Classic Reactive Fluorescent Labeling Dyes & Their Applications

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.

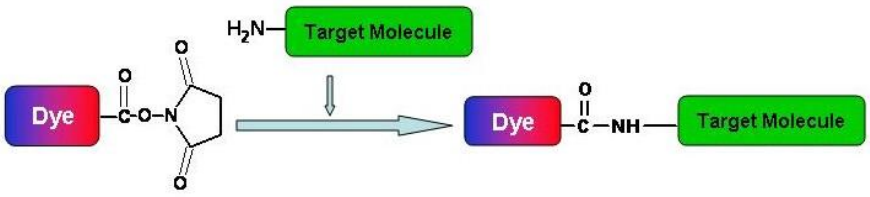
In general, the preferred bioconjugates should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules. It is quite critical to properly control the degree of substitution (DOS) when conducting a conjugation of biopolymers. A high degree of labeling may 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, and sulfonyl chlorides. AAT Bioquest offers 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, the low conjugation efficiency of FITC and the short life time of its 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 equivalent.

Fluorescent Dye Carboxylic Acids and Their Succinimidyl Esters



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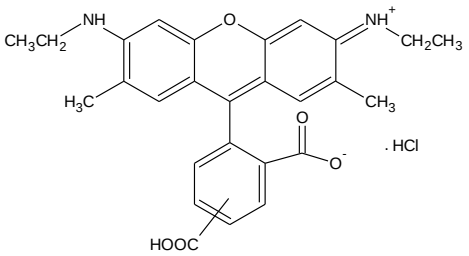
Technical Support: support@aatbio.com; Tel: 408-733-1055

Succinimidyl esters are proven to be the best reagents for amine modifications because the amide bonds 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 a few factors that should be considered when SE compounds are used for conjugation reaction:

- *Solvents:* For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- *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 at pH > 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas isothiocyanates may require a pH between 9.0 and 10.0 for optimal conjugations.
- *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.
- *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)-CR6G [5-(and-6)-Carboxyrhodamine 6G, hydrochloride]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
330	25 mg	494.97	519 nm	544 nm	DMSO	4 ºC

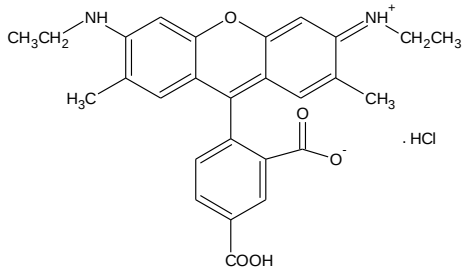


Features and Biological Applications

5(6)-CR6G is the mixture of two carboxy rhodamine 6G isomers. It is used to modify amino and hydroxy groups using EDC-mediated couplings when there are difficulties in using 5(6)-CR6G, SE. The excitation and emission wavelengths of rhodamine 6G fall between those of fluorescein and tetramethylrhodamine derivatives, making 5(6)-CR6G another color choice for the multicolor fluorescence imaging applications.

5-CR6G [5-Carboxyrhodamine 6G, hydrochloride]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
331	10 mg	494.97	518 nm	544 nm	DMSO	4 ºC



Features and Biological Applications

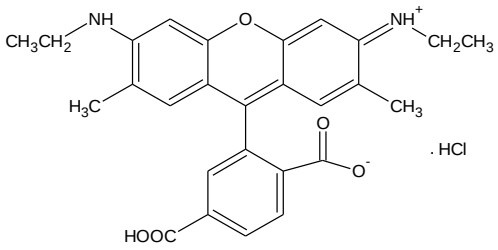
5-CR6G is the purified single isomer of 5(6)-CR6G. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-CR6G and 6-CR6G might significantly affect some biological properties of the underlying conjugates. This isomer is predominantly used to label small molecules, peptides and proteins.

References

1. Chiu, D.T., et al., Injection of ultrasmall samples and single molecules into tapered capillaries. *Anal Chem*, 997. **69**, 1801-7.
2. Hung SC, *et al.* (1997). Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Anal Biochem* 1997. **252**, 78-88.

6-CR6G [6-Carboxyrhodamine 6G, hydrochloride]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
332	10 mg	494.97	520 nm	547 nm	DMSO	4 ºC



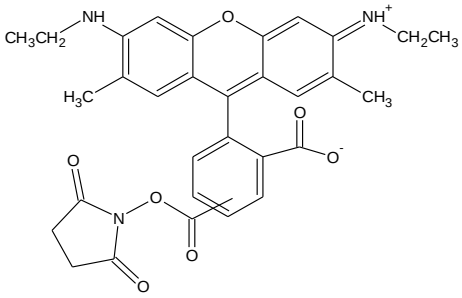
Features and Biological Applications
6-CR6G is the other purified single isomer of 5(6)-CR6G. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-CR6G and 6-CR6G might significantly affect some biological properties of the underlying conjugates. Complementary to 5-CR6G, 6-CR6G is predominantly used for labeling nucleotides and nucleic acids.

References

1. Seo, T.S., *et al.*, Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides. *Proc Natl Acad Sci U S A* 2005, **102**, 5926-31.
2. Dietrich, A., *et al.*, Fluorescence resonance energy transfer (FRET) and competing processes in donor-acceptor substituted DNA strands: A comparative study of ensemble and single-molecule data. *J Biotechnol* 2002, **82**, 211-31.
3. Arezi, B., *et al.*, Efficient and high fidelity incorporation of dye-terminators by a novel archaeal DNA polymerase mutant. *J Mol Biol* 2002, **322**, 719-29.
4. Hung SC, *et al.* (1997). Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Anal Biochem* **252**, 78-88.

5(6)-CR6G, SE [5-(and-6)-Carboxyrhodamine 6G, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
340	10 mg	555.59	522 nm	550 nm	DMSO	4 °C and desiccated



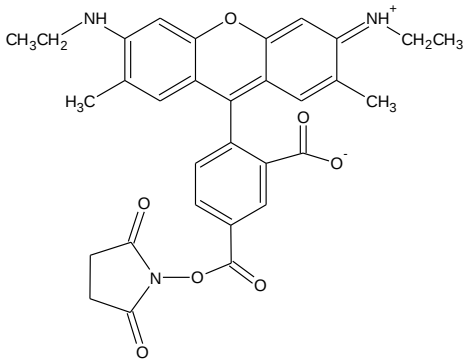
Features and Biological Applications
The absorption maxima of CR6G conjugates match well with the 514 nm spectral line of the argon-ion laser that is used in most of fluorescence instruments. In addition, the excitation and emission peaks of rhodamine 6G conjugates falling between those of fluorescein and tetramethylrhodamine provide another color choice for the multicolor imaging applications. 5(6)-CR6G, SE is the amine-reactive form of 5(6)-CR6G.

References

1. Hung SC, *et al.* (1997). Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Anal Biochem* **252**, 78-88.
2. Hung SC, *et al.* (1996). Energy transfer primers with 5- or 6-carboxyrhodamine-6G as acceptor chromophores. *Anal Biochem* 238, 165-70.

5-CR6G, SE [5-Carboxyrhodamine 6G, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
341	5 mg	555.59	524 nm	556 nm	DMSO	4 °C and desiccated



Features and Biological Applications

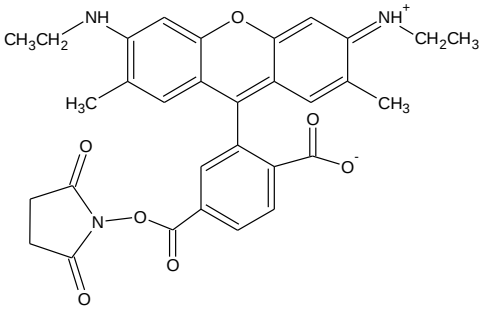
5-CR6G, SE is amine-reactive derivative of 5-CR6G. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-CR6G and 6-CR6G might significantly affect some biological properties of the underlying conjugates. In general, rhodamine 6G conjugates have higher fluorescence quantum yields than tetramethylrhodamine conjugates, as well as good photostability.

References

1. Chiu, D.T., et al., Injection of ultrasmall samples and single molecules into tapered capillaries. *Anal Chem*, 997. **69**, 1801-7.
2. Hung SC, *et al.* (1997). Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Anal Biochem* 1997. **252**, 78-88.
3. Hung SC, *et al.* (1996). Energy transfer primers with 5- or 6-carboxyrhodamine-6G as acceptor chromophores. *Anal Biochem* **238**, 165-70.

6-CR6G, SE [6-Carboxyrhodamine 6G, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
342	5 mg	555.59	524 nm	551 nm	DMSO	4 °C and desiccated



Features and Biological Applications

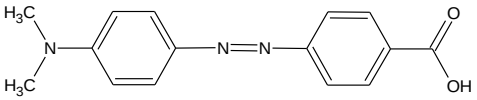
6-CR6G, SE is amine-reactive derivative of of 6-CR6G. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-CR6G and 6-CR6G might significantly affect some biological properties of the underlying conjugates. In general, rhodamine 6G conjugates have higher fluorescence quantum yields than tetramethylrhodamine conjugates, as well as good photostability.

References

1. Hung SC, *et al.* (1997). Optimization of spectroscopic and electrophoretic properties of energy transfer primers. *Anal Biochem* **252**, 78-88.
2. Hung SC, *et al.* (1996). Energy transfer primers with 5- or 6-carboxyrhodamine-6G as acceptor chromophores. *Anal Biochem* **238**, 165-70.

DABCYL acid [4-((4-(Dimethylamino)phenyl)azo)benzoic acid]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
2001	5 g	269.30	425 nm	none	DMF	4 °C



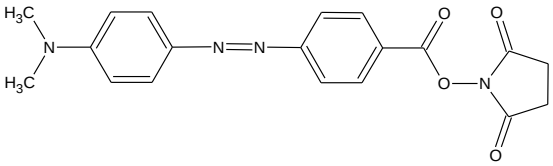
Features and Biological Applications
DABCYL acid is one of the most popular acceptors for developing FRET-based nucleic acid probes suh as Moecular Beacon-based PCR probes. It is also used to prepare a variety of FRET peptides that are often used for the detection of various proteases. It is predominantly paired with EDANS (as fluorescence donor) in FRET-based fluorescent probes.

References

1. Shengqi, W., *et al.*, A new fluorescent quantitative polymerase chain reaction technique. *Anal Biochem* 2002, **309**, 206-11.
2. Koo, K. and L.A. Jaykus, Detection of single nucleotide polymorphisms within the listeria genus using an 'asymmetric' fluorogenic probe set and fluorescence resonance energy transfer based-pcr. *Lett Appl Microbiol* 2002, **35**, 513-7.
3. Parniak, M.A., *et al.*, A fluorescence-based high-throughput screening assay for inhibitors of human immunodeficiency virus-1 reverse transcriptase-associated ribonuclease h activity. *Anal Biochem* 2003, **322**, 33-9.
4. Kuo, C.J., *et al.*, Characterization of sars main protease and inhibitor assay using a fluorogenic substrate. *Biochem Biophys Res Commun* 2004, **318**, 862-7.
5. Chen, S., *et al.*, Enzymatic activity characterization of sars coronavirus 3c-like protease by fluorescence resonance energy transfer technique. *Acta Pharmacol Sin* 2005, **26**, 99-106.

DABCYL acid, SE [4-((4-(Dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
2004	1 g	366.37	453 nm	none	DMF	4 °C and desiccated



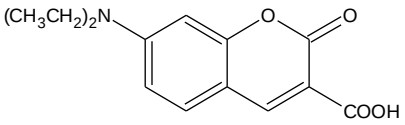
Features and Biological Applications
DABCYL, SE is the amino-reactive form of DABCYL. It is widely used to prepare a variety of FRET-based probes that contain DABCYL. DABCYL is one of the most popular acceptors for developing FRET-based nucleic acid probes and protease substrates.

