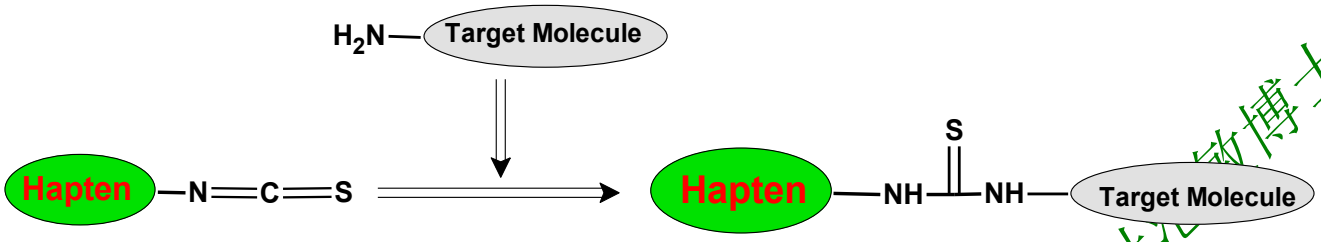
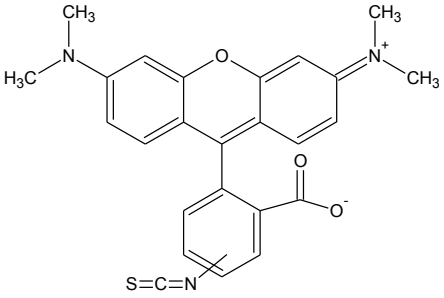
Fluorescent Dye Isothiocyanates



Isothiocyanates form thioureas upon reaction with amines. It is proven that some thiourea products (in particular, the conjugates from α-amino acids/peptides/proteins) are much less stable than the conjugates that are prepared from the corresponding succinimidyl esters. It has been reported that antibody conjugates prepared from fluorescein isothiocyanates deteriorate over time. We strongly recommend that you use succinimidy esters for your conjugations whenever possible. There are few factors that need be considered when SE compounds are used for conjugation reaction: 1). Solvents: For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO). 2). Reaction pH: The labeling reactions of amines with isothiocyanates are strongly pH dependent. Isothiocynate reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Protein modifications by isothiocyanates may require a pH 9.0-10.0 for optimal conjugations. 3). Reaction Buffers: Buffers that contain free amines such as Tris and glycine must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed before performing dye conjugations. High concentrations of nucleophilic thiol compounds should also be avoided because they may react with the labeling reagent to form unstable intermediates that could destroy the reactive dye. 4). Reaction Temperature: Isothiocynate conjugations are usually done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

5(6)-TRITC [Tetramethylrhodamine-5-(and-6)-isothiocyanate]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
410	10 mg	$145	443.52	543 nm	571 nm	DMF or DMSO	-20 °C and desiccated



Features and Biological Applications

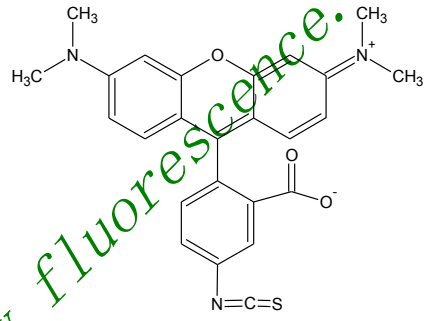
5(6)-TRITC is an amino-reactive labeling reagent that is widely used in preparing bioconjugates of proteins and nucleic acids. The resultant conjugates have similar spectral properties to those prepared from 5(6)-TAMRA, SE. However, the latter conjugates are much more stable. *Cautions must be exercised for the storage of FITC conjugates.*

References

1. Pellestor, F., *et al.*, Fast multicolor primed in situ protocol for chromosome identification in isolated cells may be used for human oocytes and polar bodies. *Fertil Steril* 2004, **81**, 408-15.
2. Gustafsson, M.K., *et al.*, No nerves and their targets in a tapeworm: An immunocytochemical study of cgmp in hymenolepis diminuta. *Parasitol Res* 2003, **90**, 148-52.
3. Takeno, S., *et al.*, Increased nitric oxide production in nasal epithelial cells from allergic patients--rt-pcr analysis and direct imaging by a fluorescence indicator: Daf-2 da. *Clin Exp Allergy* 2001, **31**, 881-8.
4. Meadows, D.L., *et al.*, Determining the extent of labeling for tetramethylrhodamine protein conjugates. *J Immunol Methods* 1991, **143**, 263-72.

