Fluorescent Peptide-Labeling Dyes

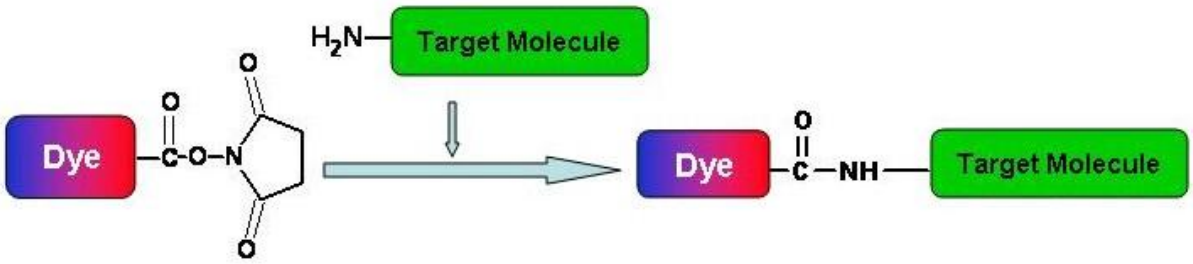
Dye-labeled fluorescent peptides are important tools in biochemical and cellular studies. The most important characteristics of fluorescent peptides are high sensitivity and non-radioactive detection. Fluorescent peptides have been widely used in fluorescence fluorimetry, fluorescence microscopy, fluorescence polarization spectroscopy, time-resolved fluorescence and fluorescence resonance energy transfer (FRET). Fluorescent peptides participating in peptide-receptor interactions can be monitored to determine the location of receptors in cells or tissues, to allow the quantification of receptors, to determine the receptor affinity for various unknown ligands (drug screening), and to identify various populations of cells endowed with peptide receptors. FRET peptides are widely used for detecting the activities of proteases and protein kinases. Other applications include receptor sorting using FACS (fluorescence-associated cell sorting) and measurement of serum peptide levels using FIA (fluorescent immunoassays) either *in vivo or in vitro* for research or diagnostic purposes.

In general, the preferred fluorescent labels should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules. A fluorescent dye can be attached to a peptide at a specific point through a covalent bond depending on the sequence of peptide. The linkage between dye and peptide is a covalent bond, which is stable and not destructive under most biological conditions. In some cases, a functional linker is introduced between dye and peptide to minimize the alteration of peptide biological activity. For all the peptide labeling, the dye needs to be attached at a defined position: N-terminus, C-terminus, or in the middle of sequence. AAT Bioquest offers a variety of fluorescent labeling reagents for facilitating the conjugation of dyes to peptides which are used for many biological studies.

N-Terminal Peptide Labeling Using Amine-Reactive Fluorescent Dyes

Amine-reactive fluorescent probes are widely used to modify peptides at the N-terminal or lysine residue. A number of fluorescent amino-reactive dyes have been developed to label various peptides, and the resultant conjugates are widely used in biological applications. Three major classes of amine-reactive fluorescent reagents are currently used to label peptides: succinimidyl esters (SE), isothiocyanates and sulfonyl chlorides. AAT Bioquest offers all the popular amine-reactive fluorescent dyes for peptide labeling. Although FITC (fluorescein isothiocyanate), one of the most popular fluorescent labeling dyes, is predominantly used for preparing a variety of fluorescent bioconjugates, its low conjugation efficiency and short shelf lifetime of FITC conjugates are still troublesome for some critical biological applications. We strongly recommend that you choose succinimidyl esters for labeling needs if other conditions and factors are equal.

Fluorescent Dye Carboxylic Acids and Their Succinimidyl Esters



Succinimidyl esters (SE) 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 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 in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

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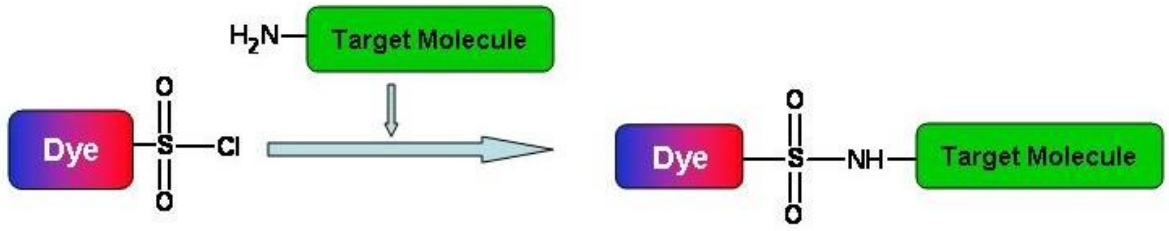
- *Reaction pH:* The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out at pH above 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.
- *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 viadialysis) 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.

