FT-R08112

FluoProbes AF NHS Ester

NHS activated dyes for fluorescent labeling of biomolecules via their amine groups

Introduction

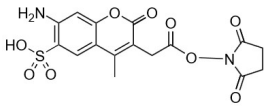
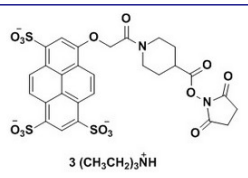
A variety of FluoProbes® **AF** dyes has been used to label proteins, nucleic acids and other biomolecules for fluorescence techniques (imaging, biochemical analysis). They replace advantageously the conventional fluorochromes such as Fluorescein (FITC) and rhodamines (TRITC, RRX). The dyes are water soluble and pH-insensitive from pH 4 to 10.

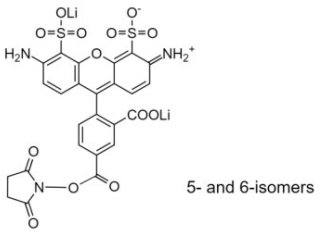
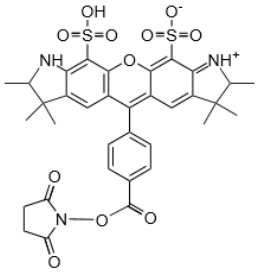
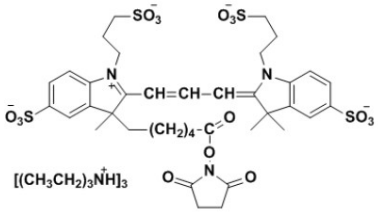
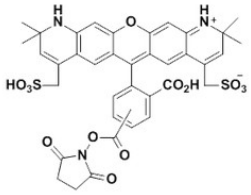
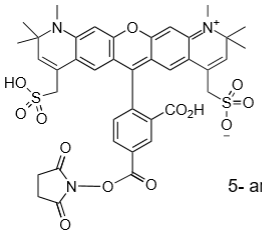
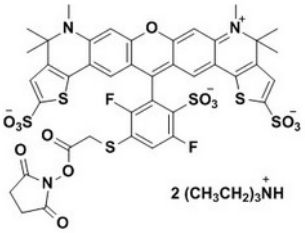
AF350: It is a blue-fluorescent dye optimal for use with the 350 nm UV laser.
AF405: It is a blue-fluorescent dye optimal for use with the 405 nm laser line.
AF488: It is a bright green-fluorescent dye optimal for use with the 488 nm Argon laser.
AF532: It is an yellow-fluorescent dye optimal for use with the frequency-doubled Nd:YAG laser line.
AF555: It is an orange-fluorescent dye optimal for use with 561 nm laser paired with a 582/15 nm bandpass filter
AF568: It is a bright green-fluorescent dye optimal for use with the 568 nm laser line.
AF594: It is a bright red-fluorescent dye optimal for use with the frequency-doubled He-Ne laser line.
AF633: It is a bright red-fluorescent dye optimal for use with the 633nm laser line.
AF647: It is a bright far red-fluorescent dye optimal for use with the 633nm laser line.

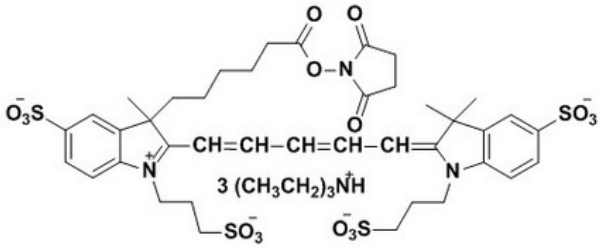
FluoProbes® **AF** NHS esters are reactive dyes for the labeling of amino-groups typically found in peptides, proteins, and some derivates such as aminoallyl-oligonucleotides. The reaction is carried out at physiological pH.

Products Description

The table below gives main physical and fluorescence characteristics of the activated dyes.

Product name cat.number/qty	**MW** g·mol^{-1} **(+added MW)**	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
AF350 – NHS FP-M17231, 1mg Soluble in DMSO	410.35	346 / 442	19 000	
AF405 – NHS FP-U90663, 1mg Soluble in DMSO	1028.3	398 / 421	35 000	

Product name cat.number/qty	**MW** g·mol^{-1} (+added MW)	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
AF488 – NHS FP-R08112, 1mg Soluble in DMSO	643.40	494 / 517	76 000 QY: 0.92	 5- and 6-isomers
AF532 – NHS FP-T82152, 1mg Soluble in DMSO	723.77	530 / 555	81 000 QY: 0.61	
AF555 – NHS FP-R08112, 1mg Soluble in DMSO	1233.62	555 / 572	155 000 QY: 0.08	
AF568 – NHS FP-R08892, 1mg Soluble in DMSO	791.80	578 / 602	88 000 QY: 0.69	
AF594 – NHS FP-R09043, 1mg Soluble in DMSO	819.85	590 / 617	96 000 QY: 0.66	 5- and 6-isomers
AF633 – NHS FP-R09171, 1mg Soluble in DMSO	1224.48	621 / 639	143 000 QY: 0.66	

Product name cat.number/qty	MW g·mol^{-1} (+added MW)	λ abs./em. nm	mol. abs. M^{-1}cm^{-1}	Comment, structure
AF647 – NHS FP-R09353, 1mg Soluble in DMSO	1269.56	651 / 672	270 000 QY: 0.33	

Storage: –20°C, protected from light (+4°C possible for short term) (M)
Avoid prolonged exposure to light. Desiccate.
Stable for 24 months after receival at -20°C in the dark. Transportation: at room temperature for up to 3 weeks.