5-TRITC [Tetramethylrhodamine-5-isothiocyanate]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
415	5 mg	$145	443.52	543 nm	571 nm	DMF or DMSO	-20 °C and desiccated



Features and Biological Applications

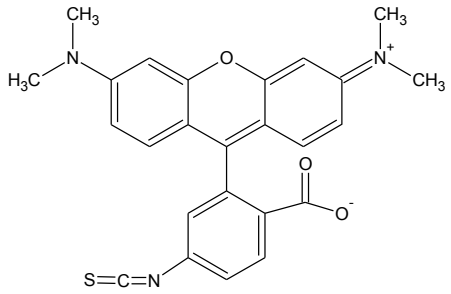
5-TRITC (also called G isomer) is a purified single isomer of the 5(6)-TRITC mixed isomers. This labeling reagent is predominantly used in labeling peptides and proteins. *Cautions must be exercised for the storage of TRITC conjugates.*

References

1. Pellestor, F., *et al.*, Fast multicolor primed in situ protocol for chromosome identification in isolated cells may be used for human oocytes and polar bodies. *Fertil Steril* 2004, **81**, 408-15.
2. Takeno, S., *et al.*, Increased nitric oxide production in nasal epithelial cells from allergic patients--rt-pcr analysis and direct imaging by a fluorescence indicator: Daf-2 da. *Clin Exp Allergy* 2001, **31**, 881-8.
3. Newkirk RF and Mack J (1992). Improved indirect fluorescence immunocytochemical method using counter stains. *Biotechniques* **13,** 536-8.

6-TRITC; R isomer [Tetramethylrhodamine-6-isothiocyanate]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
417	5 mg	$145	443.52	544 nm	572 nm	DMF or DMSO	-20°C and desiccated



Features and Biological Applications

6-TRITC (also called R isomer) is the other isomer of the TRITC labeling reagent that is widely used in preparing bioconjugates of proteins and nucleic acids. Complimentary to 5-TRITC, the 6-isomer is predominantly used in labeling nucleotides and nucleic acids. *Cautions must b exercised for the storage of TRITC conjugates.*

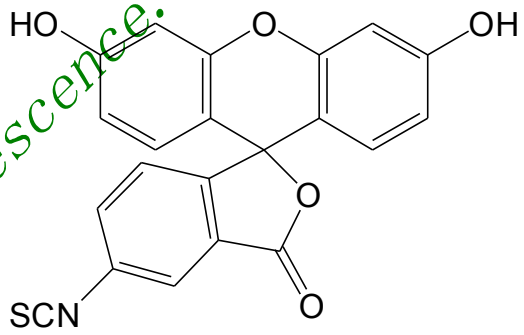
References

1. Kahn E, *et al.* (1999). Confocal-multilabeling, ultrasensitive TUNEL analysis of DNA breaks in individual cells. *Anal Quant Cytol Histol* **21,** 1-7.
2. Nederlof PM, *et al.* (1992). Fluorescence ratio measurements of double-labeled probes for multiple in situ hybridization by digital imaging microscopy. *Cytometry* **13**, 839-45.
3. Meadows, D.L., *et al.*, Determining the extent of labeling for tetramethylrhodamine protein conjugates. *J Immunol Methods* 1991, **143**, 263-72.

FITC

中文名：异硫氰酸荧光素； **英文名**：Flourescein iso-thiocyanate； CAS#：3326-32-7

分子式：$C_{21}H_{11}NO_5S$；分子量：389.38；储存条件：-20℃干燥避光保存，有效期一年。



结构式：

性质：

1. 外观：黄色粉末
2. 纯度：≥95% (HPLC)

3. 产品描述：

FITC 能和各种抗体蛋白结合，结合后的抗体不丧失与一定抗原结合的特异性，并在碱性溶液中具有强烈的黄绿色荧光。通过在荧光显微镜下观察或流式细胞仪分析可对相应抗原进行定性、定位或定量的检测。用于医学，农学和畜牧等方面，可对由地细菌病毒和寄生虫等所致疾病进行快速诊断。

4. FITC 标记抗体流程：

(1) 将待交联的蛋白（浓度≥1mg/mL）对交联反应液透析三次 4℃，至 pH＝9.0。交联反应液配制方法：7.56g NaHCO3，1.06g Na2CO3，7.36g NaCl，加水定容至 1 L。

(2) 将 FITC 溶于 DMSO 中，浓度为 1mg/mL。每次交联使用的 FITC 均应新鲜配制，避光。

(3) 按 P:F（蛋白质:FITC）=1mg:150μg 的比例将 FITC 缓慢加入于抗体溶液中，边加边轻轻晃动使其与抗体混合均匀，暗处 4℃反应 8h。

(4) 加入 5mol/L 的 NH4Cl 至终浓度 50mmol/L，4℃终止反应 2h。

(5) 将交联物在 PBS 中透析四次以上，至透析液清亮。

(6) 交联物的鉴定

蛋白浓度(mg/mL) = [A280－ 0.31×A495] / 1.4

F/P 比例: 3.1×A495 / [A280 － 0.31×A495]，该值应介于 2.5~6.5 之间。

(7) FITC 交联的蛋白应置于 pH 7.4 的磷酸盐缓冲液中，加入 0.1% NaN3、1% BSA，4℃避光保存。