Fluorescent Dye Sulfonyl Chlorides

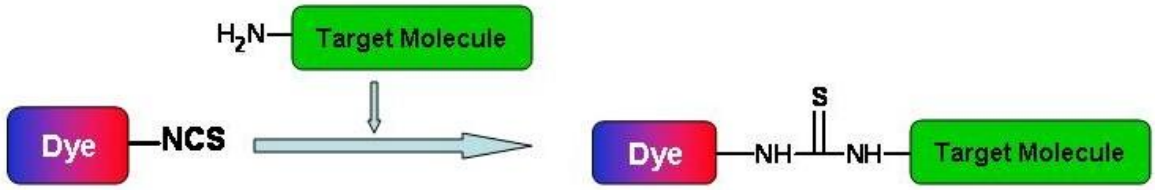


Sulfonyl chlorides are highly reactive. These reagents are unstable in water, especially at the higher pH required for reaction with aliphatic amines. Molecular modifications by sulfonyl chlorides need to be carefully carried out preferably at low temperature. Sulfonyl chlorides can also react with phenols (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.

Fluorescent Dye Isothiocyanates



Isothiocyanates form thioureas upon reaction with amines. It is proven that some thiourea products (in particular, the conjugates prepared from α-amino acids/peptides/proteins) are much less stable than the conjugates 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 succinimidyl 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 in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- *Reaction pH:* The labeling reactions of amines with isothiocyanates are strongly pH dependent. Isothiocyanate 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:* Isothiocyanate conjugations are usually done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

Table 1. *AAT Bioquest Amine-Reactive Dyes for Labeling Peptides*

Cat. #	Product Name	Unit Size
479	California Red™ SE	1 mg
320	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed isomers*	100 mg
321	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed isomers*	1 g
322	5-CR110 [5-Carboxyrhodamine 110] *Single isomer*	5 mg
323	6-CR110 [6-Carboxyrhodamine 110] *Single isomer*	5 mg
350	5(6)-CR110, SE [5-(and 6)-Carboxyrhodamine 110, succinimidyl ester]	5 mg
351	5-CR110, SE [5-Carboxyrhodamine 110, succinimidyl ester] *Single isomer*	5 mg
352	6-CR110, SE [6-Carboxyrhodamine 110, succinimidyl ester] *Single isomer*	5 mg
330	5(6)-CR6G [5-(and 6)-Carboxyrhodamine 6G]	25 mg
331	5-CR6G [5-Carboxyrhodamine 6G] *Single isomer*	10 mg
332	6-CR6G [6-Carboxyrhodamine 6G] *Single isomer*	10 mg
340	5(6)-CR6G, SE [5-(and 6)-Carboxyrhodamine 6G, succinimidyl ester] *Mixed isomers*	10 mg
341	5-CR6G, SE [5-Carboxyrhodamine 6G, succinimidyl ester] *Single isomer*	1 mg
342	6-CR6G, SE [6-Carboxyrhodamine 6G, succinimidyl ester] *Single isomer*	1 mg
2001	DABCYL acid [4-((4-(Dimethylamino)phenyl)azo)benzoic acid] *UltraPure grade*	5 g
2002	DABCYL acid [4-((4-(Dimethylamino)phenyl)azo)benzoic acid] *UltraPure grade*	25 g
2003	DABCYL acid [4-((4-(Dimethylamino)phenyl)azo)benzoic acid] *UltraPure grade*	100 g
2004	DABCYL acid, SE [4-((4-(Dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester]	1 g

