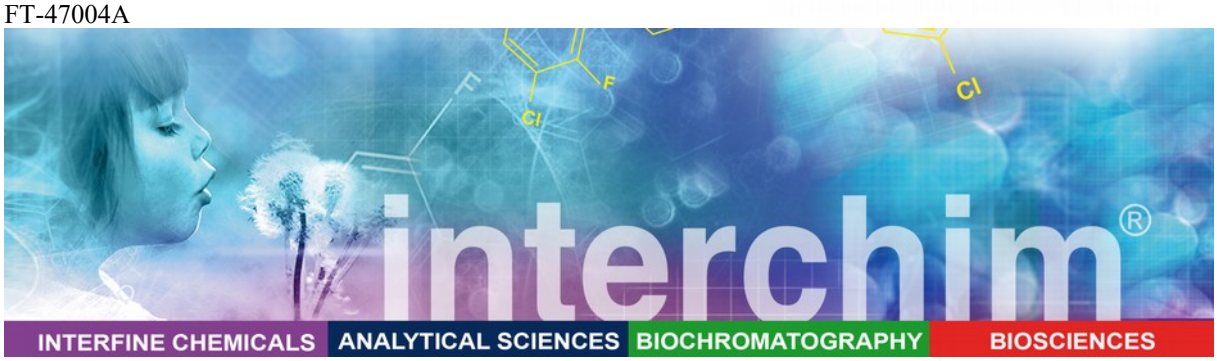
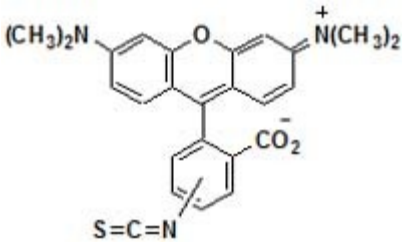
FT-47004A



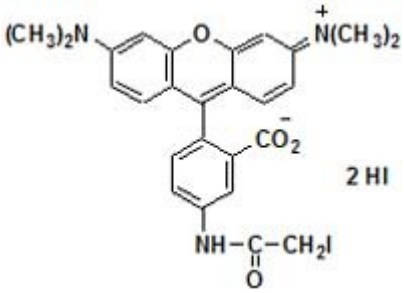
TetraMethylRhodamine (TRITC,TMRIA)

Product Description

Name :	**TRITC** Tetramethylrhodamine-5(6)-isothiocyanate Xanthylium, 9-(2-carboxyisothiocyanatophenyl)-3,6-bis(dimethylamino) -, inner salt
Catalog Number :	FP-47004A, 10mg FP-06276A, 5mg FP-17503A, 5mg
Structure :	$C_{22}H_{21}N_3O_3S$; **MW=825.22**
Soluble:	in DMSO, DMF and CH_3OH
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (MeOH)= / nm $\lambda_{exc}\backslash\lambda_{em}$ (CH_3OH)= 543 / 571 nm
Reactivity:	Amines
Extinction Coefficient :	ε =87 000 $M^{-1}cm^{-1}$
Storage:	-20°C (M)



Name :	**5-TMRIA** Tetramethylrhodamine-5-iodoacetamide dihydroiodide Xanthylium, 9-[2-carboxy-4-[(iodoacetyl)amino]phenyl]-3,6-bis(dimethylamino) -, dihyroiodide salt
Catalog Number :	FP-96468A, 5 mg
Structure :	$C_{26}H_{26}I_3N_3O_4$; **MW= 825.22**
Soluble:	in DMSO, DMF and CH_3OH
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (MeOH)= 560 / 580 nm $\lambda_{exc}\backslash\lambda_{em}$ (CH_3OH)= 543 / 567 nm
Reactivity:	Thiols
Extinction Coefficient :	ε =87 000 $M^{-1}cm^{-1}$
Storage:	-20°C (M)



Introduction

TetraMethylRhodamine (TMR) has been largely used for protein labeling in immunochemistry, for sensitive aminoacid derivatization in CE. Its carboxilic acid form (TAMRA, see below) remains prominent for oligonucleotide labeling (DNA sequencing).The absorption and emission in pH 8 buffer are red-shifted approximately 8 nm compared to MeOH, with EC ~10% lower. λabs./λem. : 543/571nm
The absorption spectrum of TMR/TRITC-labeled proteins is frequently dependent on the labeling location and on the degree of substitution, and may even show splitting into two absorption peaks at about 520 and 550 nm. Such limitation can be addressed using alternative dyes: When the fluorescence quenching by protein of the labeling dye is a serious problem, try the carboxylated version with extended spacer version TAMRA-X-SE (FP-

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33406A), or our excellent FluoProbes®547H (# FP-1H0930 or NHS ester) that has brighter and more stable fluorescence.

