Bioconjugation is essential to the modification of biomolecules with tag molecules for use in research, diagnotics and therapeutics. The classic tag molecules include fluorescent dyes, biotin and its derivatives, digioxin and other labeling molecules. This set provides highly detailed information on the chemistry, reagent systems and practical applications for creating labeled or conjugated molecules. It also describes dozens of reactions with details on hundreds of commercially available reagents and the use of these reagents for modifying or crosslinking peptides, proteins, sugars, polysaccharides, nucleic acids, oligonucleotides, lipids and synthetic polymers. Armed with the reagents described in this chapter, researchers will form unique bioconjugates that can be used for detecting, quantifying, and targeting important analytes. This set provides general guidelines for researchers to select appropriate reagents and to make highly active antibody conjugates, immunotoxins, immunogen complexes, as well as biotinylated molecules, avidin or streptavidin conjugates and tagged biomolecules.

Fluorescence is the result of a three-stage process that occurs in certain molecules (generally polyaromatic hydrocarbons or heterocycles) called fluorophores or fluorescent dyes. A fluorescent probe is a fluorophore designed to localize within a specific region of a biological specimen or to respond to a specific stimulus. Fluorescent probes enable researchers to detect particular components of complex biomolecular assemblies (including live cells) with exquisite sensitivity and selectivity. Reactive fluorescent dyes are widely used to modify amino acids, peptides, proteins (in particular, antibodies), oligonucleotides, nucleic acids, carbohydrates and other biological molecules. Yameian provides a full spectrum of fluorophores for labeling biopolymers and derivatizing low molecular weight molecules. Among the reactive dyes, amine-reactive dyes are most often used to prepare various bioconjugates for immunochemistry, histochemistry, fluorescence *in situ* hybridization (FISH), cell tracing, receptor binding and other biological applications since amino groups are either abundant or easily introduced into biomolecules. In general, thiol-reactive reagents are frequently used to develop probes for investigating some particular protein structures and functions. Additionally, some amine-containing fluorescent reagents are also used to modify biomolecules, in particular, to label glycoproteins.

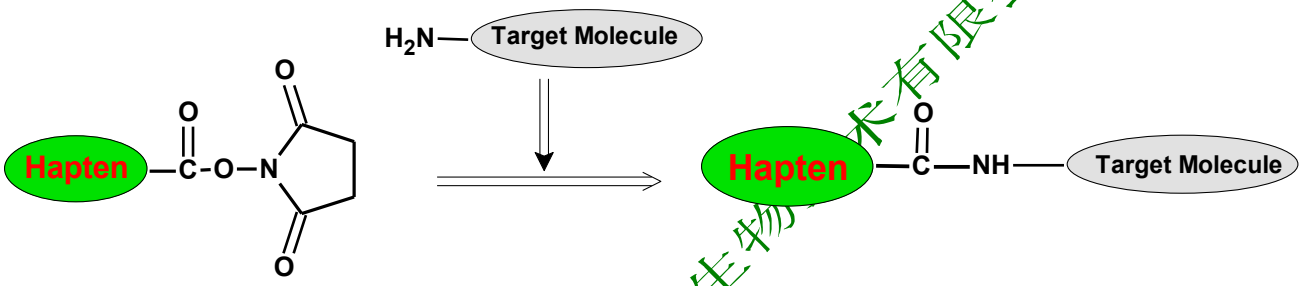
In general, the preferred bioconjugates should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules. It is quite critical to properly control the degree of substitution (DOS) when conducting a conjugation of biopolymers. A high degree of labeling may significantly decrease the water solubility and binding affinity/specificity of the target biomolecules. Although conjugating dyes to biomolecules is usually easy, preparing the optimal conjugate may require extensive experimentation. Fortunately there are some excellent publications that may provide you some important guidelines (For the technical details please read the references listed on the end of this section).

Amine-Reactive Fluorescent Dyes

Amine-reactive fluorescent probes are widely used to modify peptides, proteins, oligonucleotides, nucleic acids, ligands and other biomolecules. Amine-reactive dyes are most often used to prepare bioconjugates for immunochemistry, fluorescence *in situ* hybridization (FISH), cell tracing, receptor labeling and fluorescent analog cytochemistry. In these applications, the stability of the chemical bond between the amine-reactive dye and biomolecule is particularly important because the fluorescent conjugates are often subjected to rigorous incubation, hybridization and washing steps.