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2005	DABCYL acid, SE [4-((4-(Dimethylamino)phenyl)azo)benzoic acid, succinimidyl ester]	5 g
2030	DABSYL chloride [4-Dimethylaminoazobenzene-4-sulfonyl chloride]	1 g
811	Dansyl chloride [5-Dimethylaminonaphthalene-1-sulfonyl chloride]	100 mg
812	Dansyl-X, acid	5 g
813	Dansyl-X, SE	1 g
505	DEAC [7-Diethylaminocoumarin-3-carboxylic acid, succinimidyl ester]	100 mg
506	DEAC,SE [7-Diethylaminocoumarin-3-carboxylic acid, succinimidyl ester]	25 mg
2020	DNP-X acid [6-(2,4-Dinitrophenyl)aminohexanoic acid]	100 mg
2021	DNP-X acid, SE [6-(2,4-Dinitrophenyl)aminohexanoic acid, succinimidyl ester]	25 mg
100	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Mixed isomers*	1 g
101	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for labeling peptides and oligos*	10 g
102	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for labeling peptides and oligos*	25 g
103	5-FAM [5-Carboxyfluorescein] *Single isomer*	100 mg
104	5-FAM [5-Carboxyfluorescein] *Validated for labeling peptides*	1 g
105	5-FAM [5-Carboxyfluorescein] *Validated for labeling peptides*	5 g
106	6-FAM [6-Carboxyfluorescein] *Single isomer*	100 mg
107	6-FAM [6-Carboxyfluorescein] *Validated for labeling oligos*	1 g
108	6-FAM [6-Carboxyfluorescein] *Validated for labeling oligos*	5 g
110	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester] *Mixed isomers*	25 mg
111	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester]	100 mg
112	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester]	1 g
113	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester] *Single isomer*	10 mg
114	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester]	100 mg
115	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester]	1 g
116	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Single isomer*	10 mg
117	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Validated for labeling oligos*	100 mg
118	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Validated for labeling oligos*	1 g
120	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure Grade*	100 mg
121	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure Grade*	1 g
122	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure Grade*	10 g
125	6-FITC [FITC Isomer II, fluorescein-6-isothiocyanate] *UltraPure Grade*	5 g
820	Fluorescamine *UltraPure Grade*	25 mg
554	7-Hydroxy-4-methylcoumarin-3-acetic acid	100 mg
556	7-Hydroxy-4-methylcoumarin-3-acetic acid, succinimidyl ester	25 mg
550	7-Hydroxycoumarin-3-carboxylic acid	250 mg
551	7-Hydroxycoumarin-3-carboxylic acid, succinimidyl ester	50 mg
470	Lissamine Rhodamine B Sulfonyl Chloride [Sulforhodamine B sulfonyl chloride]	100 mg
560	7-Methoxycoumarin-3-carboxylic acid	1 g
561	7-Methoxycoumarin-3-carboxylic acid	5 g
563	7-Methoxycoumarin-3-carboxylic acid, succinimidyl ester	100 mg
825	NBD-Cl [4-Chloro-7-nitrobenzofurazan] *UltraPure grade*	25 mg
821	NBD-F [4-Fluoro-7-nitrobenzofurazan] *UltraPure grade*	5 mg
828	NBD-X acid	1 g
829	NBD-X, succinimidyl ester	100 mg
1360	NIR Fluor™ 780 acid	5 mg