References

1. Melnyk, R.A., *et al.*, Transmembrane domain mediated self-assembly of major coat protein subunits from ff bacteriophage. *J Mol Biol* 2002, **315**, 63-72.
2. Koo, K. and L.A. Jaykus, Detection of listeria monocytogenes from a model food by fluorescence resonance energy transfer-based pcr with an asymmetric fluorogenic probe set. *Appl Environ Microbiol* 2003, **69**, 1082-8.
3. Chen, S., *et al.*, Enzymatic activity characterization of sars coronavirus 3c-like protease by fluorescence resonance energy transfer technique. *Acta Pharmacol Sin* 2005, **26**, 99-106.

DEAC, acid [7-Diethylaminocoumarin-3-carboxylic acid]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
505	100 mg	261.27	432 nm	472 nm	DMF	4 °C and desiccated



Features and Biological Applications

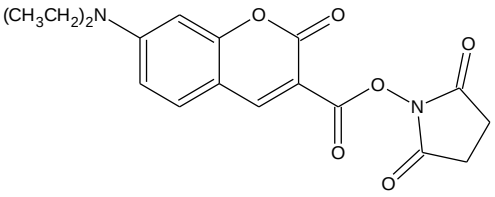
7-Diethylaminocoumarin-3-carboxylic acid can be used to create blue-fluorescent bioconjugates. It has quite strong blue fluorescence. When compared with AMCA conjugates, conjugates of the UV-light-excitable 7-dialkylaminocoumarin fluorophore have much longer-wavelength excitation and slightly longer-wavelength emission spectra (~470 nm).

Reference

1. Krieger F, Mourot A, Araoz R, Kotzyba-Hibert F, Molgo J, Bamberg E, Goeldner M. (2008) Fluorescent agonists for the Torpedo nicotinic acetylcholine receptor. Chembiochem, 9, 1146.
2. Webb MR, Reid GP, Munasinghe VR, Corrie JE. (2004) A series of related nucleotide analogues that aids optimization of fluorescence signals in probing the mechanism of P-loop ATPases, such as actomyosin. Biochemistry, 43, 14463.
3. Webb MR, Corrie JE. (2001) Fluorescent coumarin-labeled nucleotides to measure ADP release from actomyosin. Biophys J, 81, 1562.

DEAC, SE [7-Diethylaminocoumarin-3-carboxylic acid, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
506	25 mg	358.35	432 nm	472 nm	DMF	-20 °C and desiccated



Features and Biological Applications

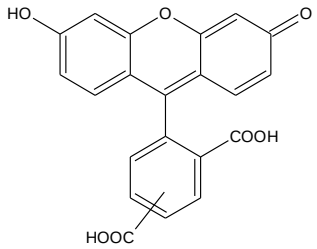
The amine-reactive 7-diethylaminocoumarin-3-carboxylic acid, succinimidyl ester can be used to create blue-fluorescent bioconjugates. It has quite strong blue fluorescence. When compared with AMCA conjugates, conjugates of the UV-light-excitable 7-dialkylaminocoumarin fluorophore have much longer-wavelength excitation and slightly longer-wavelength emission spectra (~470 nm). This fluorescent dye is quite hydrophobic, and might be used for labeling live cells.

Reference

1. Webb MR and Corrie JE (2001). Fluorescent coumarin-labeled nucleotides to measure ADP release from actomyosin. *Biophys J* **81**, 1562-1569.
2. Arguello JM, Kaplan JH. (1994) Glutamate 779, an intramembrane carboxyl, is essential for monovalent cation binding by the Na,K-ATPase. J Biol Chem, 269, 6892.
3. Arguello JM, Kaplan JH. (1991) Evidence for essential carboxyls in the cation-binding domain of the Na,K-ATPase. J Biol Chem, 266, 14627. 1362.

5(6)-FAM [5-(and-6)-Carboxyfluorescein]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
100	1 g	376.32	494 nm	519 nm	DMF	4 °C and desiccated



Features and Biological Applications

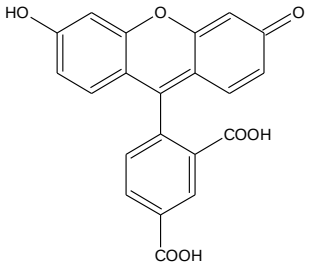
Carboxyfluorescein (commonly called FAM) and its amine-reactive succinimidyl esters are favored over FITC in bioconjugations. FAM reagents give carboxamides that are more resistant to hydrolysis. In addition, FAM reagents require less stringent conjugation conditions and give better conjugation yields, and the resulted conjugates have superior stability. FITC-labeled nucleotides and peptides tend to deteriorate more quickly than the corresponding FAM conjugates. We found that FAM reagents can be used to substitute FITC reagents in most biological applications.

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. Hung SC, *et al*. (1996). Cyanine dyes with high absorption cross section as donor chromophores in energy transfer primers. *Anal Biochem* **243**, 15-27.
3. Banks PR and Paquette DM (1995). Comparison of three common amine reactive fluorescent probes used for conjugation to biomolecules by capillary zone electrophoresis. *Bioconjug Chem* **6**, 447-458.

5-FAM [5-Carboxyfluorescein]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
103	100 mg	376.32	492 nm	518 nm	DMF	4 °C and desiccated



Features and Biological Applications

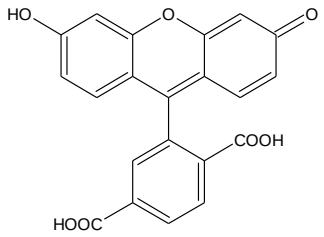
5-FAM is the purified single isomer of carboxyfluorescein. It is one of the most popular green fluorescent reagents used for labeling peptides, proteins and nucleotides. It has been predominantly used to develop a variety of green fluorescent peptides that can be excited with the 488 nm line of the Ar laser. It has also been used to prepare various small fluorescent molecules.

References

1. Adamczyk, M., *et al.*, Preparation of succinimidyl and pentafluorophenyl active esters of 5- and 6-carboxyfluorescein. *Bioconjug Chem* 1997, **8**, 253-5.
2. Yefimov, S., *et al.*, Sequential electroelution and mass spectroscopic identification of intact sodium dodecyl sulfate-proteins labeled with 5(6)-carboxyfluorescein-n-hydroxysuccinimide ester. *Electrophoresis* 2001, **22**, 2881-7.
3. Walker, B., *et al.*, Carboxyfluorescein and biotin neuromedin c analogues: Synthesis and applications. *Peptides* 1995, **16**, 255-61.

6-FAM [6-Carboxyfluorescein]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
106	100 mg	376.32	495 nm (pH > 9.0)	517 nm (pH > 9.0)	DMSO or DMF	4 °C and desiccated

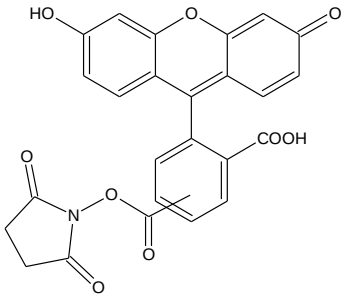


Features and Biological Applications
6-FAM is the other purified isomer of 5(6)-carboxyfluorescein. Complementary to 5-FAM isomer, 6-FAM is mainly used for labeling nucleotides and nucleic acids.

References
1. Brandis JW (1999). Dye structure affects Taq DNA polymerase terminator selectivity. *Nucleic Acids Res* **27**, 1912-8.
2. Witham PK, *et al.* (1996). A PCR-based assay for the detection of Escherichia coli Shiga-like toxin genes in ground beef. *Appl Environ Microbiol* **62**, 1347-53.

5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
110	25 mg	473.39	494 nm (pH > 9.0)	519 nm (pH > 9.0)	DMSO or DMF	4 °C and desiccated

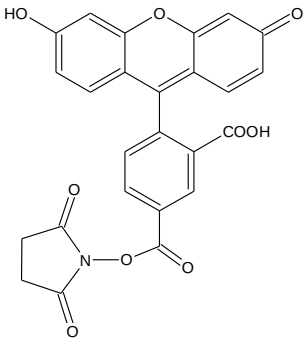


Features and Biological Applications
5(6)-FAM, SE is the amine-reactive succinimidyl ester of FAM acid. It is favored over FITC in bioconjugations. FAM reagents give carboxamides that are more resistant to hydrolysis. In addition, FAM reagents require less stringent conjugation conditions and give better conjugation yields, and the resulted conjugates have superior stability. FITC-labeled nucleotides and peptides tend to deteriorate more quickly than the corresponding FAM conjugates. We found that FAM reagents can be used to substitute FITC reagents in most biological applications.

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. Sanders SJ (2000). Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. *Mol Cell Probes* **14,** 249-53.
3. Brandis JW (1999). Dye structure affects Taq DNA polymerase terminator selectivity. *Nucleic Acids Res* **27,** 1912-8.

5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
113	10 mg	473.39	492 nm	518 nm	DMF	4 °C and desiccated



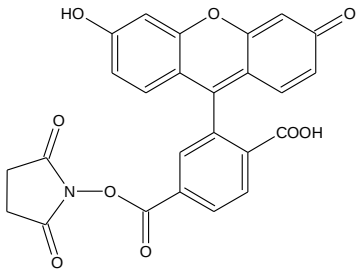
Features and Biological Applications
5-FAM, SE is the amine-reactive succinimidyl ester of single isomer 5-FAM acid. It is one of the most popular green fluorescent reagents used for labeling peptides, proteins and nucleotides. It has also been used to prepare various small fluorescent molecules.

References

1. Sakamoto M, *et al.* (2003). Application of terminal RFLP analysis to characterize oral bacterial flora in saliva of healthy subjects and patients with periodontitis. *J Med Microbiol* **52,** 79-89.
2. 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.
3. Araie, M., Carboxyfluorescein. A dye for evaluating the corneal endothelial barrier function in vivo. *Exp Eye Res* 1986, 42, 141-50.

6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
116	10 mg	473.39	495 nm	517 nm	DMF	4 °C and desiccated



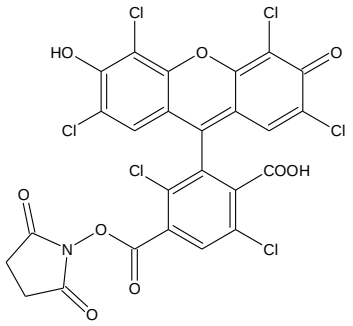
Features and Biological Applications
6-FAM, SE is the amine-reactive succinimidyl ester of single isomer 6-FAM acid. It is one of the most popular green fluorescent reagents used for labeling nucleotides and nucleic acids. Compared to 5-FAM, 6-FAM is less often used to prepare small molecules.

References

1. Sakamoto M, *et al.* (2003). Application of terminal RFLP analysis to characterize oral bacterial flora in saliva of healthy subjects and patients with periodontitis. *J Med Microbiol* **52,** 79-89.
2. Jordan JA, *et al.* (2001). TaqMan-based detection of Trichomonas vaginalis DNA from female genital specimens. *J Clin Microbiol* **39**, 3819-22.
3. Brandis JW (1999). Dye structure affects Taq DNA polymerase terminator selectivity. *Nucleic Acids Res* **27**, 1912-8.
4. Mornet, D. and K. Ue, Incorporation of 6-carboxyfluorescein into myosin subfragment 1. *Biochemistry* 1985, **24**, 840-6.

6-HEX, SE [6-Carboxy-2',4,4',5',7,7'- hexachlorofluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
202	5 mg	680.06	533 nm	550 nm	DMSO or DMF	4 °C and desiccated



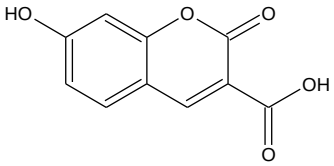
Features and Biological Applications
6-HEX, SE is the amine-reactive succinimidyl ester of 6-HEX acid. It is widely used for nucleic acid sequencing and related research.

