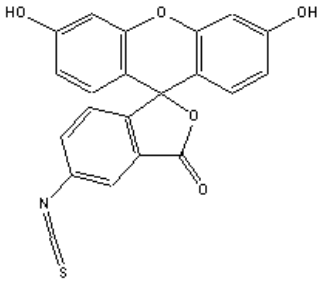
Catalog Number: 100276

Fluorescein Isothiocyanate

Structure:



Molecular Formula: $C_{21}H_{11}NO_5S$

Molecular Weight: 389.4

CAS #: 3326-32-7

Form: Isomer I

Synonyms: Fluorescein-5-Isothiocyanate; FITC

Fluorescence: Conjugated FITC has an excitation maximum of 492 nm. It fluoresces with an apple-green light with maximum emission at 515 nm. Extinction Coefficient: 75,000.

Solubility: Soluble in DMF, DMSO (5 mg/ml), acetone (1 mg/ml - clear, yellow solution), ethanol (20 mg/ml) and in aqueous solution pH > 6. Do not store FITC in aqueous media because it is unstable in water. Keeps solutions aliquoted and stored at -20°C and protected from light. Dilute in aqueous buffers immediately before use.

Description: A amine-reactive fluorescein derivative suitable for protein labeling. Attaches to the protein via an amine group.

Adsorbing FITC onto celite has been reported to increase the efficiency of dispersing FITC in a protein solution.[11] FITC on celite reportedly reacts very quickly with proteins,[11] so much faster that antibody titer may be lost by overacylation of the free amino groups. The FITC on celite does permit weighing manageable quantities when working with small amounts of protein and avoids the use of organic solvents. FITC on celite has been used for the labeling of fibrinogen.[14]
Note: FITC contributes partial negative charges at normal pH. Antibodies and fragments over-conjugated with FITC can have net negative charges which result in high background fluorescence because of nonspecific bindings to positive surfaces.

Typical Procedure for Labelling Protein with FITC[5,6]

1. Prepare a solution of at least 2 mg/ml of protein in 0.1 M sodium carbonate buffer, pH 9. Do not store sodium bicarbonate buffer more than 1 week at 2-8°C. The pH of the buffer may change upon storage. It is advised that fresh buffer be made just before use. The protein to be conjugated should be free of contaminating proteins, and protein solutions should not be prepared in buffers containing sodium azide or amines such as Tris or glycine since they inhibit the labeling reaction. If the buffer contains amines or sodium azide, dialyze protein solution against PBS, pH 7.4, overnight at 2-8°C. Avoid dialysis at high pH values (> 8.0) as this may be harmful to some proteins.

2. Dissolve the FITC in anhydrous DMSO at 1 mg/ml. For optimal results, this should be prepared fresh for each labeling reaction.

3. For each 1 ml of protein solution, add 50 ul of FITC solution, very slowly in 5 ul aliquots while gently and continuously stirring the protein solution.

4. After all of the required amount of FITC solution has been added, incubate the reaction in the dark for 8 hours at 2-8°C.

5. Add NH_4Cl to a final concentration of 50 mM and incubate for 2 hours at 2-8°C.

6. Add xylene cyanol to 0.1% concentration and glycerol to 5% concentration.

7. Separate the unbound FITC from the conjugate by gel filtration using a fine-sized gel matrix with an exclusion limit of 20,000 to 50,000 (for globular proteins such as antibodies). With the column flow stopped, carefully layer the reaction mixture onto the top of the column. Then open the column, allowing the reaction mixture to flow into the column. Just as it all enters the column bed, carefully add PBS to the top of the column and connect to a buffer supply.

Two bands will form on the column. The faster moving band, which is the conjugated protein, elutes first and can usually be seen

under room light. The slower moving band is the unreacted (free) FITC and xylene cyanol and will elute only with subsequent PBS washes.

8. Store the conjugate at 2-8°C in the column buffer in a light-proof container. Sodium azide may be added as a preservative (final concentration 0.05%). If the protein concentration is loq (< 1 mg/ml), bovine serum albumin may be added to a final concentration of 1% w/v.

9. The ratio of fluorescein to protein can be estimated by measuring the absorbance at 495 nm and 280 nm. The final F/P ratio should be between 0.3 and 1.0. Lower ratios will yield low signals; higher ratios will give high background.

If the ratios are too low, repeat the conjugation using lower levels of antibody and higher levels of FITC. If higher levels are found , either repeat the labeling with appropriate changes or purify the labeled protein further on a DEAE column. Equilibrate and load the column with 10 mM potassium phosphate (pH 8.0). Elute with increasing salt concentrations. Measure the ratios (495/280) of each fraction and select and pool the appropriate fractions.

Calculating the Fluorescein/Protein Molar Ratio

The F/P molar ratio is defined as the ratio of moles of FITC to moles of protein in the conjugate. To determine this ratio, it is necessary to first determine the absorbance of the conjugate sample at 280 nm and then at 495 nm.