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1368	NIR Fluor™ 780 succinimidyl ester	1 mg
380	5(6)-ROX [5-(and 6)-Carboxy-X-rhodamine] *Mixed isomers*	100 mg
381	5-ROX [5-Carboxy-X-rhodamine] *Single isomer*	5 g
382	6-ROX [6-Carboxy-X-rhodamine] *Single isomer*	25 mg
394	Sunnyvale Red™ SE *Superior 6-ROX Replacement*	5 mg
395	6-ROX Plus™, acid *Enhanced stability*	100 mg
397	6-ROX Plus™, succinimidyl ester *Enhanced stability*	5 mg
398	6-ROX Plus™, succinimidyl ester *Enhanced stability*	50 mg
390	5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, succinimidyl ester] *Mixed isomers*	25 mg
391	5-ROX, SE [5-Carboxy-X-rhodamine, succinimidyl ester] *Single isomer*	1 g
392	6-ROX, SE [6-Carboxy-X-rhodamine, succinimidyl ester] *Single isomer*	5 mg
480	Sulforhodamine 101 sulfonyl chloride	10 mg
360	5-(and 6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed isomers*	100 mg
361	5-(and 6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine]	1 g
362	5-(and 6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine]	5 g
363	5-TAMRA [5-Carboxytetramethylrhodamine] *Single isomer*	10 mg
364	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for labeling peptides*	100 mg
365	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for labeling peptides*	1 g
366	6-TAMRA [6-Carboxytetramethylrhodamine] *Single isomer*	10 mg
367	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling oligos*	100 mg
368	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling oligos*	1 g
370	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester]	25 mg
371	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester]	100 mg
372	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester]	1 g
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester] *Single isomer*	5 mg
374	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester]	100 mg
375	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester]	1 g
377	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester]	100 mg
376	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester] *Single isomer*	5 mg
378	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester]	1 g
2238	Tide Fluor™ 1 acid [TF1 acid] *Superior replacement to EDANS*	100 mg
2244	Tide Fluor™ 1, succinimidyl ester [TF1 SE]*Superior replacement to EDANS*	5 mg
2245	Tide Fluor™ 2 acid [TF2 acid] *Superior replacement to fluorescein*	25 mg
2248	Tide Fluor™ 2, succinimidyl ester [TF2 SE]*Superior replacement to fluorescein*	5 mg
2268	Tide Fluor™ 3 acid [TF3 acid] *Superior replacement to Cy3*	25 mg
2271	Tide Fluor™ 3, succinimidyl ester [TF3 SE]*Superior replacement to Cy3*	5 mg
2285	Tide Fluor™ 4 acid [TF4 acid] *Superior replacement to ROX and Texas Red*	10 mg
2289	Tide Fluor™ 4, succinimidyl ester [TF4 SE]*Replacement to ROX and Texas Red*	5 mg
2278	Tide Fluor™ 5 acid [TF5 acid] *Superior replacement to Cy5*	10 mg
2281	Tide Fluor™ 5, succinimidyl ester [TF5 SE]*Superior replacement to Cy5*	5 mg
2190	Tide Quencher™ 1 acid [TQ1 acid]	100 mg
2230	Tide Quencher™ 3 succinimidyl ester [TQ3 SE]	25 mg
410	5(6)-TRITC [Tetramethylrhodamine-5-(and-6)-isothiocyanate]	5 mg
415	5-TRITC; G isomer [Tetramethylrhodamine-5-isothiocyanate]	1 mg
417	6-TRITC; R isomer [Tetramethylrhodamine-6-isothiocyanate]	1 mg

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C-Terminal Labeling Using Amine-Containing Fluorescent Dyes

Amine-containing dyes are used to modify peptides using water-soluble carbodiimides (such as EDC) to convert the carboxy groups of the peptides 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 large peptide solutions at low pH to reduce intra- and inter-protein coupling to lysine residues, a common side reaction.