References

1. Winograd E, *et al.* (2004). Chemical modifications of band 3 protein affect the adhesion of Plasmodium falciparum-infected erythrocytes to CD36. *Mol Biochem Parasitol* **136,** 243-8.
2. Pesquet, E., *et al.*, Multiple gene detection by in situ rt-pcr in isolated plant cells and tissues. *Plant J* 2004, **39**, 947-59.
3. Shin JH, *et al.* (1999). Rapid identification of up to three Candida species in a single reaction tube by a 5' exonuclease assay using fluorescent DNA probes. *J Clin Microbiol* **37,** 165-70.
4. Lindqvist AK, *et al*. (1996). Chromosome-specific panels of tri- and tetranucleotide microsatellite markers for multiplex fluorescent detection and automated genotyping: evaluation of their utility in pathology and forensics. *Genome Res* **6**, 1170-6

7-Hydroxycoumarin-3-carboxylic acid

Cat. #	Size	MW	Abs	Em	Solvent	Storage
550	250 mg	206.15	387 nm	448 nm	DMSO	4 °C and desiccated



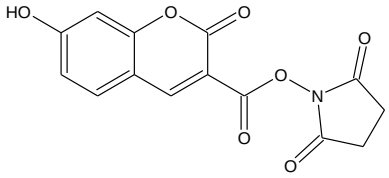
Features and Biological Applications
7-Hydroxycoumarin-3-carboxylic acid is one of the most popular blue fluorophores for labeling proteins and nucleic acids mostly through the *in situ* formation of its succinimidyl ester catalyzed by EDAC.

References

1. Higai K, *et al.* (1999). A fluorometric assay for glycosyltransferase activities using sugars aminated and tagged with 7-hydroxycoumarin-3-carboxylic acid as substrates and high performance liquid chromatography. *Biol Pharm Bull* **22**, 333-8.
2. Li H, *et al.* (1996). Properties and regulation of gap junctional hemichannels in the plasma membranes of cultured cells. *J Cell Biol* **134**, 1019-30.

7-Hydroxycoumarin-3-carboxylic acid, succinimidyl ester

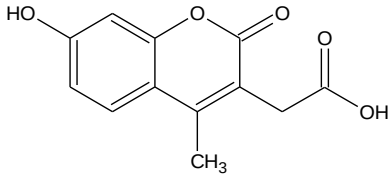
Cat. #	Size	MW	Abs	Em	Solvent	Storage
551	50 mg	303.23	363 nm	447 nm	DMSO	4 °C and desiccated



Features and Biological Applications
7-Hydroxycoumarin-3-carboxylic acid, SE is the amine-reactive succinimidyl ester of 7-Hydroxycoumarin-3-carboxylic acid. It is one of the most popular blue fluorescent dyes for labeling proteins and nucleic acids. This coumarin is also increasingly used to label peptides, nucleotides and carbohydrates.

7-Hydroxy-4-methylcoumarin-3-acetic acid

Cat. #	Size	MW	Abs	Em	Solvents	Storage
554	100 mg	234.21	360 nm	455 nm	DMSO or DMF	4 °C and desiccated



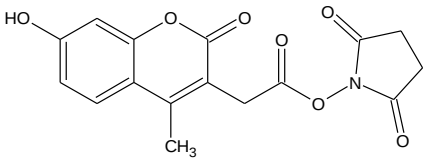
Features and Biological Applications
7-Hydroxy-4-methylcoumarin-3-acetic acid is a blue fluorophore that has pH-dependent and environment-sensitive fluorescence. This coumarin is increasingly used to label peptides, nucleotides and carbohydrates.

References

1. Gabor G, *et al*. (1995). Sensitivity enhancement of fluorescent pH indicators using pH-dependent energy transfer. *Anal Chim Acta* **313**, 131.
2. Exley D and Ekeke GI (1981). Fluoroimmunoassay of 5α-dihydrotestosterone. *J Steroid Biochem* **14**, 1297-1302.

7-Hydroxy-4-methylcoumarin-3-acetic acid, succinimidyl ester

Cat. #	Size	MW	Abs	Em	Solvent	Storage
556	25 mg	331.28	364 nm	458 nm	DMSO	4 °C and desiccated



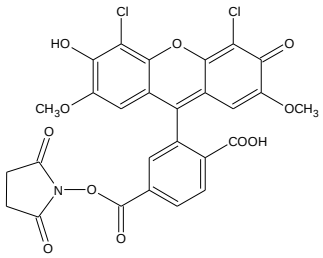
Features and Biological Applications
7-Hydroxy-4-methylcoumarin-3-acetic acid, SE is an amine-reactive blue fluorophore that has pH-dependent and environment-sensitive fluorescence. It is widely used for preparing bioconjugates of blue fluorescence.

References

1. Gabor G, *et al*. (1995). Sensitivity enhancement of fluorescent pH indicators using pH-dependent energy transfer. *Anal Chim Acta* **313**, 131.
2. Exley D and Ekeke GI (1981). Fluoroimmunoassay of 5α-dihydrotestosterone. *J Steroid Biochem* **14**, 1297-1302.

6-JOE, SE [6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
203	5 mg	602.34	520 nm	548 nm	DMF	4 °C and desiccated



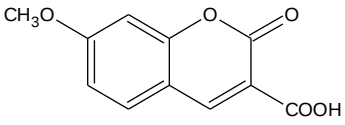
Features and Biological Applications
6-JOE has similar absorption and emission spectra to those of rhodamine 6G, and its SE is predominantly used for automated DNA sequencing. It is also used to label nucleotides.

References

1. Petersen, K., *et al.*, Short pna molecular beacons for real-time pcr allelic discrimination of single nucleotide polymorphisms. *Mol Cell Probes* 2004, **18**, 117-22.
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.

7-Methoxycoumarin-3-carboxylic acid

Cat. #	Size	MW	Abs	Em	Solvent	Storage
560	250 mg	220.18	336 nm	402 nm	DMF	4 °C and desiccated



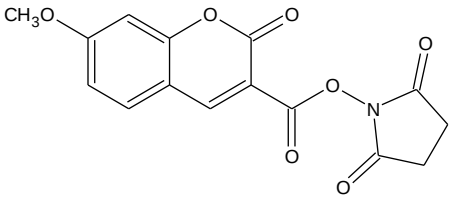
Features and Biological Applications
7-Methoxycoumarin-3-carboxylic acid is used for HPLC derivatization. It is also used to develop FRET probes for analyzing protease activities.

References

1. Berthelot, T., *et al.*, Synthesis of nepsilon-(7-diethylaminocoumarin-3-carboxyl)- and nepsilon-(7-methoxycoumarin-3-carboxyl)-l-fmoc lysine as tools for protease cleavage detection by fluorescence. *J Pept Sci* 2005, **11**, 153-60.
2. Tisljar U, *et al*. (1990). An alternative quenched fluorescence substrate for Pz-peptidase. *Anal Biochem* **186**, 112-5.

7-Methoxycoumarin-3-carboxylic acid, succinimidyl ester

Cat. #	Size	MW	Abs	Em	Solvent	Storage
563	250 mg	317.25	358 nm	410 nm	DMF	4 °C and desiccated



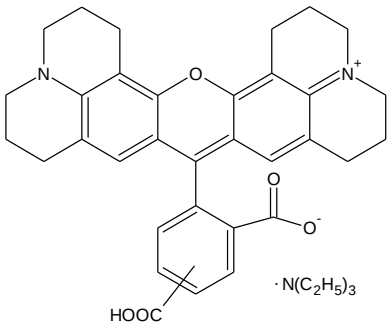
Features and Biological Applications
7-Methoxycoumarin-3-carboxylic acid, succinimidyl ester is an excellent amino-reactive tag that has strong blue fluorescence. It is used to label peptides and nucleotides. It is also used to label cell membranes although its fluorescence is quite short.

References

1. Berthelot, T., *et al.*, Synthesis of nepsilon-(7-diethylaminocoumarin-3-carboxyl)- and nepsilon-(7-methoxycoumarin-3-carboxyl)-l-fmoc lysine as tools for protease cleavage detection by fluorescence. *J Pept Sci* 2005, **11**, 153-60.
2. Tisljar U, *et al*. (1990). An alternative quenched fluorescence substrate for Pz-peptidase. *Anal Biochem* **186**, 112-5.
3. Mita H, *et al*. (1989). Quantitation of platelet-activating factor by high performance liquid chromatography with fluorescent detection. *Anal Biochem* **180**, 131-5.

5(6)-ROX [5-(and-6)-Carboxy-X-rhodamine]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
380	100 mg	635.80	568 nm	591 nm	DMF	-20 °C and desiccated



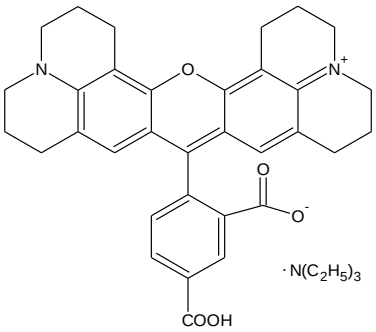
Features and Biological Applications
ROX dyes are strongly red fluorescent. They have longer excitation and emission wavelengths than the 'conventional' rhodamines. These dyes are used to label peptides, proteins, and other biological ligands. 5-ROX, 6-ROX and their mixture 5(6)-ROX are used to label biomolecules by EDC-mediated reactions. Compared to other rhodamines, ROX is very unstable.
Cautions must be exercised to prevent the deterioration during storage.

References

1. Seo, T.S., *et al.*, Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides. *Proc Natl Acad Sci U S A* 2005, **102**, 5926-31.
2. Seo, T.S., *et al.*, Photocleavable fluorescent nucleotides for DNA sequencing on a chip constructed by site-specific coupling chemistry. *Proc Natl Acad Sci U S A* 2004, **101**, 5488-93.
3. 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.
4. Li Y and Glazer AN (1999). Design, synthesis and spectroscopic properties of peptide-bridged fluorescence energy-transfer cassettes. *Bioconjug Chem* **10**, 241-5.

5-ROX [5-Carboxy-X-rhodamine]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
381	10 mg	635.80	567 nm	591 nm	DMF	-20 °C and desiccated



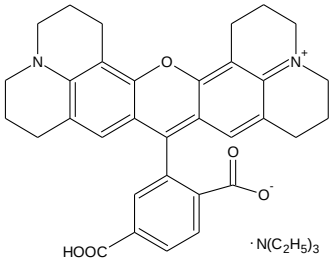
Features and Biological Applications
5-ROX is the purified single isomer of 5(6)-ROX. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-ROX and 6-ROX might significantly affect some biological properties of the underlying conjugates. 5-ROX is predominantly used to label peptides and proteins. *Cautions must be exercised to prevent the deterioration during storage.*

References

1. Bartlett JM. (1999). Modification of the GeneScan 2500 fluorescent dye standard for accurate product sizing. *Mol Biotechnol* **13**, 185-9.
2. Hung SC, *et al.* (1998). Comparison of fluorescence energy transfer primers with different donor-acceptor dye combinations. *Anal Biochem* **255**, 32-8.

