FluoProbes®

FT-BB7493

CY$_{anine}$ NHS

NHS activated CY$_{anine}$ agents for fluorescent labeling of biomolecules via their amine groups

Introduction

A variety of **Cyanine 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).

CY$_{anine}$3 : An orange-to-red fluorescence-emitting dye. Excitation/emission maximum 555/567 nm
Cy$_{anine}$3 is one of the most broadly used fluorophores which can be detected by various fluorometers, imagers, and microscopes. Due to inherently high extinction coefficient, this dye is also easily detected by naked eye on gels, and in solution. Can replace TRITC, AF555, DL550, Chromeo546.
See also alternative superior dye: FluoProbes547H.

CY$_{anine}$3.5 can replace SulfoRhodamine 101.
See also alternative superior dye: FluoProbes594.

CY$_{anine}$5 : A far-red fluorescence-emitting dye. Excitation/emission maximum 647/657 nm
CY$_{anine}$5 fluorophore has become an incredibly popular label in life science research and diagnostics. Fluorophore emission has maximum in red region, where many CCD detectors have maximum sensitivity, and biological objects have low background. Dye color is very intense, therefore quantity as small as 1 nanomol can be detected in gel electrophoresis by naked eye. Can replace far red red fluorescent dyes.
See also alternative superior dye: FluoProbes647H

CY$_{anine}$5.5 : A near-infrared (IR) fluorescence-emitting dye. Excitation/emission maximum 678/694 nm
can replace near infrared fluorescent dyes.
See also alternative superior dye: FluoProbes682.

CY$_{anine}$7: A near-IR fluor that is invisible to the naked eye. Excitation/emission maximum 750/776 nm
It is used in in vivo imaging applications.
See also alternative superior dye: FluoProbes752.

CY$_{anine}$7.5 is a near infrared red fluorophores used for *in vivo* imaging applications.
See also alternative superior dye: FluoProbes800.

CY$_{anine}$ 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.

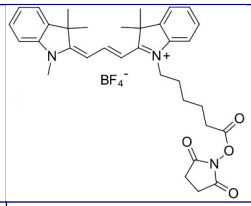
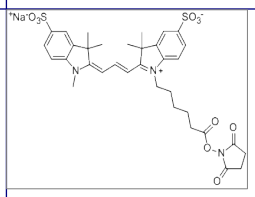
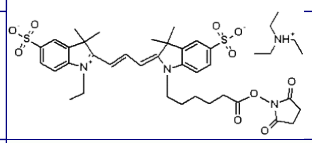
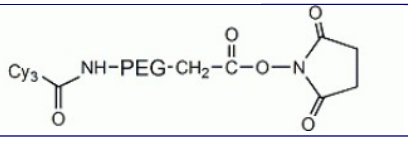
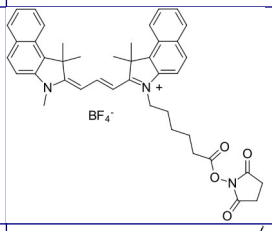
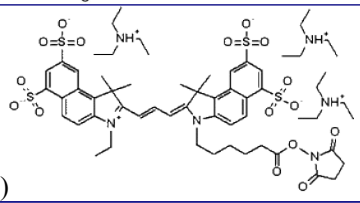
Sulfo NHS – CY$_{anine}$ are water-soluble derivatives of the CY$_{anine}$ dyes, allowing more convenient use, and to achieve higher coupling ratio and coupled ratio. DiSulfonated forms are the most classic, but some mono-, tri- and quadri-sulfonated forms are available as well, for even lower or higher hydrosolubility.
PEG-CY$_{anine}$ derivatives are also water soluble, but they also confer hydrophylicity to the conjugate via the PEG spacer.