***Table 2**. AAT Bioquest carbonyl-reactive dyes for labeling peptides*

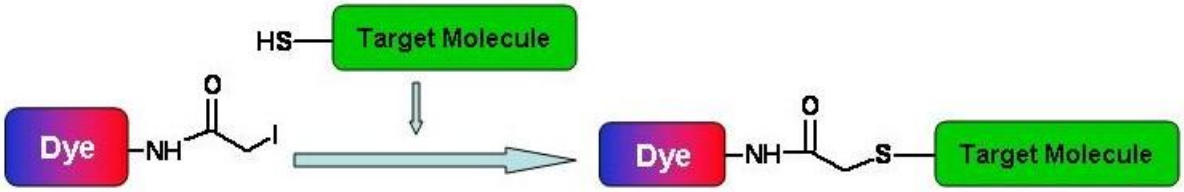
Cat. #	Product Name	Unit Size
2006	DABCYL C2 amine	100 mg
810	Dansyl cadaverine [5-Dimethylaminonaphthalene-1-(N-(5-aminopentyl))sulfonamide]	25 mg
2025	DNP amine	25 mg
610	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	1 g
611	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	10 g
615	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	1 g
616	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	10 g
127	5(6)-FAM cadaverine	100 mg
128	5-FAM cadaverine	100 mg
129	5-FITC cadaverine	100 mg
4010	*N*-BOC-cadaverine	5 g
4011	*N*-BOC-cadaverine	25 g
4013	*N*-BOC-ethylenediamine	5 g
4014	*N*-BOC-ethylenediamine	25 g
4018	*N*-FMOC-cadaverine	5 g
4019	*N*-FMOC-cadaverine	25 g
4016	*N*-FMOC-ethylenediamine	5 g
4017	*N*-FMOC-ethylenediamine	25 g
355	5(6)-TAMRA cadaverine	25 mg
356	5-TAMRA cadaverine	5 mg
357	6-TAMRA cadaverine	5 mg
2239	Tide Fluor™ 1 amine [TF1 amine] *Superior replacement to EDANS*	5 mg
2246	Tide Fluor™ 2 amine [TF2 amine] *Superior replacement to fluorescein*	1 mg
2269	Tide Fluor™ 3 amine [TF3 amine] *Superior replacement to Cy3*	1 mg
2286	Tide Fluor™ 4 amine [TF4 amine] *Superior replacement to ROX and Texas Red*	1 mg
2279	Tide Fluor™ 5 amine [TF5 amine] *Superior replacement to Cy5*	1 mg
2192	Tide Quencher™ 1 amine [TQ1 amine]	5 mg
2202	Tide Quencher™ 2 amine [TQ2 amine]	5 mg
2222	Tide Quencher™ 3 amine [TQ3 amine]	5 mg
482	TR cadaverine [Sulforhodamine 101 cadaverine sulfonamide]	5 mg
481	TR hydrazide [Sulforhodamine 101 sulfonyl hydrazide]	5 mg

Labeling Cysteine Residue Using Thiol-Reactive Fluorescent Dyes

Because free thiol (SH) groups, also called mercapto groups, are not present as abundantly as amino groups in most peptides, 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 for probing biological structures, functions and interactions. Thiol-reactive dyes have been used to develop peptide 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.

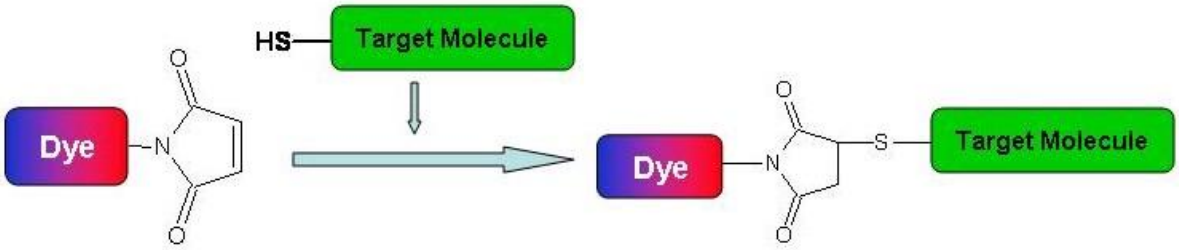
Fluorescent Dye Iodoacetamides (IA)



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 react with thiol groups (such as cysteine and reduced glutathione) more readily 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, decreasing 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.
- *Oxygen Sensitivity:* Air oxidation of thiol compounds (to disulfides) is a major competing reaction for the iodoacetamide modifications of thiol compounds. We recommended that air exposure of reaction solution be minimized whenever possible.

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. Maleimides are generally much less light-sensitive than iodoacetamides. The latter compounds are known to be very light liable, especially in solution.

The conjugation conditions required by maleimides are less stringent than those required by 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.