6-ROX [6-Carboxy-X-rhodamine]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
382	10 mg	635.80	570 nm	591 nm	DMF	-20 °C and desiccated



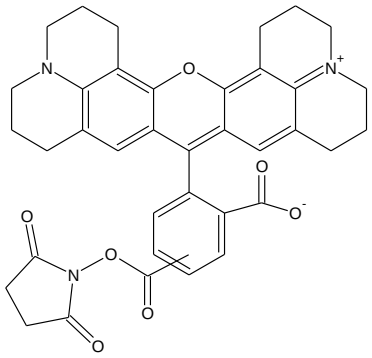
Features and Biological Applications
6-ROX is the other purified single isomer of 5(6)-ROX. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-ROX and 6-ROX might significantly affect some biological properties of the underlying conjugates. 6-ROX is predominantly used for labeling nucleotides and sequencing DNA. *Cautions must be exercised to prevent the deterioration during storage.*

References

1. Seo, T.S., *et al.*, Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides. *Proc Natl Acad Sci U S A* 2005, **102**, 5926-31.
2. Seo, T.S., *et al.*, Photocleavable fluorescent nucleotides for DNA sequencing on a chip constructed by site-specific coupling chemistry. *Proc Natl Acad Sci U S A* 2004, **101**, 5488-93.
3. Lu H, *et al.* (1994). High-speed and high-accuracy DNA sequencing by capillary gel electrophoresis in a simple, low cost instrument. Two-color peak-height encoded sequencing at 40°C. *J Chromatogr A* **680**, 497-501.

5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
390	25 mg	631.67	576 nm	601 nm	DMSO	-20 °C and desiccated



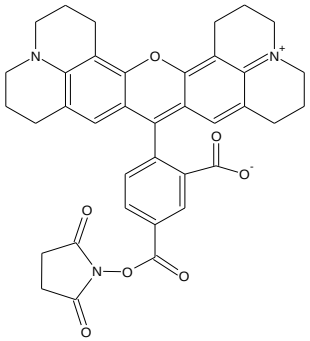
Features and Biological Applications
5(6)-ROX is the succinimidyl esters of 5(6)-ROX. This product is the primary labeling reagents for preparing various ROX-based bioconjugates. Compared to other rhodamines, ROX is very unstable. *Cautions must be exercised to prevent the deterioration during storage.*

References

1. Bertram JG, *et al.* (2000). Molecular mechanism and energetics of clamp assembly in Escherichia coli. The role of ATP hydrolysis when gamma complex loads beta on DNA. *J Biol Chem* **275**, 28413-20.
2. Rodionov VI, *et al.* (1994). Microtubule dynamics in fish melanophores. *J Cell Biol* **126**, 1455-64.

5-ROX, SE [5-Carboxy-X-rhodamine, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
391	5 mg	631.67	573 nm	602 nm	DMF or DMSO	-20 °C and desiccated



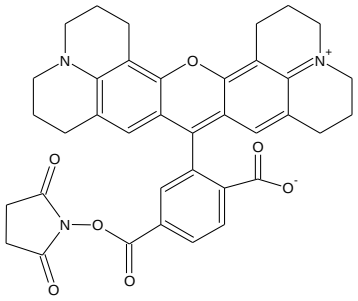
Features and Biological Applications
Single isomers of ROX are increasingly preferred for labeling peptides and nucleotides because they give better resolution in HPLC purifications that are often required in bioconjugations. Variable ratios of the 5- and 6-isomers often cause complications in the interpretation of labeling results and assay performances. 5-ROX is predominantly used to label peptides and proteins. Compared to other rhodamines, ROX is very unstable. *Cautions must be exercised to prevent the deterioration during storage.*

References

1. Bertram JG, *et al.* (2000). Molecular mechanism and energetics of clamp assembly in Escherichia coli. The role of ATP hydrolysis when gamma complex loads beta on DNA. *J Biol Chem* **275**, 28413-20.
2. Li, Y. and A.N. Glazer, Design, synthesis, and spectroscopic properties of peptide-bridged fluorescence energy-transfer cassettes. *Bioconjug Chem* 1999, **10**, 241-5.
3. Rodionov VI, *et al.* (1994). Microtubule dynamics in fish melanophores. *J Cell Biol* **126**, 1455-64.

6-ROX, SE [6-Carboxy-X-rhodamine, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
392	5 mg	631.67	575 nm	602 nm	DMSO	-20 °C and desiccated



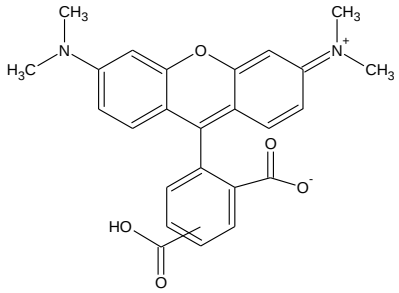
Features and Biological Applications
6-ROX, SE is the other purified single isomer of 5(6)-ROX, SE. 6-ROX is predominately used for labeling nucleotides and sequencing nucleic acids. Compared to other rhodamines, ROX is very unstable. *Cautions must be exercised to prevent the deterioration during storage.*

References

1. Dominko T, *et al*. (2000). Dynamic imaging of the metaphase II spindle and maternal chromosomesin bovine oocytes: implications for enucleation efficiency verification, avoidance of parthenogenesis, and successful embryogenesis. *Biol Reprod* **62**, 150-4.
2. Yoshikawa, Y., *et al.*, Differential display with carboxy-x-rhodamine-labeled primers and the selection of differentially amplified cdna fragments without cloning. *Anal Biochem* 1998, **256**, 82-91.
3. Hung, S.C., *et al.*, Cyanine dyes with high absorption cross section as donor chromophores in energy transfer primers. *Anal Biochem* 1996, **243**, 15-27.
4. Li, Y. and A.N. Glazer, Design, synthesis, and spectroscopic properties of peptide-bridged fluorescence energy-transfer cassettes. *Bioconjug Chem* 1999, **10**, 241-5.

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

Cat. #	Size	MW	Abs	Em	Solvent	Storage
360	100 mg	430.45	541 nm	565 nm	DMF or DMSO	4 °C and desiccated



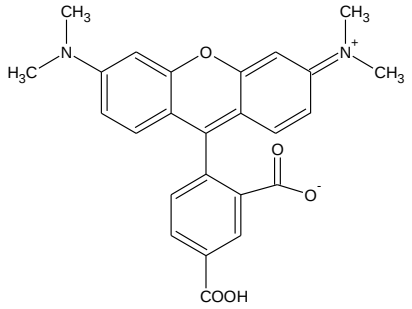
Features and Biological Applications
TAMRA is one of the most popular fluorophores used in various bioconjugations. TMR is a bright organe fluorophore. 5(6)-TAMRA is the mixture of two carboxy tetramethylrhodamine (TMR) isomers. It is used to modify amino and hydroxy groups using EDC-mediated couplings. 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	MW	Abs	Em	Solvents	Storage
363	10 mg	430.45	541 nm	568 nm	DMF or DMSO	4 °C and desiccated



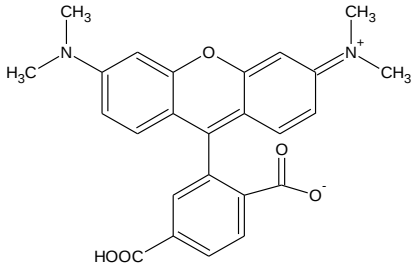
Features and Biological Applications
5-TAMRA is the purified single isomer of 5(6)-TAMRA. It is widely used to label peptides and proteins. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between 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	MW	Abs	Em	Solvent	Storage
366	10 mg	430.45	541 nm	568 nm	DMF or DMSO	4 °C and desiccated



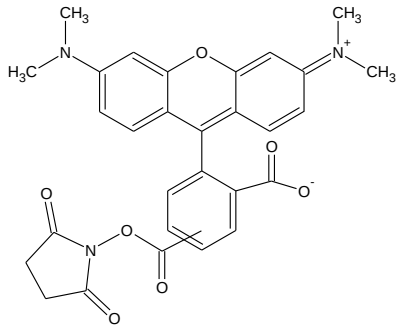
Features and Biological Applications
6-TAMRA is the other purified single isomer of 5(6)-TAMRA. It is predominantly used for nucleotide labeling. It is also used in fluorescence *in situ* hybridization (FISH).

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	MW	Abs	Em	Solvent	Storage
370	25 mg	527.53	546 nm	575 nm	DMSO	4 °C and desiccated



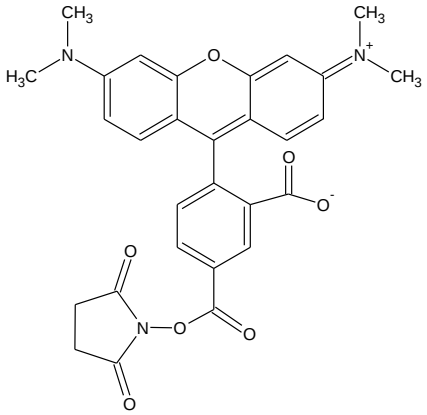
Features and Biological Applications
The succinimidyl esters of 5-TAMRA, 6-TAMRA or the mixed isomers are the primary labeling reagents for the preparation of orange fluorescent bioconjugates, including peptide, protein, nucleotide and nucleic acid conjugates, especially fluorescent antibodies and avidin 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	MW	Abs	Em	Solvent	Storage
373	5 mg	527.53	547 nm	574 nm	DMSO	4 °C and desiccated



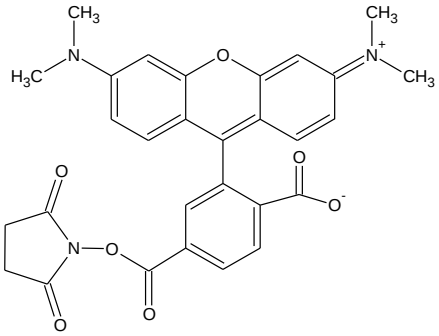
Features and Biological Applications
5-TAMRA, SE is predominantly used for labeling peptides and proteins while 6-TAMRA, SE is often used for labeling nucleotides and sequencing nucleic acids. The single TAMRA isomers are increasingly preferred for labeling peptides and nucleotides because they give 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	MW	Abs	Em	Solvent	Storage
376	5 mg	527.53	547 nm	573 nm	DMSO	4 °C and desiccated



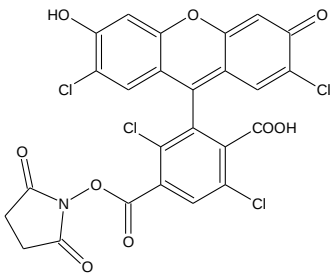
Features and Biological Applications
6-TAMRA, SE is the other purified single isomer of 5(6)-TAMRA, SE. It is predominantly used for nucleotide labeling and DNA sequencing.

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.

6-TET, SE [6-Carboxy-2',4,7,7'- tetrachlorofluorescein, succinimidyl ester]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
211	5 mg	611.17	521 nm	536 nm	DMSO	4 °C and desiccated

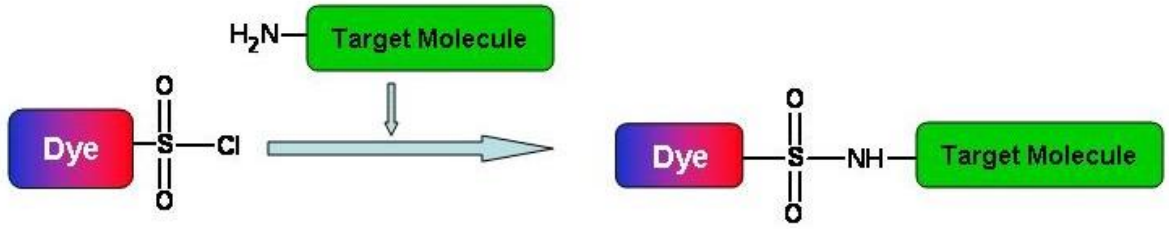


Features and Biological Applications
6-TET, SE is a popular amino-reactive fluorescent probe that is widely used in nucleic acid sequencing and related research.

References

1. Sanders SJ (2000). Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. *Mol Cell Probes* **14**, 249-53.
2. Gibson UE, *et al* (1996). A novel method for real time quantitative RT-PCR. *Genome Res* **6**, 995-1001.

