

Description	Tetramethylrhodamine isothiocyanate isomer R
Product code	TRITC-R
Molecular weight	443.53
Cas-no.	107347-53-5
Formula	C ₂₅ H ₂₁ N ₃ O ₃ S
Synthesis	A proprietary procedure is used for the synthesis of crystalline isomer R. TRITC is analogous to fluorescein isothiocyanate with two dimethylamino groups replacing the two hydroxyls. Two forms are mentioned in the literature, amorphous and crystalline isomer-R.
Purity	The isothiocyanate content of TRITC can be measured by infrared absorption. The purity is checked by thin layer chromatography. Experiences using protein conjugates prepared from different forms of TRITC vary, partly due to variations in the purity of the dye. Racemic TRITC is less efficient as compared to the crystalline isomer R. Solutions of TRITC are not stable and should be used immediately after preparation.
Optical characteristics	Crystalline isomer R in PBS (pH 7.1) has a single absorption maximum at approximately 555 nm with a maximum orange-red fluorescence emission a wavelength 620 nm There is an indication of a minor peak at approximately 515 nm. The intensity of the fluorescence emission is determined by excitation intensity, absorption and quantum yield. Fluorescence efficiency as a function of wavelength is quite constant up to the main absorption wavelength, but decreases rapidly above it. Technical considerations sometimes prevent the use of the primary excitation in microscopy, making it necessary to use a lower wavelength. There is usually a small shift in the wavelength of absorption of the conjugated fluorochrome.
Conjugation characteristics	Protein contain different groups which can react with the TRITC. 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. When conjugating different proteins, different amino acids may be involved in the process. When conjugating TRITC 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 TRITC to protein in the reaction mixture (1:40 to 1:20). The total amount of protein to be conjugated may also influence the optimal amount of the Fluorochrome required in the coupling process. TRITC is not readily soluble in water. After dissolving it in a small volume of dimethylformamide or dimethylsulphoxide it can be diluted in the carbonate/bicarbonate buffer (0.5 M, pH 9.0) . It is also soluble in acetone. To prevent precipitation, the acetone solution should be added with caution to the protein solution at 0° to 2°C, with a final volume of acetone not exceeding 1/30 of the volume of the protein solution. 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 TRITC-conjugated goat and rabbit IgG was found to be at a molecular TRITC-protein (F/P) ratio of approximately 2. At a molecular ratio above 2.5, the fluorescence intensity is rapidly reduced. This may be due o a reduction in antibody affinity with the increasing F/P ratios and an increase in non-specific staining as a result of he elevated net-charge of the 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. The TRITC is readily soluble in dimethylformamide, dimethylsulphoxide and in 0.05 M carbonate-bicarbonate buffer pH 9.6.
Intended use	Together with FITC, TRITC is still widely used in fluorescence microscopy. Stable covalent bonds are obtained without destroying the fluorescents the fluorescent structure Unreacted material can easily be removed by dialysis or gel filtration. Fluorescence efficiency is only slightly effected on conjugation to proteins. TRITC-antibody conjugates are stable under normal storage conditions, while immunological properties of antigens and antibodies remain reserved. TRITC, especially the racemic form, may have cytotoxic properties. TRITC-R has been tested for the absence of toxicity. Although its fluorescence efficiency is less than that of FITC, TRITC has the additional advantage of significantly less photo decomposition during excitation which makes it more suitable for cytochemical techniques requiring prolonged microscopic observation. If non-specific staining in histochemistry or cytochemistry is troublesome, a lower F/P ratio may be advisable. This is achieved by reducing the amount of the fluorochrome.
Packing	Vial with 10 mg.
Storage / shelf life	Do not breathe dust, avoid contact with skin and eyes. TRITC isomer-R should be stored in the dark in a desiccator at -20°C. 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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