Table 3*. AAT Bioquest thiol-reactive dyes for labeling peptides*

Cat. #	Product Name	Unit Size
222	5-IAF [5-Iodoacetamidofluorescein]	25 mg
413	5-TMRIA [Tetramethylrhodamine-5-iodoacetamide]	5 mg
634	*b*BBr [Dibromobimane] *UltraPure Grade*	5 mg
633	*m*BBr [Monobromobimane] *UltraPure Grade*	25 mg
2026	DNP maleimide	25 mg
617	EDANS C2 maleimide	25 mg
618	EDANS iodoacetamide	25 mg
130	Fluorescein-5-maleimide	25 mg
1366	NIR Fluor™ 780 maleimide	1 mg
412	Tetramethylrhodamine-5-(and-6)-maleimide *Mixed isomers*	5 mg
421	Tetramethylrhodamine-5-maleimide *Single isomer*	1 mg
419	Tetramethylrhodamine-6-maleimide *Single isomer*	1 mg
2242	Tide Fluor™ 1 maleimide [TF1 maleimide] *Superior replacement to EDANS*	5 mg
2247	Tide Fluor™ 2 maleimide [TF2 maleimide] *Superior replacement to fluorescein*	1 mg
2270	Tide Fluor™ 3 maleimide [TF3 maleimide] *Superior replacement to Cy3*	1 mg
2287	Tide Fluor™ 4 maleimide [TF4 maleimide] *Replacement to ROX and Texas Red*	1 mg
2280	Tide Fluor™ 5 maleimide [TF5 maleimide] *Superior replacement to Cy5*	1 mg
2196	Tide Quencher™ 1 maleimide [TQ1 maleimide]	5 mg
2206	Tide Quencher™ 2 maleimide [TQ2 maleimide]	5 mg
2226	Tide Quencher™ 3 maleimide [TQ3 maleimide]	5 mg

Labeling Aspartic Acid Residue Using FMOC-Asp(Dye)-OH Derivatives

Although there are many fluorescent reagents developed for labeling N-terminals of peptides, very few reagents are available for labeling in-sequence amino acids such as aspartic acid, glutamic acid and lysine residues. AAT Bioquest now offers a variety of fluorescent FMOC-Asp (Dye)-OH derivatives and amine-containing labeling reagents to facilitate the in-sequence labeling of aspartic acid residues of peptides.

Table 4. *AAT Bioquest fluorescent dyes for labeling in-sequence aspartic acid residues of peptides*

Cat. #	Product Name	Unit Size
2006	DABCYL C2 amine	100 mg
810	Dansyl cadaverine [5-Dimethylaminonaphthalene-1-(N-(5-aminopentyl))sulfonamide]	25 mg
2025	DNP amine	25 mg
610	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	1 g
611	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	10 g
615	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	1 g
616	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	10 g
127	5(6)-FAM cadaverine	100 mg
128	5-FAM cadaverine	100 mg
129	5-FITC cadaverine	100 mg
5003	FMOC-Asp(5/6-FAM)-OH	100 mg
5005	FMOC-Asp(5/6-TAMRA)-OH	100 mg
5004	FMOC-Asp(5-FAM)-OH	100 mg
5006	FMOC-Asp(5-TAMRA)-OH	100 mg
5001	FMOC-Asp(EDANS)-OH	1 g
5002	FMOC-Asp(EDANS)-OH	5 g
4010	*N*-BOC-cadaverine	5 g
4011	*N*-BOC-cadaverine	25 g
4013	*N*-BOC-ethylenediamine	5 g
4014	*N*-BOC-ethylenediamine	25 g
4018	*N*-FMOC-cadaverine	5 g
4019	*N*-FMOC-cadaverine	25 g
4016	*N*-FMOC-ethylenediamine	5 g
4017	*N*-FMOC-ethylenediamine	25 g
355	5(6)-TAMRA cadaverine	25 mg
356	5-TAMRA cadaverine	5 mg
357	6-TAMRA cadaverine	5 mg
2239	Tide Fluor™ 1 amine [TF1 amine] *Superior replacement to EDANS*	5 mg
2246	Tide Fluor™ 2 amine [TF2 amine] *Superior replacement to fluorescein*	1 mg
2269	Tide Fluor™ 3 amine [TF3 amine] *Superior replacement to Cy3*	1 mg
2286	Tide Fluor™ 4 amine [TF4 amine] *Superior replacement to ROX and Texas Red*	1 mg
2279	Tide Fluor™ 5 amine [TF5 amine] *Superior replacement to Cy5*	1 mg
2192	Tide Quencher™ 1 amine [TQ1 amine]	5 mg
2202	Tide Quencher™ 2 amine [TQ2 amine]	5 mg
2222	Tide Quencher™ 3 amine [TQ3 amine]	5 mg
482	TR cadaverine [Sulforhodamine 101 cadaverine sulfonamide]	5 mg
481	TR hydrazide [Sulforhodamine 101 sulfonyl hydrazide]	5 mg

Labeling Glutamic Acid Residue Using FMOC-Glu(Dye)-OH Derivatives

Although there are many fluorescent reagents developed for labeling N-terminals of peptides, very few reagents are available for labeling in-sequence amino acids such as aspartic acid, glutamic acid and lysine residues. AAT Bioquest now offers a variety of fluorescent FMOC-Glu(Dye)-OH derivatives and amine-containing labeling reagents to facilitate the in-sequence labeling of glutamic acid residues of peptides.