Directions for use

Protocol for Labeling of Amino-Biomolecules

Introduction:
NHS (N-HydroxySuccinimide) esters and other activated esters (sulfo-NHS, sulfotetrafluorophenyl - STP) are highly reactive compounds suitable for the modification of amino groups. NHS is most common type of activated esters.

Usual modifications are fluorescent labels, fluorescence quenchers, and other reporter groups. Alkyne and azido group can be attached using activated esters to adapt biomolecules to Click Chemistry.

Since amino groups are nearly always contained in proteins and peptides, modification of these biopolymers is especially common. Other examples are amino-oligonucleotides, amino-modified DNA, and amino-containing sugars.

The reaction of NHS esters with amines is strongly pH-dependent: at low pH, the amino group is protonated, and no modification takes place. At higher-than-optimal pH, hydrolysis of NHS ester is quick, and modification yield diminishes. Optimal pH value for modification is 8.3-8.5.

Water is most common solvent for the labeling. If NHS ester is poorly soluble, it can be added as a solution in DMSO or DMF to a solution of protein in water, adjusted to pH 8.3-8.5. Note that DMF must not contain amines.

We recommend using the following general protocol for the labeling of biomolecules with NHS esters. See related products for auxiliary reagents.

Protocol:

1. Calculate required amount of NHS ester:

NHS_ester_weight [mg] =
8 × amino_compound_weight [mg] × NHS_ester_molar_weight [Da] / amino_compound_molar_weight [Da].

8 is molar excess of NHS ester. It is experimental value for mono-labeling, suitable for many common proteins and peptides. However, in some cases using less or more NHS ester is required. It depends on protein structure.

The molar weights of NHS esters are displayed in the product description at first page *(note that molar weights of reagents produced by other vendors may vary).*

2. Determine volume of reaction mixture. The labeling can be performed on any scale from nanomols to dozens of grams. When the scale is low, use minimal volume (10-20 uL). Higher concentrations (1-10 mg of amino-biomolecule per mL of mixture) are optimal.

3. Dissolve NHS ester in 1/10 reaction volume of DMF or DMSO. Amine-free DMF is preferred solvent. After the reaction, NHS ester can be stored in solution for 1-2 months at -20ºC.

4. Dissolve biomolecule in 9/10 reaction volume of buffer with pH 8.3-8.5.

0.1 M Sodium bicarbonate solution has appropriate pH. Other alternatives are 0.1 M Tris buffer (although Tris has amino group, it is hindered and does not react with NHS esters), or 0.1 M phosphate buffer. Note pH is most important thing.

When doing large-scale labeling (hundreds of milligrams of NHS ester), note that the mixture tends to acidify with time because of hydrolysis of NHS ester. Monitor pH, or use more concentrated buffer then.

5. Add NHS ester solution to the solution of biomolecule, and vortex well. Keep on ice overnight, or at room temperature during at least 4 hours.

6. Purify the conjugate using appropriate method: gel-filtration for macromolecules is most universal. Precipitation and chromatography is another alternative. Organic impurities (such as N-hydroxysuccinimide, NHS ester, acid produced by hydrolysis) are almost always easily separated.

Related products

* **CY$_{anine}$ dyes** functionalized by **NHS** (BB7493), **Maleimide** (JO6660), **Azide** (HO7250), **Alkyne** (1A6320), **Hydrazide** (LQV050), **DBCO** (DQP790: CycloAlkynes, for strain-promoted Click reactions), **Amino** group (CY3AM0), **Carboxyl** group (CY3CA0). 2D DI GE **3Dye labeling kit** (CY$_{anine}$2/CY$_{anine}$3/CY$_{anine}$5) (EV0870)

* Superior **FluoProbes fluorescent dyes**
- activated by –NHS (list), i.e. FP488-NHS #BA6800
- activated by –Maleimide (list), i.e. FP488-MAL #BA6810
- activated by –Azide, i.e. FP488-Azide #YE4970

* Classic dyes such as **FAM, R110, JOE TAMRA,** and **ROX.**

* Fluorescently labeled ligands:
• **Labeled lectins**, i.e. ConA-CY$_{anine}$3 #FT-WT868.
• **Labeled secondary antibodies**
• **Labeled tags**, i.e. CY$_{anine}$3-polylysine #FT-WT8550

* Other labeling/conjugation chemistries: • Click Chemistry reagents

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06