Fluorescent Dye Sulfonyl Chlorides

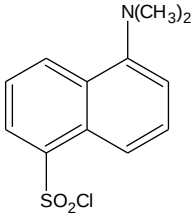


Sulfonyl chlorides are highly reactive. These reagents are unstable in water, especially at the higher pH required for reaction with aliphatic amines. Molecular modifications by sulfonyl chlorides need to be carefully carried out preferably at low temperature. Sulfonyl chlorides can also react with phenols (including tyrosine), aliphatic alcohols (including polysaccharides), thiols (such as cysteine) and imidazoles (such as histidine), but these reactions are not common in proteins or in aqueous solution. There are a few factors that need to be considered when SC compounds are used for conjugation reaction:

- *Solvents*: SC dyes are generally hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF). *Sulfonyl chlorides are unstable in dimethylsulfoxide (DMSO) and should never be used in this solvent.*
- *Reaction pH*: The labeling reactions of amines with SC reagents are strongly pH dependent. SC reagents react with non-protonated amine groups. On the other hand, the sulfonylation reagents tend to hydrolyze in the presence of water, with the rate increasing as the pH increases. Thus sulfonylation-based conjugations may require a pH 9.0-10.0 for optimal conjugations. In general, sulfonylation-based conjugations have much lower yields than the succinimidyl ester-based conjugations.
- *Reaction Buffers*: Buffers that contain free amines such as Tris and glycine must be avoided when using an amine-reactive reagent. Ammonium sulfate and ammonium must 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.
- *Reaction Temperature*: Most SC conjugations are done at room temperature. However, reduced temperature may be required for a particular SC labeling reaction.

Dansyl chloride [5-Dimethylaminonaphthalene-1-sulfonyl chloride]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
811	100 mg	269.75	372 nm	none	DMF	4 °C and desiccated



Features and Biological Applications

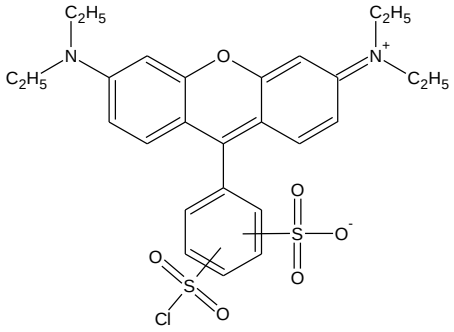
Dansyl chloride is non-fluorescent until it reacts with amines. The resulting Dansyl amides have environmentally sensitive fluorescence quantum yields and emission maxima along with large Stokes shifts. This environment-sensitive fluorescence property has made Dansyl chloride an important tool for biophysical studies. It is particularly useful for preparing fluorescent drug or ligand analogs that are expected to bind to hydrophobic sites in proteins, membranes or other biological receptors. Dansyl protein conjugates have fluorescence lifetimes in the range of 10 – 20 nanoseconds.

References

1. Bartzatt R (2001). Fluorescent labeling of drugs and simple organic compounds containing amine functional groups, utilizing Dansyl chloride in Na_2CO_3 buffer. *J Pharmacol Toxicol Methods* **45**, 247-53.
2. Kumar TK, *et al.* (1995). Fluorescent staining for proteins on polyacrylamide gels with 5-dimethylamino-1-naphthalenesulfonyl chloride (Dansyl chloride). *J Biochem Biophys Methods* **30**, 79-84.

Lissamine™ rhodamine B sulfonyl chloride

Cat. #	Size	MW	Abs	Em	Solvent	Storage
470	100 mg	577.11	568 nm	584 nm	DMF	4 °C and desiccated



Features and Biological Applications

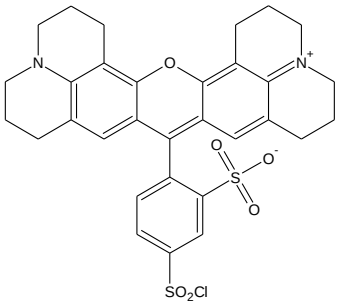
Lissamine™ rhodamine B sulfonyl chloride is a mixture of three isomeric sulfonyl chlorides. Its fluorescence emission spectrum lies between those of tetramethylrhodamines and X-rhodamines, and it can be well excited by 568 nm line of the Ar-Kr mixed gas laser that is used in many confocal laser-scanning microscopes. Like FITC reagents, LRB-SC is relatively inexpensive. However, it is quite labile in basic solutions, making it somewhat difficult to achieve reproducible conjugations.

References

1. Smith SN and Steer RP (2001). The photophysics of Lissamine rhodamine–B sulfonyl chloride in aqueous solution: implications for fluorescent protein–dye conjugates. *J Photochem Photobiol, A* **139**, 151.
2. Neves C, *et al.* (2000). Novel method for covalent fluorescent labeling of plasmid DNA that maintains structural integrity of the plasmid. *Bioconjug Chem* **11**, 51-5.

Sulforhodamine 101 sulfonyl chloride

Cat. #	Size	MW	Abs	Em	Solvent	Storage
71	10 mg	625.16	588 nm	601 nm	DMF	4 °C and desiccated



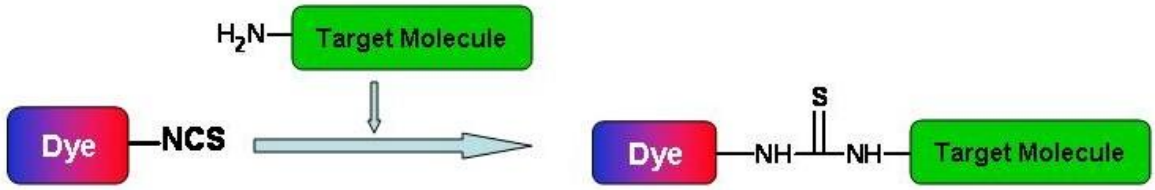
Features and Biological Applications

Sulforhodamine 101 sulfonyl chloride (also called Texas Red®) is the most popular labeling reagent of sulfonyl chloride. It is quite unstable in water, especially at the higher pH required for reaction with aliphatic amines. Protein modification by this reagent is frequently done at low temperature (preferably at 4 °C). This reagent reacts with amine compounds such as amino acids, peptides and proteins to give bright red fluorescent conjugates that are extremely stable, and resistant to protease-catalyzed hydrolysis. Unlike succinimidyl esters, sulfonyl chlorides react with both aliphatic amines and aromatic amines undiscriminatingly.

References

1. Larramendy ML, *et al.* (1998). Comparison of fluorescein isothiocyanate- and Texas red-conjugated nucleotides for direct labeling in comparative genomic hybridization. *Cytometry* **31**, 174-9.
2. Lefevre, C., *et al.*, Texas res-x and rhodamine red-x, new derivatives of sulforhodamine 101 and lissamine rhodamine b with improved labeling and fluorescence properties. *Bioconjug Chem* 1996, **7**, 482-9.
3. Schneider H (1989). Differential intracellular staining of identified neurons in Locusta with Texas Red and Lucifer Yellow. J Neurosci Methods **30**, 107-15.

Fluorescent Dye Isothiocyanates

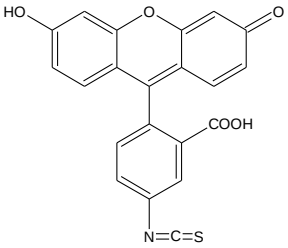


Isothiocyanates form thioureas upon reaction with amines. It is proven that some thiourea products (in particular, the conjugates from α-amino acids/peptides/proteins) are much less stable than the conjugates that are prepared from the corresponding succinimidyl esters. It has been reported that antibody conjugates prepared from fluorescein isothiocyanates deteriorate over time. We strongly recommend that you use succinimidy esters for your conjugations whenever possible. There are a few factors that need to be considered when SE compounds are used for conjugation reaction:

- *Solvents:* For the most part, reactive dyes are hydrophobic molecules and should be dissolved either in anhydrous dimethylformamide (DMF) or in dimethylsulfoxide (DMSO).
- *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.
- *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.
- *Reaction Temperature:* Most isothiocynate conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

FITC 'Isomer I' [5-FITC; fluorescein-5-isothiocyanate]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
120	100 mg	389.38	494 nm	519 nm	DMF	4 °C and desiccated



Features and Biological Applications

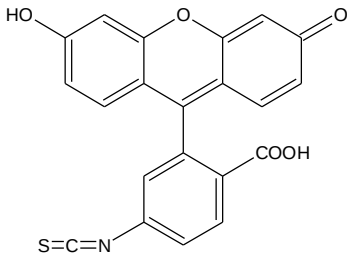
Despite the availability of alternative amine-reactive fluorescein derivatives that yield conjugates with superior stability and comparable spectra, fluorescein isothiocyanate (FITC) remains one of the most popular fluorescent labeling reagents, probably due to the low material cost. It appears that 5-FITC is more widely used than the 6-FITC isomer. FITC reagents are prominently used to label proteins. In addition, FITC has also been used to label peptides, oligonucleotides and other small organic ligands. *Cautions must be exercised for the storage of FITC conjugates.*

References

1. Hattori S, *et al*. (2002). Real-time zymography and reverse zymography: a method for detecting activities of matrix metalloproteinases and their inhibitors using FITC- labeled collagen and casein as substrates. *Anal Biochem* **301**, 27-34.
2. Thiagarajah JR, *et al*. (2001). Evidence of amiloride-sensitive fluid absorption in rat descending colonic crypts from fluorescence recovery of FITC-labeled dextran after photobleaching. *J Physiol* **536**, 541-53.

FITC 'Isomer II' [6-FITC, fluorescein-6-isothiocyanate]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
125	100 mg	389.38	494 nm	520 nm	DMF	4 °C and desiccated



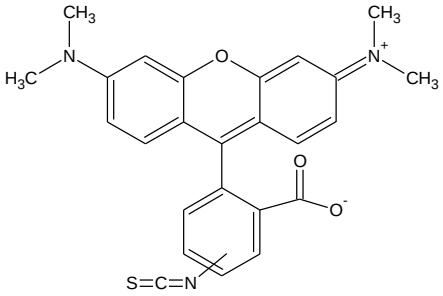
Features and Biological Applications
5-FITC and 6-FITC have very similar absorption and fluorescence spectra. However, the isomers may differ in their binding and reactivities to proteins, and the conjugates may migrate differently in electrophoretic gels. Thus, we offer highly purified single isomers. *Cautions must be exercised for the storage of FITC conjugates.*

References

1. Balogh, P., *et al.*, Hapten-mediated identification of cell membrane antigens using an anti-FITC monoclonal antibody. *J Immunol Methods* 1994, **169**, 35-40.
2. Korting, H.J. and E. Schubert, Preparation of FITC-labeled permanent preparations for fluorescent antibody technic]. *Z Med Lab Diagn* 1977, **18**, 387.
3. Gunduz, N., The use of FITC-conjugated monoclonal antibodies for determination of s-phase cells with fluorescence microscopy. *Cytometry* 1985, **6**, 597-601.

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

Cat. #	Size	MW	Abs	Em	Solvents	Storage
410	10 mg	443.52	543 nm	571 nm	DMF or DMSO	4 °C and desiccated



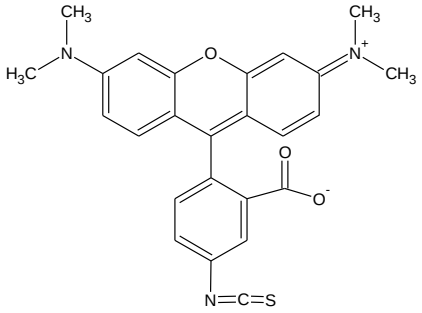
Features and Biological Applications
5(6)-TRITC is an amino-reactive labeling reagent that is widely used in preparing bioconjugates of proteins and nucleic acids. The resultant conjugates have similar spectral properties to those prepared from 5(6)-TAMRA, SE. However, the latter conjugates are much 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	MW	Abs	Em	Solvents	Storage
415	5 mg	443.52	543 nm	571 nm	DMF or DMSO	4 °C and desiccated



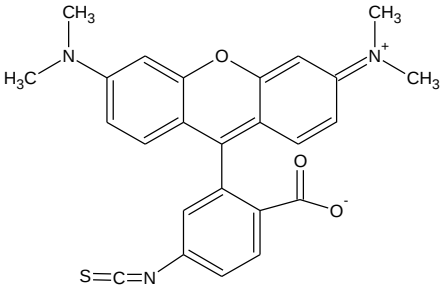
Features and Biological Applications
5-TRITC (also called G isomer) is a purified single isomer of the 5(6)-TRITC mixed isomers. This labeling reagent is predominantly used in labeling peptides and proteins. *Cautions must be exercised for the storage of TRITC conjugates.*

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	MW	Abs	Em	Solvent	Storage
417	5 mg	443.52	544 nm	572 nm	DMF	4 °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. Complementary to 5-TRITC, the 6-isomer is predominantly used in labeling nucleotides and nucleic acids. *Cautions must be 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.