Table 5. AAT Bioquest fluorescent dyes for labeling in-sequence glutamic acid residues of peptides

Cat. #	Product Name	Unit Size
2006	DABCYL C2 amine	100 mg
810	Dansyl cadaverine [5-Dimethylaminonaphthalene-1-(N-(5-aminopentyl))sulfonamide]	25 mg
2025	DNP amine	25 mg
610	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	1 g
611	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	10 g
615	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	1 g
616	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	10 g
127	5(6)-FAM cadaverine	100 mg
128	5-FAM cadaverine	100 mg
129	5-FITC cadaverine	100 mg
5012	FMOC-Glu(5/6-FAM)-OH	100 mg
5014	FMOC-Glu(5/6-TAMRA)-OH	100 mg
5013	FMOC-Glu(5-FAM)-OH	100 mg
5015	FMOC-Glu(5-TAMRA)-OH	100 mg
5010	FMOC-Glu(EDANS)-OH	1 g
5011	FMOC-Glu(EDANS)-OH	5 g
4010	*N*-BOC-cadaverine	5 g
4011	*N*-BOC-cadaverine	25 g
4013	*N*-BOC-ethylenediamine	5 g
4014	*N*-BOC-ethylenediamine	25 g
4018	*N*-FMOC-cadaverine	5 g
4019	*N*-FMOC-cadaverine	25 g
4016	*N*-FMOC-ethylenediamine	5 g
4017	*N*-FMOC-ethylenediamine	25 g
355	5(6)-TAMRA cadaverine	25 mg
356	5-TAMRA cadaverine	5 mg
357	6-TAMRA cadaverine	5 mg
2239	Tide Fluor™ 1 amine [TF1 amine] *Superior replacement to EDANS*	5 mg
2246	Tide Fluor™ 2 amine [TF2 amine] *Superior replacement to fluorescein*	1 mg
2269	Tide Fluor™ 3 amine [TF3 amine] *Superior replacement to Cy3*	1 mg
2286	Tide Fluor™ 4 amine [TF4 amine] *Superior replacement to ROX and Texas Red*	1 mg
2279	Tide Fluor™ 5 amine [TF5 amine] *Superior replacement to Cy5*	1 mg
2192	Tide Quencher™ 1 amine [TQ1 amine]	5 mg
2202	Tide Quencher™ 2 amine [TQ2 amine]	5 mg
2222	Tide Quencher™ 3 amine [TQ3 amine]	5 mg
482	TR cadaverine [Sulforhodamine 101 cadaverine sulfonamide]	5 mg

Labeling Lysine Residue Using FMOC-Lys(Dye)-OH Derivatives

Although there are many fluorescent reagents developed for labeling N-terminals of peptides, very few reagents are available for labeling in-sequence amino acids such as aspartic acid, glutamic acid and lysine residues. AAT Bioquest now offers a variety of fluorescent FMOC-Lys (Dye)-OH derivatives to facilitate the in-sequence labeling of lysine residues of peptides. In addition, all our amine-reactive fluorescent dyes can also be used to label in-sequence lysine residues if the N-terminal amino group is blocked.

Table 5. AAT Bioquest fluorescent dyes for labeling in-sequence aspartic acid residues of peptides

Cat. #	Product Name	Unit Size
5042	FMOC-Lys(5/6-FAM)-OH	1 g
5044	FMOC-Lys(5/6-TAMRA)-OH	100 mg
5043	FMOC-Lys(5-FAM)-OH	100 mg
5070	FMOC-Lys(5-FITC)-OH	100 mg
5045	FMOC-Lys(5-TAMRA)-OH	100 mg
5040	FMOC-Lys(DABCYL)-OH	1 g
5041	FMOC-Lys(DABCYL)-OH	5 g
5050	FMOC-Lys(TF2)-OH	100 mg
5060	FMOC-Lys(TQ2)-OH	100 mg

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