Place the conjugate sample in a quartz cuvette. Read the absorbance of the conjugate sample at 280 nm and 495 nm. The absorbance reading of the conjugate sample should be between 0.2 and 1.4 at 280 nm. If the absorbance reading is outside this range, adjust the sample dilution accordingly.

For FITC-IgG conjugates:

From the absorbance readings (A_{280} and A_{495}) of the conjugate sample, calculate the F/P ratio of the conjugate according to the equation:

$$\text{Molar F/P} = \frac{2.77 \times A_{495}}{A_{280} - (0.35 \times A_{495})}$$

The protein concentration of the FITC-IgG conjugate is calculated from the following formula:

$$\text{IgG (mg/ml)} = \frac{A_{280} - (0.35 \times A_{495})}{1.4}$$

Where 1.4 is the A_{280} of IgG from most species at a concentration of 1.0 mg/ml at pH 7.0.

For Other FITC-Protein conjugates:

When any protein other than IgG is conjugated to FITC, use the general formula below, substituting the appropriate values for the particular protein:

$$\text{Molar F/P} = \frac{MW}{389} \quad X \quad \frac{A_{495}/195}{A_{280} - [(0.35 \times A_{495})]/E^{0.1\%}} = \frac{A_{495} \times C}{A_{280} - (0.35 \times A_{495})}$$

$$\text{Where: } C = \frac{MW \times E^{0.1\%}_{280}}{389 \times 195}$$

C is a constant value given for a protein.
MW is the molecular weight of the protein.
389 is the molecular weight of FITC.
195 is the absorption $E^{0.1\%}$ of bound FITC at 490 nm at pH 13.0
(0.35 X A_{495}) is the correction factor due to the absorbance of FITC at 280 nm.[11]
$E^{0.1\%}$ is the absorption at 280 nm of a protein at 1.0 mg/ml.

Availability:

Catalog Number	Description	Size
100276	Fluorescein Isothiocyanate, Isomer I	100 mg 250 mg 500 mg 1 g

Also Available:

Catalog Number	Description	Size
101713	Fluorescein Isothiocyanate On Celite (10%)	250 mg 1 g
191368	Fluorescein Isothiocyanate-Avidin	1 mg 5 mg 10 mg
191294	Fluorescein Isothiocyanate- Casein-Agarose	5 ml
158062	Fluorescein Isothiocyanate-Dextran, Average MW 4000, MW:Number Av. Mol. Wt. not more than 1.5; degree of substitution 0.003-0.020 mole FITC per mole of glucose	100 mg 250 mg
158063	Fluorescein Isothiocyanate-Dextran, Average MW 20000; degree of substitution 0.003-0.020 mole FITC per mole of glucose	100 mg 250 mg
158064	Fluorescein Isothiocyanate-Dextran, Average MW 40000, MW:Number Av. Mol. Wt. not more than 1.25; degree of substitution 0.003-0.020 mole FITC per mole of glucose	100 mg 250 mg 1 g
158065	Fluorescein Isothiocyanate-Dextran, Average MW 70000, MW:Number Av. Mol. Wt. not more than 1.25; degree of substitution 0.003-0.020 mole FITC per mole of glucose	100 mg 250 mg
158066	Fluorescein Isothiocyanate-Dextran, Average MW 150000, MW:Number Av. Mol. Wt. not more than 1.35; degree of substitution 0.003-0.020 mole FITC per mole of glucose	100 mg 250 mg 1 g
191393	Fluorescein Isothiocyanate- Streptavidin, supplied in PBS at approximately 0.5 mg/ml	0.25 ml 1 ml
797061	Protein A- Fluorescein Isothiocyanate	1 ml
55881	Protein A- Fluorescein Isothiocyanate, supplied in PBS	2 mg
622801	Protein A- Fluorescein Isothiocyanate	5 mg
154095	Protein A- Fluorescein Isothiocyanate-Colloidal Gold, 5 nm	0.25 ml 0.50 ml 1 ml
191418	Dimethyl Sulfoxide, ACS Reagent Grade	100 ml 500 ml 1 liter
191406	Ammonium Chloride, ACS Reagent Grade	500 g 1 kg 5 kg
806801 806803	Xylene Cyanole F.F	10 g 25 g
800687 800688 800689	Glycerol, ultra pure	500 ml 1 liter 4 liters
195588	Sephadex G-75, Globular Proteins 3000-80,000, dextrans 1000-50,000	10 g 50 g 100 g
195581	Sephadex G-50, fine, globular proteins 1500-30,000, dextrans 500-10,000	10 g 50 g 100 g
102891	Sodium Azide	25 g 100 g 500 g
103700	Bovine Serum Albumin	1 g 5 g 10 g

		25 g 100 g
158394	*Antibodies: A laboratory manual*, by Ed Harlow and David Lane (1988)	1 book

FITC conjugated antibodies also available.

References:

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5. Goding, J.W., *J. Immunol. Methods*, **v. 13**, 215-226 (1976).
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