

Description	Fluorescein isothiocyanate isomer 1
Product code	FITC-1
Molecular weight	389.39
Cas-no.	3326-32-7
Formula	C ₂₁ H ₁₇ NO ₅ S
Synthesis	Aminofluorescein is obtained from nitrofluorescein by catalytic hydrogenation and is then treated with thiophosgene to obtain the isothiocyanate. Two isomers are formed which can be separated by chromatography. The purified isomer-1 is a bright yellow powder; it is more fluorescent than isomer-2. In solution according to pH, different tautomers and ionic forms of FITC are possible. The pure crystalline isomer-1 is superior to other grades for the labeling of proteins. It is included in the list of dyes certified by the U.S. Biological Stain Commission.
Purity	The purity of nitrofluorescein, aminofluorescein and the FITC are checked by thin layer chromatography. Isothiocyanate content may be measured by infrared absorption.
Optical characteristics	The main absorption peak of FITC is at 490 nm and its greenish-yellow fluorescence emission at 520 nm. Between 350 and 420 nm absorption is very low, but there are two small peaks at 280 and 325 nm. The absorption at 280 nm results in a relatively low intensity fluorescence emission at approximately 355 nm. The value for the fluorescence intensity is determined by excitation intensity, absorption and quantum yield. Evidence is available for the constancy of the fluorescence efficiency as a function of wavelength up to the main absorption wavelength, above which it decreases rapidly. This is of practical importance when selecting the best exciting wavelength, since technical considerations sometimes prevent the use of the primary exciting band in microscopy, making it necessary to use wavelengths below 400 nm.
Conjugation characteristics	Proteins contain different groups which can react with the FITC. Free amino and carboxyl groups at the ends of each protein chain may be involved under weakly alkaline conditions, in addition to many other free amino groups in the lysine side chains and the free carboxyl groups in the aspartic and glutamic acid residues. When conjugating different proteins, different amino acids may be involved in the process. When conjugating FITC to IgG-class antibodies, the epsilon-amino group of lysine is the most probable site of reaction, followed by the terminal amino groups at a relatively higher degree of conjugation. The degree of conjugation can be varied by using different ratios of FITC to protein in the reaction mixture (1:100 to 1:50) in the reaction mixture. The total amount of protein to be conjugated may also influence the optimal amount of the fluorochrome required in the coupling process. However, the fluorescence intensity of a labelled protein does not vary linearly with the degree of conjugation. It reaches a maximum at a relatively low degree of conjugation. The lowest degree of conjugation which gives maximum fluorescence is to be preferred because it will cause least changes in the physical and biological properties of the native protein. This optimal degree of conjugation of FITC-conjugated goat and rabbit IgG was found to be at a molecular TRITC-protein (F/P) ratio of approximately 1.5 to 2. At a molecular ratio of 2.8 to 3.6, the fluorescence intensity was only 20% of that of an equivalent amount of FITC. The reduction in fluorescence efficiency with the increasing degree of conjugation may be due to increased interaction between the protein and the FITC molecules. These observations have been confirmed by immunohistological findings which can be partly explained by a reduction of antibody affinity with increasing F/P ratios and an increase in non-specific staining as a result of the elevated net-charge of the FITC-protein complex. Protein solutions intended for conjugation should not contain sodium azide as a preservative. If it is present it should be removed prior to conjugation because it interferes with the coupling of the fluorochrome.
Intended use	FITC is the fluorochrome of choice to prepare histological protein and antibody conjugates for use in fluorescence microscopy. Stable covalent bonds are obtained without destroying the fluorescent structure. Unreacted material can easily be removed by dialysis or gel filtration. Fluorescence efficiency is high and is only slightly affected on conjugation to proteins. It allows unambiguous histological and cytological interpretation because of good colour contrast with the tissue background, even offsetting reduced fluorescence under some conditions. FITC-antibody conjugates are stable under normal storage conditions, while immunological properties of antigens and antibodies are normally preserved. FITC-antibody conjugates are non-toxic and the fluorochrome does not add significantly to the antigenicity of the complex. Simple and rapid conjugation procedures which are relatively insensitive to experimental variables have been described.
Packing	Vial with 100 mg.
Storage / shelf life	Do not breathe dust, avoid contact with skin and eyes. FITC isomer-1 should be stored in the dark in a desiccator at -20°C and is stable for at least 3 years. Before opening the vial it should be allowed to come to ambient temperature to avoid condensation of moisture.
Caution	This product should be handled only by qualified persons and appropriate precautions should be taken in its handling and disposal, and of all associated materials. For <i>in vitro</i> laboratory research purposes only.

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