteins; conjugation time and effect of the concentrations of reactants.

The average number of FITC labels per BSA molecule was predicted using absorption and fluorescence data. Absorption spectra of FITC show a prominent peak at 490 nm and several peaks in the UV region, BSA has maximum absorption at 280 nm. The fluorochrome, FITC can be estimated at  $\lambda_{ex}=490$  nm and  $\lambda_{em} = 525$  nm. Absorption data can be used for fairly higher concentration of FITC and BSA. F/P (fluorochrome to protein) ratio is calculated on the basis of the absorption values 280 nm for protein, and 490 nm for FITC.

Semipermeable membranes can be used to control the reaction rate. Therefore, well characterised fluorescence labelled proteins (lower fluorochrome to protein ratio) can be obtained within 1 h dialysis time when equal concentration of BSA and FITC are employed.

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