Other Amine-Reactive Fluorescent Reagents

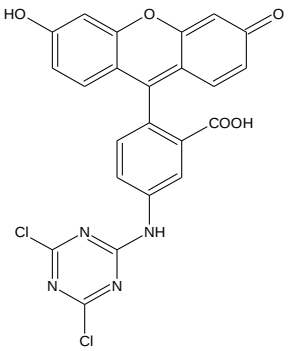
Electron-deficient aryl halides are effective amine-reactive labeling reagents for the preparation of various bioconjugates. AAT Bioquest offers fluorescamine, NBD chloride, NBD fluoride, AAT fluoride, SBD fluoride, 5- and 6-fluorescein dichlorotriazines. Due to their low selectivity, these dyes also react with thiol moieties besides amino groups. 5- and 6-fluorescein dichlorotriazines may even react with hydroxy compounds such as carbohydrates.

Non-fluorescent NBD chloride readily reacts with primary aliphatic amines (such as amino acids), generating bright yellow fluorescent amine adducts. NBD also reacts with thiols, although these adducts absorb and emit at shorter wavelengths and are less fluorescent than amine derivatives. NBD fluoride usually yields the same products as NBD chloride but is much more reactive. NBD fluoride may even react with hydroxy group under harsh conditions when amino or thiol groups are not available. The absorption and fluorescence emission spectra, quantum yields and extinction coefficients of NBD conjugates are all markedly dependent on the surrounding environment. Like the Dansyl dye adducts, the fluorescence quantum yield of NBD adducts of amines in water are very low (<0.01). NBD adducts of secondary amines are less fluorescent than that of primary amines while the adducts of aromatic amines with NBD are essentially non-fluorescent. There are a few factors that need to be considered when electron-deficient aryl halides are used for conjugation reaction.

- *Solvents:* For the most part, electron-deficient aryl halides are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- *Reaction pH:* The labeling reactions of amines with electron-deficient aryl halides are strongly pH dependent. A pH of 7.5–9.5 is usually optimal for modifying lysine residues.
- *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 readily react with the labeling reagent.
- *Reaction Temperature:* Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

5-DTAF [5-(4,6-Dichlorotriazinyl)aminofluorescein]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
200	25 mg	495.28	492 nm	517 nm	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

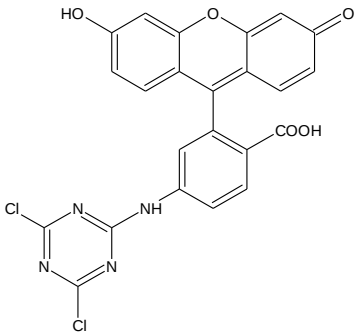
The 5-isomer of fluorescein dichlorotriazine (5-DTAF) is highly reactive with proteins. Unlike other reactive fluoresceins, 5-DTAF not only reacts with amino groups, but also reacts with thiol groups and even directly reacts with hydroxy groups such as polysaccharides and other alcohols in aqueous solution at pH above 9. Due to its high reactivity, 5-DTAF is also used to label carbohydrates.

References

1. Prigent-Richard S, et al. (1998). Fluorescent and radiolabeling of polysaccharides: binding and internalization experiments on vascular cells. *J Biomed Mater Res* **40**, 275-81.
2. Blakeslee D (1977). Immunofluorescence using dichlorotriazinylaminofluorescein (DTAF). II. Preparation, purity and stability of the compound. *J Immunol Methods* **17**, 361-4.

6-DTAF [6-(4,6-Dichlorotriazinyl)aminofluorescein]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
204	25 mg	495.28	492 nm	517 nm	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

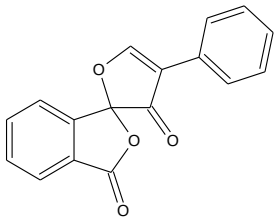
Compared to 5-DTAF, the 6-isomer is more preferably used for labeling oligonucleotides and nucleic acids. Like 5-DTAF, 6-DTAF not only reacts with amino groups, but also reacts with thiol groups and even directly reacts with hydroxy groups such as polysaccharides and other alcohols in aqueous solution at pH above 9. Due to its high reactivity, 6-DTAF is also used to label carbohydrates.

References

1. Prigent-Richard S, *et al.* (1998). Fluorescent and radiolabeling of polysaccharides: binding and internalization experiments on vascular cells. *J Biomed Mater Res* **40**, 275-81.
2. Blakeslee D (1977). Immunofluorescence using dichlorotriazinylaminofluorescein (DTAF). II. Preparation, purity and stability of the compound. *J Immunol Methods* **17**, 361-4.
3. Blakeslee D and Baines MG (1976). Immunofluorescence using dichlorotriazinylaminofluorescein (DTAF). I. Preparation and fractionation of labeled IgG. *J Immunol Methods* 13, 305-20.

Fluorescamine

Cat. #	Size	MW	Abs	Em	Solvent	Storage
820	25 mg	278.26	315 nm	none	DMSO	4 °C and desiccated



Features and Biological Applications

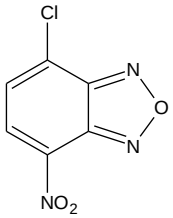
Fluorescamine is a popular amine-fluorogenic reagent for determining protein concentrations in solutions and on gels. It is also used to analyze low molecular weight amines by TLC, HPLC and capillary electrophoresis. Fluorescamine is nonfluorescent but readily reacts with primary aliphatic amines, including those in peptides and proteins, to yield a strongly blue-green fluorescent adduct that can be excited by UV light. The amine adduct has absorption maximum at 385 nm and fluorescence maximum at 486 nm.

References

1. Bantan-Polak, T., *et al.*, A comparison of fluorescamine and naphthalene-2,3-dicarboxaldehyde fluorogenic reagents for microplate-based detection of amino acids. *Anal Biochem* 2001, **297**, 128-36.
2. Guzman, N.A., *et al.*, Assay of protein drug substances present in solution mixtures by fluorescamine derivatization and capillary electrophoresis. *J Chromatogr* 1992, **598**, 123-31.
3. Larsson, L.I., *et al.*, Fluorescamine as a histochemical reagent: Demonstration of polypeptide hormone-secreting cells. *Histochemistry* 1975, **44**, 245-51.
4. Nakamura, H., Thin-layer chromatography of histidine, histamine and histidyl peptides at picomole level using a unique fluorogenic reaction with fluorescamine. *J Chromatogr* 1977, **131**, 215-22.

NBD-Cl [4-Chloro-7-nitrobenzofurazan]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
825	100 mg	199.55	337 nm	none	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

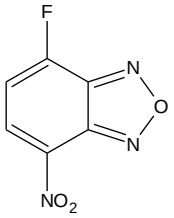
NBD-Cl is widely used to label peptides, proteins, drugs and other biomolecules. It is also a popular derivatizing reagent for HPLC analysis. NBD-Cl is non-fluorescent, and generates fluorescent adducts upon reacting with aliphatic amines or thiol compounds. It reacts with amino groups such as aliphatic amines, amino acids, peptides, and proteins to form highly fluorescent compounds. The NBD-amine adducts have λ_{ex} = 464 nm and λ_{em} = 512 nm in aqueous solutions. The fluorescence spectra of NBD-amine adducts are highly environment-sensitive, and the fluorescence intensity decreases significantly in aqueous solutions. NBD-Cl also reacts with thiol group to form fluorescent adducts, but the thiol adducts are much less fluorescent than the amine adducts.

References

1. Santa, T., *et al.*, Design and synthesis of a hydrophilic fluorescent derivatization reagent for carboxylic acids, 4-n-(4-n-aminoethyl)piperazino-7-nitro-2,1,3-benzoxadiazole (NBD-PZ-NH2), and its application to capillary electrophoresis with laser-induced fluorescence detection. *Biomed Chromatogr* 2002, **16**, 523-8.
2. Brenner, B., *et al.*, Fluorescence of NBD-labelled troponin-i as a probe for the kinetics of thin filament activation in skeletal muscle fibers. *Adv Exp Med Biol* 1998, **453**, 177-84.
3. Cowley, D.J. and A.J. Schulze, Conformational dynamics and kinetics of peptide antagonist interactions with interleukin-1 receptor. Fluorescence studies using the NBD-labelled peptide af12415. *J Pept Res* 1997, **49**, 444-54.

NBD-F [4-Fluoro-7-nitrobenzofurazan]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
821	5 mg	183.10	337 nm	none	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

NBD-F has properties and applications similar to those of NBD-Cl. Upon reacting with amines and thiol compounds it generates the same fluorescent adducts as those of NBD-Cl. Compared with NBD-Cl, NBD-F is much more reactive, and should be more carefully stored. For example, the reaction of NBD fluoride with glycine is reported to be 500 times faster than the reaction of NBD chloride with glycine. Both NBD chloride and NBD fluoride are extensively used as derivatization reagents for chromatographic analysis of amino acids and other low molecular weight amines.

References

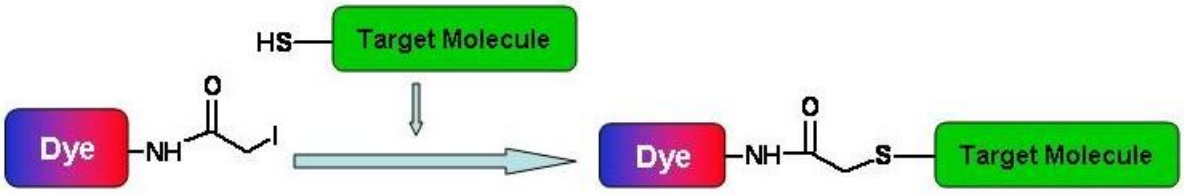
1. Suzuki S, *et al*. (2001). Rapid analysis of amino sugars by microchip electrophoresis with laser-induced fluorescence detection. *Electrophoresis* **22**, 4023-31.
2. Tani M, *et al*. (1998). Enzymatic synthesis of omega-amino-ceramide: preparation of a sensitive fluorescent substrate for ceramidase. *Anal Biochem* **263**, 183-8.

Thiol-Reactive Fluorescent Dyes

Because free thiol (SH) groups, also called mercapto groups, are not present as abundantly as amino groups in most biopolymers such as proteins and nucleic acids, thiol-reactive reagents often provide a means of selectively modifying a protein at a defined site. Therefore thiol-reactive dyes are often used to prepare fluorescent peptides, proteins and oligonucleotides for probing biological structures, functions and interactions. Thiol-reactive dyes have been used to develop probes for analyzing the topography of proteins in biological membranes, determining distances within the protein or between the proteins and monitoring the changes in protein conformation using environment-sensitive probes.

There are many types of thiol-reactive dyes reported in the literature, including iodoacetamides, disulfides, maleimides, vinyl sulfones and various electron-deficient aryl halides and sulfonates. Iodoacetamides and maleimides are by far the most popular thiol-reactive moieties.

