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Datasheet

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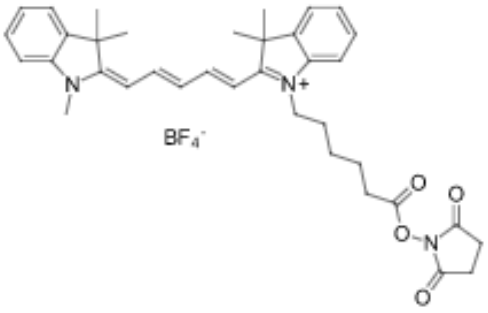
Cyanine5 NHS ester

Cat# C5101– 10 mg

Storage under -20°C

INTFORMATION

Product Name	Cyanine5 NHS ester
Cat NO.	C5101
Size	10 mg
Description	During the last years, Cyanine5 has become an incredibly popular label in life science research and diagnostics. The fluorophore has its emission maximum in the red region, where many CCD detectors exhibit maximum sensitivity, and biological objects show low background. The dye color is very intense, therefore quantities as small as 1 nmol can be detected in gel electrophoresis by naked eye. This Cyanine5 NHS ester is a reactive dye for the labeling of amino-groups in peptides, proteins, and oligonucleotides. This dye requires a small amount of organic co-solvent (such as DMF or DMSO) to be used in labeling reactions (please see our recommended protocol for more details). This reagent is ideal for very cost-efficient labeling of soluble proteins as well as all kinds of peptides and oligonucleotides. This reagent also works well in organic solvents for small molecule labeling. For more sophisticated targets such as easily degradable proteins, when the use of DMF or DMSO is undesirable, consider using water-soluble sulfo-Cyanine 5 NHS ester which does not require any co-solvent, and features very similar fluorescent properties. Cyanine5 fluorophore is compatible with various instrumentation including many fluorescent microscopes, imagers, scanners, and fluorescence readers. A number of various Cyanine5 analogs exist - Cyanine5 NHS ester can replace activated esters of Cy5®, Alexa Fluor 647, and DyLight 649.
Excitation	646 nm
Emission	662 nm
Molecular Formulation	$C_{36}H_{42}N_3BF_4O_4$
Molecular Weight	667.54
CAS No.	1263093-76-0 (tetrafluoroborate), 1032678-42-4 (chloride), 350686-88-3 (without anion)
Solubility	very poorly soluble in water (0.19 mM = 127 mg/L), good in polar (DMSO, DMF) and chlorinated (DCM, chloroform) organic solvents

Image	

STORAGE

Powder	-20°C	1 years, in the dark

PROTOCOL

NHS (N-HydroxySuccinimide) esters and other activated esters (sulfo-NHS, sulfotetrafluorophenyl — STP) are 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 (and thus should have no odor).

We recommend using the following general protocol for the labeling of biomolecules with NHS esters produced by Lumiprobe.

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, reagent, and solubility. Molar weight of Lumiprobe products can be found on corresponding product pages.

For example, to label 3 mg of BSA (molar weight 69300 Dalton) with Cy5 NHS ester (molar weight 616 Dalton), and obtain maximum yield of mono-labeled product, one should use 8 × 3 mg × 616 Da / 69300 Da = 0.21 mg of Cy5 dye NHS ester.

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 −20°C.
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. Another alternative is 0.1 M phosphate buffer. Note pH is the most important thing. Avoid using buffers containing amines (Tris can sometimes be used but not recommended).

   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. For proteins and nucleic acids, ethanol or acetone precipitation can be used.

PRODUCT USE LIMITATION

These products are intended for research use only.