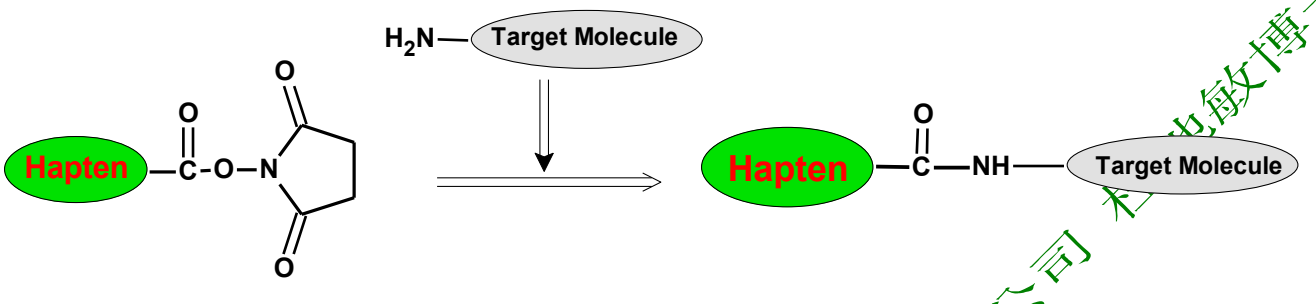
Fluorescent Dye Carboxylic Acids and Their Succinimidyl Esters



Succinimidyl esters are proven to be the best reagents for amine modifications because the amide bonds that are formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. There are few factors that need be considered when SE compounds are used for conjugation reaction:

1). *Solvents:* For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

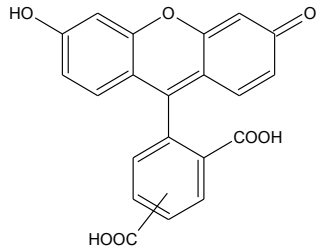
2). *Reaction pH:* The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas isothiocyanates may require a pH 9.0-10.0 for optimal conjugations.

3). *Reaction Buffers:* Buffers that contain free amines such as Tris and glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed (such as viadinlysis) before performing dye conjugations.

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

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

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
100	250 mg	$49	376.32	494 nm (pH>9.0)	519 nm (pH>9.0)	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

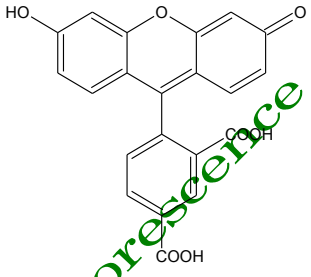
Carboxyfluorescein (commonly called FAM) and its amine-reactive succinimidyl esters are favored over FITC in bioconjugations. FAM reagents give carboxamides that are more resistant to hydrolysis. In addition, FAM reagents require less stringent conjugation conditions and give better conjugation yields, 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	Price	MW	Abs	Em	Soluble in	Storage
103	100 mg	$49	376.32	492 nm (pH>9.0)	518 nm (pH>9.0)	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

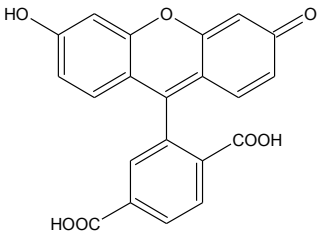
5-FAM is the purified single isomer of carboxyfluorescein. It is one of the most popular green fluorescent reagents used for labeling peptides, proteins and nucleotides. It has been predominantly used to develop a variety of green fluorescent peptides that can be excited with the 488 nm line of the Ar 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.
4. Kemenes, G., *et al.*, Photoinactivation of neurones axonally filled with the fluorescent dye 5(6)-carboxyfluorescein in the pond snail, lymnaea stagnalis. *J Neurosci Methods* 1991, **39**, 207-16.

6-FAM [6-Carboxyfluorescein]

Cat#	Size	Price	MW	Abs	Em	Soluble in	Storage
106	100 mg	$49	376.32	495 nm (pH>9.0)	517 nm (pH>9.0)	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

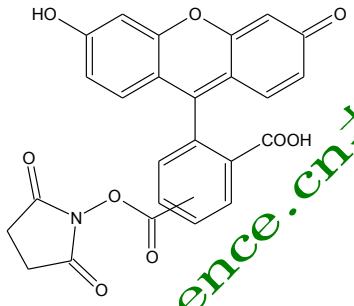
6-FAM is the other purified isomer of 5(6)-carboxyfluorescein. Complimemtary to 5-FAM isomer, 6-FAM is mainly used in labeling nucleotides and nucleic acids.

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	Price	MW	Abs	Em	Soluble in	Storage
110	25 mg	$49	473.39	494 nm (pH>9.0)	519 nm (pH>9.0)	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

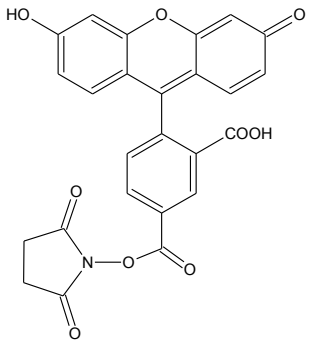
5(6)-FAM, SE is the amine-reactive succinimidyl ester of FAM acid. It is favored over FITC in bioconjugations. FAM reagents give carboxamides that are more resistant to hydrolysis. In addition, FAM reagents require less stringent conjugation conditions and give better conjugation yields, and the resulted 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	Price	MW	Abs	Em	Soluble in	Storage
113	10 mg	$79	473.39	492 nm (pH>9.0)	518 nm (pH>9.0)	DMSO or DMF	4 °C and desiccated



Features and Biological Applications

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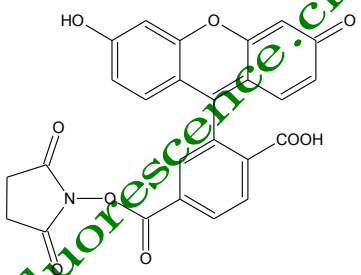
1. Sakamoto M, *et al.* (2003). Application of terminal RFLP analysis to characterize oral bacterial flora in saliva of healthy subjects and patients with periodontitis. *J Med Microbiol* **52,** 79-89.
2. Hahn M, *et al.* (2001). Influence of fluorophore dye labels on the migration behavior of polymerase 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	Price	MW	Abs	Em	Soluble in	Storage
116	10 mg	$127	473.39	495 nm (pH>9.0)	517 nm (pH>9.0)	DMSO or DMF	Refrigerated 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.