3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

FLUORESCEIN ISOTHIOCYANATE

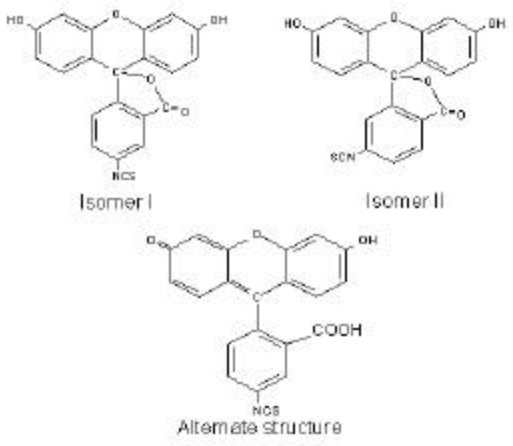
Product Numbers:
F4274, Isomer I
F7250, Isomer I
F1628, Isomer I on Celite
F4002, Isomer II
F3651, Mixed Isomers

Storage Temperature 2-8 °C

Synonym: FITC

CAS #: 3326-32-7

Product Description



Isomer I has the thiocyanate group on the 4 carbon of the benzene ring, whereas isomer II has the thiocyanate on the 5 carbon. The two isomers are

Appearance: powder
Molecular Formula: $C_{21}H_{11}NO_5S$
Molecular Weight: 389.4
Excitation: λmax = 495 nm
Emission: λmax = 525 nm
The full absorbance spectrum has been reported.[1]

Fluorescein isothiocyanate (FITC) is widely used to attach a fluorescent label to proteins via the amine group. The isothiocyanate group reacts with amino terminal and primary amines in proteins. It has been used for the labeling of proteins including antibodies and lectins.[2-6]

indistinguishable spectrally, either by wavelength or intensity. Isomer I is more easily isolated in pure form, so is less expensive. This may explain why isomer I is more commonly used for labeling. For many purposes, however, the mixed isomers of FITC will be perfectly suitable.[7] The geometry of binding to biomolecules and resulting properties related to elution under electrophoretic or HPLC conditions may, however, require the use of a single pure isomer. The coupling procedure is straightforward experimentally, and the reaction is rapid. A method for determining the degree of substitution is reported.[2] A similar method is described in the Sigma bulletin for the FluoroTag™ FITC conjugation kit, FITC-1.

Adsorbing FITC onto Celite (diatomaceous earth), offered as product F1628, has been reported to increase the efficiency of dispersing FITC in a protein solution.[8] FITC on Celite (isomer I) reportedly reacts very quickly with proteins,[8] so much faster that antibody titer may be lost by overacylation of the free amino groups. Sigma has not confirmed this claim; however, 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.[9]

Preparation Instructions

FITC is tested for solubility and solution appearance at 1 mg/ml in acetone. It is soluble in anhydrous dimethyl sulfoxide (DMSO) at 5 mg/ml.[10] It is soluble in water at less than 0.1 mg/ml in water, at 20 mg/ml in ethanol and at 9 mg/ml in 2-methoxyethanol.[1] An organic solvent for stock solution is advised, since FITC decomposes in water. FITC is diluted in basic buffer for coupling procedures immediately prior to use.[11]

Storage/Stability

The products are light-sensitive, and should be stored dry and in the dark at 2 °C to 8 °C.

Procedure

Labeling of Protein with FITC[3, 5, 6]

1. Prepare a solution of at least 2 mg/ml of protein in 0.1 M sodium carbonate buffer, pH 9.
   Notes: Do not store sodium carbonate-bicarbonate buffer more than 1 week at 0-5 °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 0 - 5 °C. Avoid dialysis at high pH values (> 8.0-8.5) as this may be harmful to some proteins.
2. Dissolve the FITC in anhydrous DMSO at 1 mg/ml. **Note:** This should be prepared fresh for each labeling reaction.
3. For each 1 ml of protein solution, add 50 µl of FITC solution, very slowly in 5 µl aliquots while gently and continuously stirring the protein solution.
4. After all the required amount of FITC solution has been added, incubate the reaction in the dark for 8 hours at 4 °C.
5. Add NH_4Cl to a final concentration of 50 mM and incubate for 2 hours at 4 °C.
6. Add xylene cyanol to 0.1% and glycerol to 5%.
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 4 °C in the column buffer in a light-proof container. Sodium azide may be added as a preservative (final concentration 15 mM). If the protein concentration is low (< 1 mg/ml), bovine serum albumin (BSA) may be added to a final concentration of 1%.
9. The ratio of fluorescein to protein of the product can be estimated by measuring the absorbance at 495 nm and 280 nm. The F/P ratio should be between 0.3 and 1.0. Lower ratios will yield low signals; higher ratios will give high background.

Determination of Fluorescein/Protein Molar Ration (F/P)

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

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

The protein concentration of the fluorescein-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:

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

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.[8]
$E^{0.1\%}$ is the absorption at 280 nm of a protein at 1.0 mg/ml.

F1628 is approximately 10 % FITC (by weight) on Celite
Celite is a trademark of Manville Service Corp.

References

1. Green, F.J., Sigma-Aldrich Handbook of Stains, Dyes and Indicators, p. 377 (1990).
2. Schreiber, A.B., and Haimovich, J., Methods Enzymol., **93,** 147-155 (1983).
3. Harlow, E. and Lane, D., Antibodies a Laboratory Manual, pp. 353-355 (Cold Spring Harbor Laboratory, 1988).
4. Bridges, C.D. and Fong, S.L., Methods Enzymol., **81,** 65-77 (1982).
5. The, T.H., and Feltkamp, T.E.W., Immunology, **18,** 865-873 and 875-881 (1970).
6. Goding, J.W., J. Immunol. Methods, **13,** 215-226 (1976).
7. NCCLS Approved Standard:ASM-1, 2nd Ed. (June 1975). Standard test for labeling efficiency of FITC with BSA (albumin).
8. Rinderknecht, H., Experientia, **16,** 430 (1960).
9. Xia, Z., et al., Br. J. Haematol., **93,** 204-214 (1996).
10. Current Protocols in Immunol., Coligan, J.E. et al. (Eds.), pp. 5.3.3 (Wiley & Sons).
11. McKinney, R.M., et al., Anal. Biochem., **14,** 421-428 (1966).

AJH 8/08