TMR is available with several derivatives for labeling various chemical targets by standard chemistry:

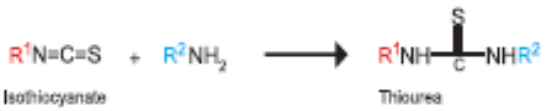
Reactive group	Targetted chemicals group
Isothiocyate	Amines (lower specificity)
NHS (SE)	Amines
IodoAcetamide	Sulfhydryls

Directions for use

TRITC

TRITC is a popular red fluorescent agent, even the carboxylated and succinimidyl derivatives may be preferable for labeling applications, less sensitive fluorescence and higher amine specificity.

- The **Isothiocyanates (R–NCS)** group , which are moderately reactive but quite stable in water and most solvents, form thioureas upon reaction with amines.



This has made isothiocyanates products convenient labeling agents for proteins. However,
-although the thiourea product is reasonably stable, it has been reported that it can deteriorate over time (succinimidyl esters are preferable to that point). The thiourea is also susceptible to conversion to a guanidine by concentrated ammonia (Banks 1995).
-water competes in reaction
-additionnaly, IsothioCyanates gives reactions, generally not stable,with other frous, uncluding tyrosyls, carbonyls, sulfhydryls., and heavy metals (Fe3+; Cu+; Ag+). Succinimidyl esters are again preferable to that specificity point.
Now, isothiocyanates like FITC remains widely used, and in some applications. For example, they can modify aromatic amines.

Protocols may found in the literature.

References - TRITC

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TMRIA

TMRIA, thiol-selective reactive dyes, is used to label proteins via the cystein residues.
Protein structural studies (1), protein-protein (2,5) and protein-DNA interactions are the main applications.
The pure 5-isomer of TMRIA has been reported to predominantly label better than the isomer 6 [r], so it is preferred for some particular applications when the mixed isomers of TMRIA may give different results from batch to batch due to the varying ratios and different reactivities of the two isomers.

- The iodoacetyl group is among the most frequently used reagents for thiol modification. However, the specificity of reaction may be insufficiently specific, and Maleimide or MTS reaction then preferred. They react with sulfhydryls by nucleophilic substitution. In most proteins, the site of reaction is at cysteine residues that either are intrinsically present or result from reduction of cystines. In addition, methionines can sometimes react with haloalkyl reagents.

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The reaction with the free sulfhydryl proceeds by nucleophilic substitution of iodine with a thiol group, forming a stable thioether linkage. The reaction can occur with other groups, but the specificity of reaction with sulfhydryls can be driven using slight stoichiometric excess of iodoacetyl groups over the present number of free sulfhydryls, and by keeping a pH 7.5 and 8.5 (optimally at pH8.3) for the reaction.
If there is a gross excess of iodoacetyl group over the number of free sulfhydryls (or absence of free sulfhydryls), the iodoacetyl group can cross-react with amino acids. I.e. reaction occurs with Imidazoles at pH 6.9-7.0, for over a week [r]. Histidyl side chains and amino groups (unprotonated form) react with iodoacetyl groups above pH 5 and pH 7, respectively [r].
The iodoacetyl group is much more stable to hydrolysis as compared to the ester, so this reaction is usually performed in second step for conjugations in aqueous buffers. Reducing agents containing buffers should be precluded (i.e. DTT, mercaptoethylamine).
Finally, Iodoacetamides in solution undergo rapid photodecomposition to unreactive products. The iodoacetyl reactions should thujs be carried out in the dark to limit the generation of free iodine, which has the potential for reacting with tyrosine, histidine, and tryptophan amino-acids [r].

Protocols may found in the literature.

References - TMRIA

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