Fluorescent Dye Iodoacetamides (IA)

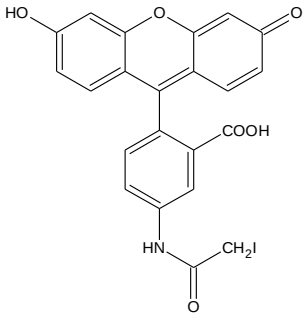


Iodoacetamides are one of the most popular thiol-reactive moieties for labeling biopolymers and small biomolecules. Iodoacetamides readily react with thiol moieties of biopolymers and small biomolecules to form thioether conjugates. The thioether bond formed is quite stable. Although iodoacetamides generally have good selectivity to thiol groups, they may react with histidine or potentially tyrosine under higher pH if free thiols are not readily available. The bioconjugation reactions of thiol-reactive probes can be quenched by the addition of cysteine, glutathione or mercaptosuccinic acid to the reaction mixture, forming highly water-soluble adducts that are easily removed by dialysis or gel filtration. There are quite a few factors that need to be considered when iodoacetamides are used for conjugation reaction:

- *Solvents:* Most iodoacetamide dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF). Dimethyl sulfoxide (DMSO) should be avoided whenever possible since some particular iodoacetamides may be oxidized in DMSO at elevated temperature.
- *Reaction pH:* The labeling reactions of thiol compounds with iodoacetamides are strongly pH dependent. Thiol-reactive reagents more readily react with thiol groups (such as cysteine and reduced glutathione) at higher pH. However, higher pH also increases the oxidative dimerization of thiol compounds. Thus thiol conjugations of iodoacetamides are often run in carbonate buffers with a pH ranging from 7.5 to 9.5. A pH of 8.5–9.5 is usually optimal for modifying cysteine residues.
- *Reaction Buffers:* High concentrations of nucleophilic thiol compounds should also be avoided because they compete for the labeling reagent and, as a result, decrease conjugation yields. Buffers that contain free amines such as Tris and glycine should be avoided whenever possible since some iodoacetamides may also react with amines. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation should be removed before performing dye conjugations.
- *Reaction Temperature:* Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.
- *Light Sensitivity:* Iodo compounds are known to be very light-sensitive, especially in solution. Thus, we recommend the reactions of iodoacetamides with biomolecules be carried out under subdued light.
- *Avoiding Oxygen:* Air oxidation of thiol compounds (to disulfides) is a major competing reaction for the iodoacetamide modifications of thiol componunds. It is recommended that air exposure of reaction solution should be minimized whenever possible.

5-IAF [5-Iodoacetamidofluorescein]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
222	25 mg	515.25	493 nm	515 nm	DMSO	4 °C and desiccated



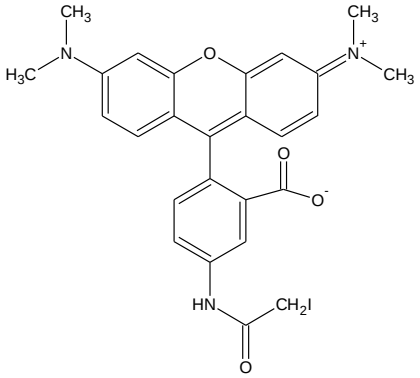
Features and Biological Applications
5-IAF is one of the most popular green fluorescent dyes for thiol modifications of proteins along with fluorescein –5-maleimide.

References

1. Hughes, E.A., *et al.*, Fine specificity analysis indicates that the primary and secondary fluorescein-specific cytotoxic t cell receptor repertoires are indistinguishable. *Immunol Cell Biol* 1995, **73**, 153-7.
2. Marya, P.K., *et al.*, Characterization of an active, fluorescein-labelled kinesin. *Eur J Biochem* 1990, **193**, 39-45.
3. Bishop, J.E., *et al.*, (iodoacetamido)fluorescein labels a pair of proximal cysteines on the ca2+-atpase of sarcoplasmic reticulum. *Biochemistry* 1988, **27**, 5233-40.
4. Bock, P.E., *et al.*, Protein-protein interactions in contact activation of blood coagulation. Binding of high molecular weight kininogen and the 5-(iodoacetamido) fluorescein-labeled kininogen light chain to prekallikrein, kallikrein, and the separated kallikrein heavy and light chains. *J Biol Chem* 1985, **260**, 12434-43.

5-TMRIA [Tetramethylrhodamine-5-iodoacetamide]

Cat. #	Size	MW	Abs	Em	Solvents	Storage
413	5 mg	569.39	541 nm	567 nm	DMF or DMSO	4 °C and desiccated

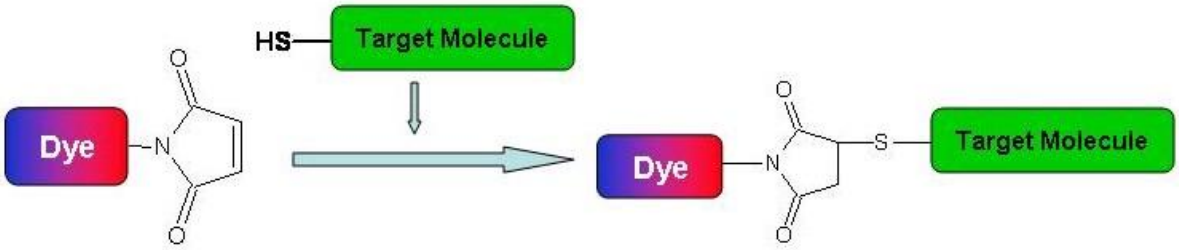


Features and Biological Applications
Tetramethylrhodamine iodoacetamides (TMRIA) are thiol-selective reactive dyes that are used to label proteins *via* the cysteine residues. 5-TMRIA, the pure 5-isomer of TMRIA, is increasingly preferred for some particular applications since the mixed isomers of TMRIA may give different results from batch to batch due to the varying ratios and different reactivities of the two isomers. For example, 5-TMRIA is reported to predominantly label SH-1 (Cys-707) of the myosin heavy chain in skinned muscle fibers.

References

1. Martyn DA, *et al.* (2001). Ca^{2+}- and cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle. *Biophys J* **80**, 360-70.
2. Ajtai K and Burghardt TP (1995). Conformation of xanthene dyes in the sulfhydryl 1 binding site of myosin. 2. *Biochemistry* **34**, 15943-52.

Fluorescent Dye Maleimides

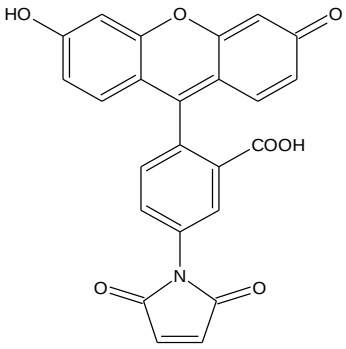


Maleimides and iodoacetamides are by far the most popular thiol-reactive moieties. Maleimides readily react with thiol moieties of biopolymers to form thioether conjugates even under neutral conditions. The thioether bond formed is quite stable. Maileimides are generally much less light-sensitive than iodoacetamides. The latter compounds are known to be very light liable, especially in solution.

Maleimides require conjugation conditions less stringent than those of iodoacetamides as described above. Unlike iodoacetamides, maleimides do not react with histidine and methionine under physiological conditions. For example, most conjugations can be done at room temperature at neutral pH. However, either elevated or reduced pH or temperature may be required for a particular labeling reaction.

Fluorescein-5-maleimide

Cat. #	Size	MW	Abs	Em	Solvents	Storage
130	25 mg	427.36	493 nm	515 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications

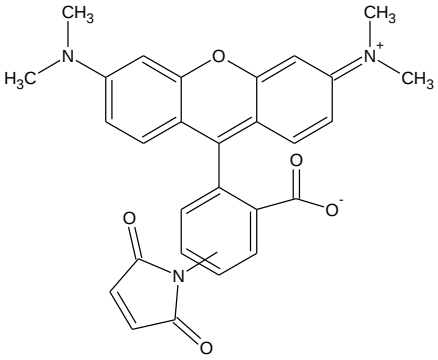
Maleimides are among the most frequently used reagents for thiol modification. In most proteins, the site of reaction is at cysteine residues, which either are intrinsically present or result from reduction of cystines. Unlike iodoacetamides, maleimides do not react with histidine and methionine under physiological conditions. Fluorescein-5-maleimide is one of the most popular fluorescent dyes for thiol modifications of proteins along with 5-TMRIA.

References

1. Mehan, R.S., *et al.*, Mapping out regions on the surface of the aspartate receptor that are essential for kinase activation. *Biochemistry* 2003, **42**, 2952-9.
2. Bullok, K.E., *et al.*, Characterization of novel histidine-tagged tat-peptide complexes dual-labeled with (99m)tc-tricarbonyl and fluorescein for scintigraphy and fluorescence microscopy. *Bioconjug Chem* 2002, **13**, 1226-37.

Tetramethylrhodamine-5-(and-6)-maleimide

Cat. #	Size	MW	Abs	Em	Solvents	Storage
412	25 mg	481.51	540 nm	567 nm	DMF or DMSO	4 °C and desiccated



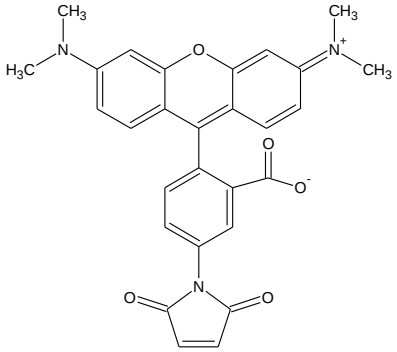
Features and Biological Applications
Tetramethylrhodamine-5-(and-6)-maleimide is an excellent reagent for preparing orange fluorescent bioconjugate through thiol modifications of peptides, nucleotides, nucleic acids and proteins. Maleimides are among the most frequently used reagents for thiol modification. In most proteins, the sites of reactions are at cysteine residues, which either are intrinsically present or result from reduction of cystines. Unlike iodoacetamides, maleimides do not react with histidines and methionines under physiological conditions.

References

1. Geibel S, *et al.* (2003). Conformational dynamics of the Na^+/K^+-ATPase probed by voltage clamp fluorometry. *Proc Natl Acad Sci USA* **100,** 964-9.
2. Cha A and Bezanilla F (1998). Structural implications of fluorescence quenching in the Shaker K^+ channel. *J Gen Physiol* **112,** 391-408.

Tetramethylrhodamine-5-maleimide

Cat. #	Size	MW	Abs	Em	Solvents	Storage
421	5 mg	481.51	540 nm	567 nm	DMF or DMSO	4 °C and desiccated



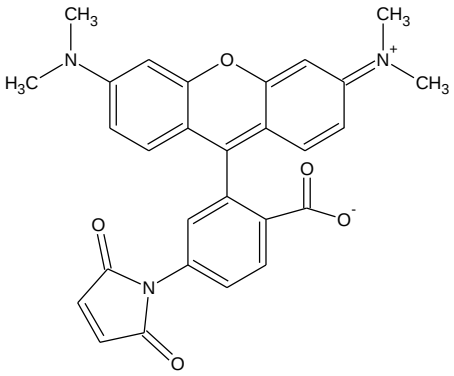
Features and Biological Applications
Tetramethylrhodamine-5-maleimide is the purified single isomer of tetramethylrhodamine-5 (and 6)-maleimide mixed isomers. It is widely used for thiol modifications of peptides and proteins. It is preferred for some complicated biological applications where reproducibility is more critical than material cost since the minor positional difference between the 5-isomer and 6-isomer might significantly affect some biological properties of the underlying conjugates. The 5-isomer is predominantly used to label peptides and proteins.