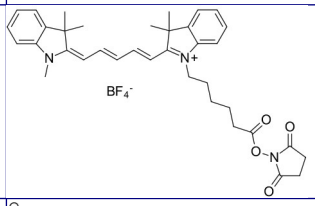
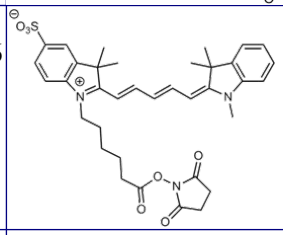
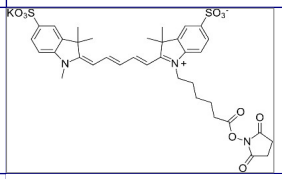
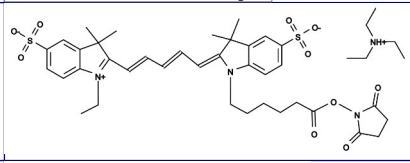
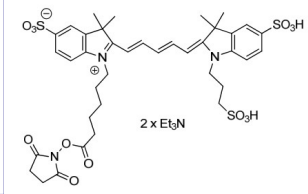
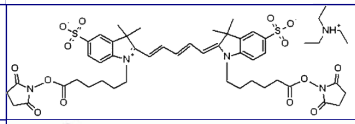
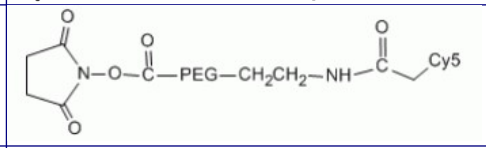
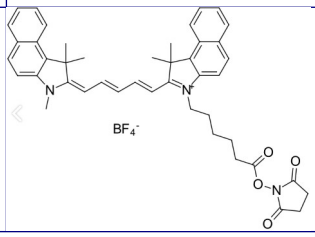
bis-NHS derivatives are available for higher labeling or bi-functional conjugate applications

The triethylammonium (TEA) salts are more soluble in DMSO and DMF than the corresponding potassium salts.

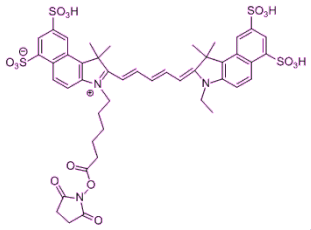
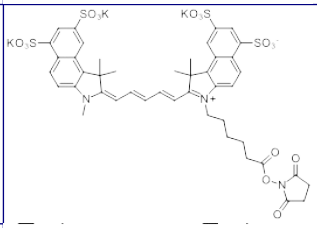
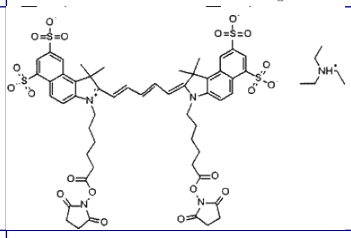
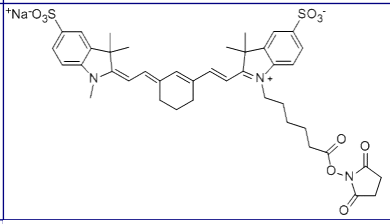
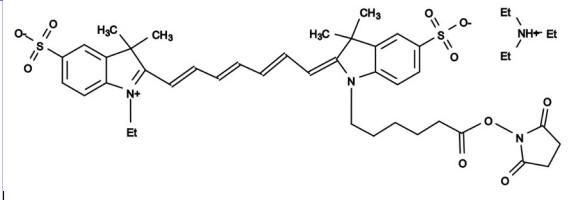
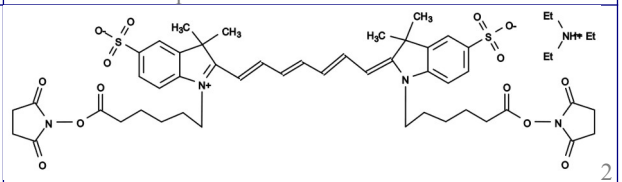
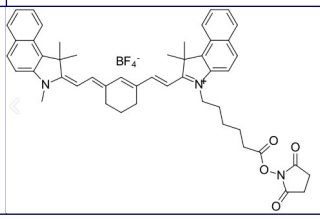
Products Description