A number of fluorescent amino-reactive dyes have been developed to label various biomolecules, and the resultant conjugates are widely used in biological applications. Three major classes of amine-reactive fluorescent reagents are currently used to label biopolymers: succinimidyl esters (SE), isothiocyanates, sulfonyl chlorides. We offer all the popular amine-reactive fluorescent dyes for peptide/protein labelings, nucleotide modifications and microarray applications. Although FITC (fluorescein isothiocyanate), one of the most popular fluorescent labeling dyes, is predominantly used for preparing a variety of fluorescent bioconjugates, its low conjugation efficiency and short shelf lifetime of FITC conjugates are still troublesome for some critical biological applications. We strongly recommend that you choose succinimidyl esters for labeling needs if other conditions and factors are equal.

Fluorescent Dye Carboxylic Acids and Their Succinimidyl Esters



Succinimidyl esters are proven to be the best reagents for amine modifications because the amide bonds that are formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. 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).

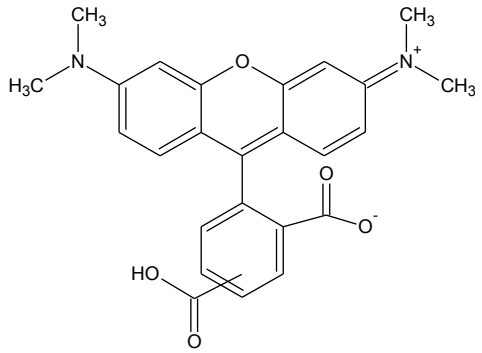
2). *Reaction pH:* The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas 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 and thiol compounds 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 (such as viadinlysis) before performing dye conjugations.

4). *Reaction Temperature:* Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
360	25 mg	$49	430.45	541 nm	565 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

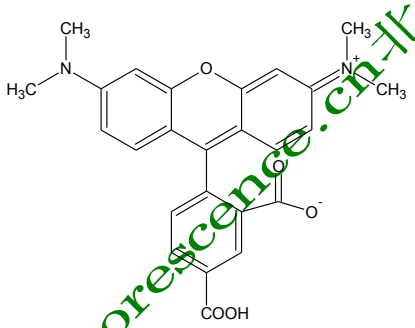
TAMRA is one of the most popular fluorophores used in various bioconjugations. TMR is a bright organic fluorophore. 5(6)-TAMRA is the mixture of two carboxy tetramethylrhodamine (TMR) isomers. It is used to modify amino and hydroxy groups using EDC-mediated couplings. It can be readily converted to the amine-reactive 5(6)-TAMRA, SE.

References

1. Evans NA, *et al.* (2001). Visualizing differences in ligand-induced beta-arrestin-GFP interactions and trafficking between three recently characterized G protein-coupled receptors. *J Neurochem* **77**, 476-85.
2. Hess KL, *et al.* (1997). A novel flow cytometric method for quantifying phagocytosis of apoptotic cells. *Cytometry* **27**, 145-52.

5-TAMRA [5-Carboxytetramethylrhodamine]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
363	10 mg	$79	430.45	541 nm	568 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

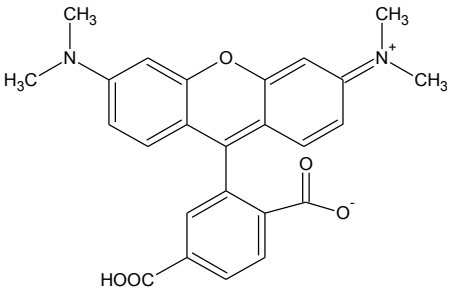
5-TAMRA is the purified single isomer of 5(6)-TAMRA. widely used to label peptides and proteins. It is preferred f some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-TAMRA and 6-TAMRA might affect some biological properties of the underlying conjugates.

References

1. Evans NA, *et al.* (2001). Visualizing differences in ligand-induced beta-arrestin-GFP interactions and trafficking between three recently characterized G protein-coupled receptors. *J Neurochem* **77**, 476-85.
2. Hahn, M., *et al.*, Influence of fluorophor dye labels on the migration behavior of polymerase chain reaction—amplified short tandem repeats during denaturing capillary electrophoresis. *Electrophoresis* 2001, **22**, 2691-700.
3. Yoo H and Juliano RL (2000). Enhanced delivery of antisense oligonucleotides with fluorophore-conjugated PAMAM dendrimers. *Nucleic Acids Res* **28**, 4225-31.

6-TAMRA [6-Carboxytetramethylrhodamine]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
366	10 mg	$79	430.45	541 nm	568 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

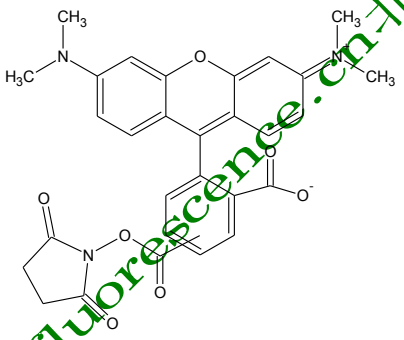
6-TAMRA is the other purified single isomer of 5(6)-TAMRA. It is predominantly used for nucleotide labeling. It is also used in fluorescence *in situ* hybridization (FISH).