References

1. Gagnon, D.G., *et al.*, Membrane topology of loop 13-14 of the na(+)/glucose cotransporter (sglt1): A scam and fluorescent labelling study. *Biochim Biophys Acta* 2005, **1712**, 173-84.
2. Chen, Y. and S.S. Lehrer, Distances between tropomyosin sites across the muscle thin filament using luminescence resonance energy transfer: Evidence for tropomyosin flexibility. *Biochemistry* 2004, **43**, 11491-9.

Tetramethylrhodamine-6-maleimide

Cat. #	Size	MW	Abs	Em	Solvent	Storage
419	5 mg	481.51	542 nm	568 nm	DMF	4 °C and desiccated



Features and Biological Applications

Tetramethylrhodamine-6-maleimide is the other purified single isomer of tetramethylrhodamine-5 (and 6)-maleimide mixed isomers. Compared to the 5-isomer, tetramethylrhodamine-6-maleimide is preferably used for thiol modifications of nucleotides and nucleic acids.

References

1. Dempski, R.E., *et al.*, The beta subunit of the na+/k+-atpase follows the conformational state of the holoenzyme. *J Gen Physiol* 2005, **125**, 505-20.
2. Loo, D.D., *et al.*, Perturbation analysis of the voltage-sensitive conformational changes of the na+/glucose cotransporter. *J Gen Physiol* 2005, **125**, 13-36.

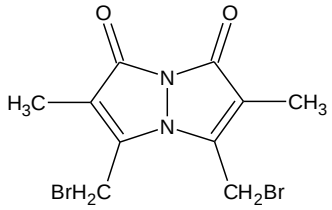
Other Thiol-Reactive Fluorescent Dyes

NBD chloride was first introduced as a fluorogenic derivatization reagent for amines. It also reacts with thiols to form adducts that absorb and emit at shorter wavelengths. NBD and SBD compounds are widely used for fluorogenic thiol modifications of biopolymers. In general, NBD compounds are more often used for modifying amino groups and SBD compounds for selective modifications of thiol groups. The absorption and fluorescence emission spectra, quantum yields and extinction coefficients of NBD-thiol conjugates are dependent on the surrounding environment. SBD is an analog of NBD. SBD fluoride has been used for the derivatization of both amino and thiol groups. Quite a few excellent reviews have been published for the applications of SBD, NBD and SBD compounds (see the reference listed on the end of this chapter). Most of NBD and SBD compounds react with both amino and thiol groups except that SBD-Cl has good selectivity to thiol groups. Additionally, NBD and SBD compounds are widely used for HPLC derivatizations.

Bromobimanes including monobromobimane and dibromobimane are another class of popular thiol-reactive fluorescent tags, and is widely used to detect various thiol-containing biomolecules such as glutathione in cells. It is fluorogenic upon reacting with thiol-containing molecules. The monobromobimanes are essentially non-fluorescent until they react with several low molecular weight thiols, including glutathione, *N*-acetylcysteine, mercaptopurine, peptides and plasma thiols, as well as with carboxylic acids. Monobromobimane is the most extensively used bimane derivative. These reagents are also useful for detecting the distribution of protein thiols in cells before and after chemical reduction of disulfides. Both monobromobimane and the more thiol-selective monochlorobimane have been extensively used for detecting glutathione in live cells. Monobromobimane can also be used to derivatize thiol-containing proteins prior to separation by isoelectric focusing without appreciably modifying the protein's electrophoretic mobility. Dibromobimane is an interesting crosslinking reagent for proteins because it is unlikely to fluoresce until *both* of its alkylating groups have reacted.

*d*BBr [Dibromobimane]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
634	25 mg	350.01	395 nm	490 nm	DMSO	4 °C and desiccated



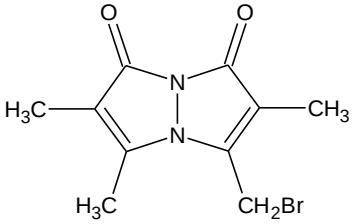
Features and Biological Applications
*d*BBr is one of the most popular thiol-reactive fluorescent tags, and is widely used to detect various thiol-containing biomolecules such as glutathione in cells. It is fluorogenic upon reacting with thiol-containing molecules. Dibromobimane is a unique fluorogenic crosslinking reagent for proteins because it is unlikely to fluoresce until *both* of its alkylating groups have reacted. It has been used to crosslink thiols in myosin, hemoglobin, *Escherichia coli* lactose permease and mitochondrial ATPase. Dibromobimane was also used to probe for the proximity of dual-cysteine mutagenesis sites in ArsA ATPase and to crosslink thiols in actin, myosin and P-glycoprotein.

References

1. Sinz, A. and K. Wang, *Mapping spatial proximities of sulfhydryl groups in proteins using a fluorogenic cross-linker and mass spectrometry.* Anal Biochem, 2004. **331**(1): p. 27-32.
2. Kim, E., et al., *Cross-linking constraints on F-actin structure.* J Mol Biol, 2000. **299**(2): p. 421-9.
3. Kosower, N.S., et al., *Bimane fluorescent labels. Characterization of the bimane labeling of human hemoglobin.* Biochim Biophys Acta, 1980. **622**(2): p. 201-9.

*m*BBr [Monobromobimane]

Cat#	Size	MW	Abs	Em	Solvent	Storage
633	25 mg	271.11	395 nm	490 nm	DMSO	4 °C and desiccated



Features and Biological Applications
*m*BBr is one of the most popular thiol-reactive fluorescent tags, and is widely used to detect various thiol-containing biomolecules such as glutathione in cells. It is fluorogenic upon reacting with thiol-containing molecules. *m*BBr is used for the determination of the redox status of low molecular weight and protein thiols in biological systems. *m*BBr-based *in situ* derivatization results in maximal recovery of both free, reduced low molecular weight and bromobimane accessible protein thiols. The quantitation of the corresponding adducts of protein thiols is achieved by fluorescence spectroscopy following protein precipitation.

References

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Carbonyl-Reactive (Amine-Containing) Fluorescent Dyes and Their Applications

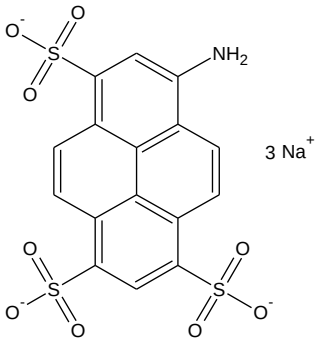
Amine-containing dyes are widely used to modify water-soluble biopolymers (such as proteins) through the formation of Schiff Base or reductive amination. Among them, fluorescently labeled cadaverine and lysine derivatives and a variety of hydrazides have been predominantly used for modifying biomolecules. These dyes are used for modifications of carbohydrates, glycoproteins and nucleic acids that are first periodate-oxidized to introduce aldehydes and ketones into the biopolymers for subsequent reductive amination. The combination of periodate oxidation with reductive amination provides an effective way for site-selective modifications of biopolymers. For example, periodate oxidation of the 3'-terminal ribose is reported to be one of the few methods of selectively modifying RNA. Periodate-oxidized ribonucleotides are converted to fluorescent nucleotide probes by reaction with fluorescent hydrazines and amines.

Amine-containing dyes are also used to modify biopolymers (such as proteins) using water-soluble carbodiimides (such as EDC) to convert the carboxy groups of the biopolymers into amide groups. Either NHS or NHSS may be used to improve the coupling efficiency of EDC-mediated protein–carboxylic acid conjugations. A large excess of the amine-containing dyes is usually used for EDC-mediated bioconjugations in concentrated protein solutions at low pH to reduce intra- and inter-protein coupling to lysine residues, a common side reaction.

The amine-containing dyes are also valuable building blocks in bioorganic and medicinal chemistry. We have used our amine-containing dyes to custom-synthesize many fluorescently labeled drugs, natural toxins and biological ligands.

APTS [8-Aminopyrene-1,3,6-trisulfonic acid, trisodium salt]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
624	10 mg	523.39	424 nm	505 nm	water	4 °C and desiccated



Features and Biological Applications

This water-soluble reagent has strong green fluorescence with great Stokes shift. It is widely used for detecting carbohydrates and labeling glycoproteins in electrophoresis. It is also used to modify other carbonyl-containing biomolecules such as aldehydes and ketones.

References

1. Dang F, *et al.* (2003). Ultrafast analysis of oligosaccharides on microchip with light-emitting diode confocal fluorescence detection. *Electrophoresis* **24,** 714-21.
2. Suzuki, S. and S. Honda, Miniaturization in carbohydrate analysis. *Electrophoresis* 2003, **24**, 3577-82.
3. Blennow, A., *et al.*, The molecular deposition of transgenically modified starch in the starch granule as imaged by functional microscopy. *J Struct Biol* 2003, **143**, 229-41.
4. Chen, F.T., *et al.*, Quantitative analysis of sugar constituents of glycoproteins by capillary electrophoresis. *Glycobiology* 1998, **8**, 1045-52.
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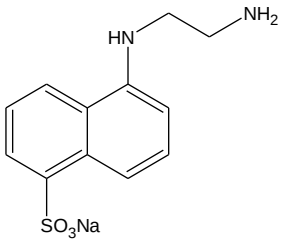
EDANS, sodium salt [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid, sodium salt]

Cat. #	Size	MW	Abs	Em	Solvent	Storage
615	1 g	288.30	335 nm	493 nm	DMSO	4 °C and desiccated



Features and Biological Applications
EDANS, sodium salt is one of the most popular donors for developing FRET-based nucleic acid probes that is widely used in real time PCR assays. In addition, it is also used for developing protease substrates. EDANS, sodium salt is often paired with DABCYL or DABSYL in FRET-based probes. Its fluorescence is environment-sensitive.

References

1. Chen, S., *et al.*, Enzymatic activity characterization of sars coronavirus 3c-like protease by fluorescence resonance energy transfer technique. *Acta Pharmacol Sin* 2005, **26**, 99-106.
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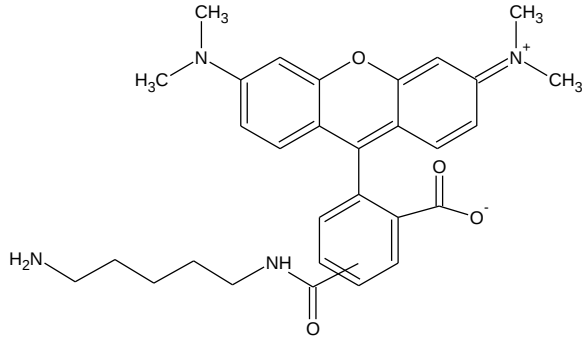
5(6)-TAMRA cadaverine [Tetramethylrhodamine 5-(and -6)-carboxamide cadaverine

Cat. #	Size	MW	Abs	Em	Solvents	Storage
355	100 mg	514.62	544 nm	570 nm	DMF or DMSO	4 °C and desiccated



Features and Biological Applications
5(6)-TAMRA cadaverine has bright orange fluorescence. It is a good glutamate transglutaminase substrate and a useful building block for small fluorescent molecules. It can be coupled to carboxy groups *via* EDC-mediated reactions.

References

1. Nurminskaya MV, *et al.* (2002). Transglutaminase factor XIIIA in the cartilage of developing avian long bones. *Dev Dyn* **223**, 24-32.
2. Patricelli MP, *et al.* (2001). Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* **1**, 1067.

TR cadaverine [Sulforhodamine 101 cadaverine sulfonamide]
TR hydrazide [Sulforhodamine 101 sulfonyl hydrazide]

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

References

1. Lakowicz JR (1991-2003). *Topics in Fluorescence Spectroscopy: Techniques (Volume 1, 1991); Principles (Volume 2, 1991); Biochemical Applications (Volume 3, 1992)*; *Probe Design and Chemical Sensing (Volume 4, 1994)*; *Nonlinear and Two-Photon Induced Fluorescence (Volume 5, 1997)*; *Protein Fluorescence (Volume 6, 2000)*; *DNA Technology (Volume 7, 2003)*, Plenum Publishing, New York.
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