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

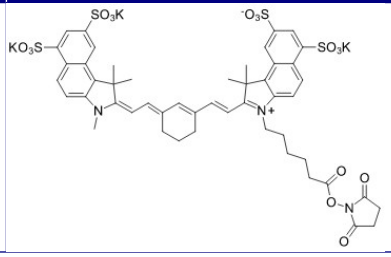
Product name cat.number/qty*	**MW** g·mol^{-1} **(+added MW)**	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
CY$_{anine}$2				Replace Fluorescein(FITC)
CY$_{anine}$2 – NHS (L) FP-LV2330, 5nmol Soluble in organic solvents (DMSO, DMF), low solubility in water	550.6	489 / 506	150 000 QY: 0.12	
CY$_{anine}$3				Replace Cy 3, AF555, DL550, Chromeo546
CY$_{anine}$3 – NHS (L) FP-BB7493, 1mg FP-1H4680, 1nmol Soluble in organic solvents (DMSO, DMF, dichloromethane); low solubility in water	590.15 (+474.2)	555 / 570	150 000 QY: 0.31	
Sulfo-CY$_{anine}$3 **bis**NHS				bears 2 amine reactive groups (2 NHS) and Sulfo group
DiSulfo-CY$_{anine}$3 – NHS FP-1B7980, 1mg FP-1B7981, 5mg Dark red powder Very high solubility in water, good in polar organic solvents (DMF, DMSO)	735.80 (598.2)	548 / 567	162 000 QY: 0.1	
DiSulfo-CY$_{anine}$3 – NHS FP-LQU980, 1mg	829.03 (TEA salt)	555/565	"	 (incl. 1 ethyl)
CY$_{anine}$3 – PEG – NHS 2000Da FP-Inquire, 1mg 3400Da FP-Inquire, 1mg 5000Da FP-Inquire, 1mg	~2500 ~3900 ~5500	649/665	"	
CY$_{anine}$3.5				
CY$_{anine}$3.5 – NHS FP-FZ8730, 1mg Dark purple powder Soluble in organic solvents (DMF, DMSO, dichloromethane), insoluble in water	690.27	591 /604	116 000 QY: 0.35	
TetraSulfo-CY$_{anine}$3.5-NHS FP-LQV060, 1mg	1291.66 (TEA salt)	581 / 596	"	 (incl. 1 ethyl)

Product name cat.number/qty*	**MW** g·mol^{-1} (+added MW)	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
CY$_{anine}$5				
CY$_{anine}$5 – NHS FP-BB2070, 1mg FP-BB2071, 5mg FP-BB2074, 25mg	616.19 (465.68)	646 / 662	250 000 QY: 0.2	
MonoSulfo-CY$_{anine}$5 – NHS FP-**1G2520, 5mg** FP-**1G2521, 25mg** Good solubility in polar (DMSO, DMF) and chlorinated (DCM, chloroform) organic solvents, low solubility in water	659.79 ()	642 / 660	180 000 QY : 0.26	
CY$_{anine}$5 – NHS ester minimal dye (L) FP-SJH900, 5nmol		645 / 660		
DiSulfo-CY$_{anine}$5 – NHS FP-**IO0510, 1mg**	777.95	646 / 662	271 000 QY: 0.28	
DiSulfo-CY$_{anine}$5 – NHS, TFA salt FP-LQU990, 1mg	855.07 (TEA salt)	649/665	"	 (incl. 1 ethyl)
TriSulfo-CY$_{anine}$5 – NHS FP-1I1951, 5mg	1050.35 (TEA salt) 847.97 (proto-nated)	649 / 670	250 000	
DiSulfo-CY$_{anine}$5 – bisNHS FP-LQV120, 1mg	1038.23 (TEA salt)	649/665	"	
CY$_{anine}$5 – PEG – NHS 2000Da FP-1I2220, 1mg 3400Da FP-1I2230, 1mg 5000Da FP-1I2240, 1mg	~2500 ~3900 ~5500	649/665	"	
CY$_{anine}$5.5				
CY$_{anine}$5.5 – NHS FP-FZ8750, 1mg Soluble in organic solvents (DMSO, DMF, dichloromethane); low solubility in water	716.33	674 / 707	209 000 QY: 0.2	