References

1. Hsu TM, *et al*. (2001). Genotyping single-nucleotide polymorphisms by the invader assay with dual-color fluorescence polarization detection. *Clin Chem* **47**, 1373-7.
2. Schutz E, *et al*. (2000). Genotyping of eight thiopurine methyltransferase mutations: three-color multiplexing, two-color/shared anchor and fluorescence-quenching hybridization probe assays based on thermodynamic nearest-neighbor probe design. *Clin Chem* **46**, 1728-37.
3. Lyttle, M.H., *et al.*, A tetramethyl rhodamine (tamra) phosphoramidite facilitates solid-phase-supported synthesis of 5'-tamra DNA. *J Org Chem* 2000, **65**, 9033-8.

5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
370	25 mg	$99	527.53	546 nm	575 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

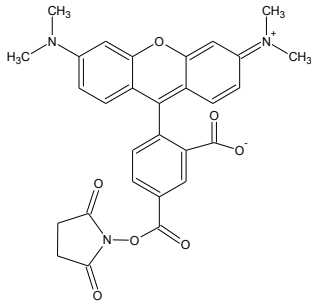
The succinimidyl esters of 5-TAMRA, 6-TAMRA or the mixed isomers are the primary labeling reagents for the preparation orange fluorescent bioconjugates, including peptide, protein, nucleotide and nucleic acid conjugates, especially fluorescent antibodies and avidin derivatives used in immunochemistry. TMR dyes have also been widely used as acceptors for FAM fluorophores in a variety of FRET studies.

References

1. Hahn M, *et al.* (2001). Influence of fluorophore dye labels on the migration behavior of polymerase chain reaction-amplified short tandem repeats during denaturing capillary electrophoresis. *Electrophoresis* **22**, 2691-700.
2. Hsu TM, *et al.* (2001). Genotyping single-nucleotide polymorphisms by the invader assay with dual-color fluorescence polarization detection. *Clin Chem* **47**, 1373-7.
3. Micka, K.A., *et al.*, Twgdam validation of a nine-locus and a four-locus fluorescent str multiplex system. *J Forensic Sci* 1999, **44**, 1243-57.

5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
373	5 mg	$99	527.53	547 nm	574 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

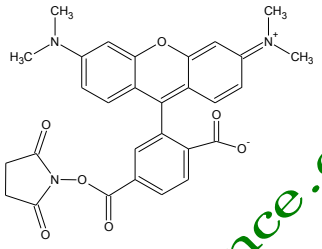
5-TAMRA, SE is predominantly used for labeling peptides and proteins while 6-TAMRA, SE is often used for labeling nucleotides and sequencing nucleic acids. The single TAMRA isomers are increasingly preferred for labeling peptides and nucleotides because they give better resolution in HPLC purification that is often required in the conjugation processes.

References

1. Evans NA, *et al.* (2001). Visualizing differences in ligand-induced beta-arrestin-GFP interactions and trafficking between three recently characterized G protein-coupled receptors. *J Neurochem* **77**, 476-85.
2. Nasarabadi S, *et al.* (1999). Simultaneous detection of TaqMan probes containing FAM and TAMRA reporter fluorophores. *Biotechniques* **27,** 1116-8.

6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
376	5 mg	$99	527.53	547 nm	573 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

6-TAMRA, SE is the other purified single isomer of 5(6)-TAMRA, SE. It is predominantly used for nucleotide labeling and DNA sequencing.

References

1. Hsu TM, *et al.* (2001). Genotyping single-nucleotide polymorphisms by the invader assay with dual-color fluorescence polarization detection. *Clin Chem* **47**, 1373-7.
2. Sanders SJ (2000). Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. *Mol Cell Probes* **14**, 249-53.
3. Sanders Sevall, J., Factor v leiden genotyping using real-time fluorescent polymerase chain reaction. *Mol Cell Probes* 2000, **14**, 249-53.
4. Micka, K.A., *et al.*, Twgdam validation of a nine-locus and a four-locus fluorescent str multiplex system. *J Forensic Sci* 1999, **44**, 1243-57.
5. Slateva, K., *et al.*, Fluorotyping of hla-a by sequence-specific priming and fluorogenic probing. *Tissue Antigens* 1998, **52**, 462-72.