FT-BB7493

Product name cat.number/qty*	**MW** g·mol^{-1} (+added MW)	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
Tetra-Sulfo-CY$_{anine}$5.5–NHS FP-LQV160, **1mg** Blue powder Soluble in water, DMF, DMSO	1014.14	678 / 694	190 000 CF_{280}: 0.18 CF_{260}: 0.05	
Tetra-Sulfo-CY$_{anine}$5.5–NHS FP-0B8400, **1mg** Blue powder Soluble in water, DMF, DMSO	1114.37	675 / 694	195 000 CF_{280}: 0.11 CF_{260}: 0. 09	
TetraSulfo-CY$_{anine}$5.5 – bisNHS FP-LQV130, 1mg	1500.86 (TEA salt)	678/701	Inquire	
CY$_{anine}$7				
CY$_{anine}$7 – NHS FP-JV9770, 1mg	682.29			Soluble in organic solvents (DMSO, DMF, dichloromethane), low solubility in water
DiSulfo-CY$_{anine}$7 – NHS FP-1B8001, **5mg** Dark green powder Soluble in water, DMF, DMSO	827.94	740 / 773	240 600	
MonoSulfo-CY$_{anine}$7 – NHS FP-LQV140, 1mg	881.11 (TEA salt)		Inquire	 Ethy l substituent and spacer C7
DiSulfo-CY$_{anine}$7 – bisNHS FP-LQV150, 1mg	1064.27 (TEA salt)	749 / 776	Inquire	 2 reactive groups (2 NHS), 2 sulfo, Ethyl, and spacer C7
CY$_{anine}$7.5				
CY$_{anine}$7.5 – NHS FP-JO3060, 1mg Soluble in organic solvents (DMSO, DMF, dichloromethane); low solubility in water	782.41 [631.9]	788 / 808	223 000	

FT-BB7493

Product name cat.number/qty*	MW g·mol^{-1} (+added MW)	λ abs./em. nm	mol. abs. M^{-1}cm^{-1}	Comment, structure
Tetra-Sulfo-CY$_{anine}$7.5 – NHS FP-AWHGX0, 1mg Soluble in water, DMF, DMSO	1180.47 [950.2]	778 / 797	222 000	

*: search online or ask for other quantities (5mg to +100g).
The different packaging have the same catalog number as the standard size above, only the last digit is changed.

Storage: –20°C, protected from light (+4°C possible for short term) (M)
Avoid prolonged exposure to light. Desiccate.
Stable for 24 months after receipt 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.

For example, to label 3 mg of insulin (molar weight 69300 Dalton) with CY$_{anine}$5 NHS ester (molar weight 616 Dalton), and obtain maximum yield of mono-labeled product, you should calculate 10 × 3 mg × 616Da / 69300Da = 0.266 mg of CY$_{anine}$5 dye NHS ester.

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 / associated products and documents

* **CY$_{anine}$ Dyes** (Cy3, Cy5, Cy5.5, Cy7…) functionalized by **NHS** (FT-BB7493), **Maleimide** (FT-JO6660), **Azide** (FT-HO7250), **Alkyne** (FT-1A6320), **DBCO** (FT-DQP790), **Hydrazide** (FT-LQV050), **DBCO** (FT-DQP790: CycloAlkynes, for strain-promoted Click reactions), **Amino** group (FT-CY3AM0), **Carboxyl** group (FT-CY3CA0). **3Dye 2D DI GE (CY2/CY3/CY5) labeling kit** (FT-EV0870)
* Superior **FluoProbes fluorescent dyes**
- activated by –NHS (list), i.e. FP488-NHS (FT-BA6800) - activated by –Azide, i.e. FP488-Azide (FT-YE4970)
- activated by –Maleimide (list), i.e. FP488-MAL (FT-BA6810)

* Classic dyes such as **FAM, CR110, JOE TAMRA,** and **ROX**, functionalized **by –Alkyne or DBCO** (FT-DQP790), or **by -Azide** (FT-EV0920).

* Fluorescently labeled ligands:
• **Labeled lectins**, i.e. ConA-CY$_{anine}$3 (#WT868/FT-47496A)
• **Labeled secondary antibodies**
• **Labeled tags**, i.e. CY$_{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

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