GeneCopoeia™
Expressway to Discovery

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CyDye® NHS Esters (Succinimidyl Esters)

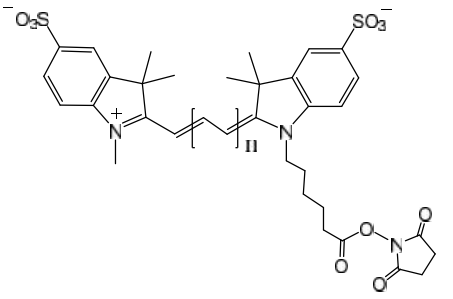
Table 1. Products and Storage

Cat. No.	Product Name	Ex/Em (nm)	Unit	Storage	Stability
C164	Cy3 NHS Ester	550/570	1 mg	-20 °C	The product is stable for at least six month when stored as directed.
C168	Cy5 NHS Ester	650/670	1 mg	-20 °C	
C170	Cy5.5 NHS Ester	675/694	1 mg	-20 °C	
C172	Cy7 NHS Ester	747/776	1 mg	-20 °C	
C181	Cy3 Bis NHS Ester	550/570	1 mg	-20 °C	
C183	Cy5 Bis NHS Ester	650/670	1 mg	-20 °C	
C184	Cy5.5 Bis NHS Ester	675/694	1 mg	-20 °C	
C185	Cy7 Bis NHS Ester	747/776	1 mg	-20 °C	

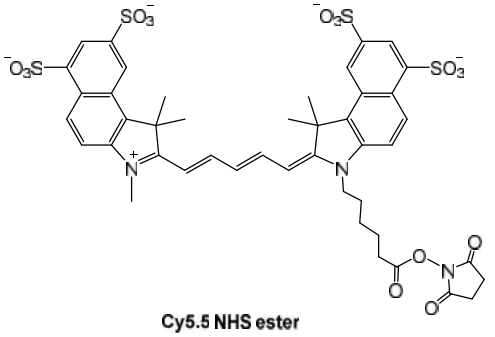
Introduction

Cyanine dyes are useful fluorescent labels for labeling biomolecules, especially proteins and nucleic acids. CyDye® conjugates exhibit brighter fluorescence and greater photostability than the conjugates of conventional fluorophores. Cyanine dyes are also highly water soluble and pH insensitive from pH 4–10, providing significant advantages over other existing fluorophores.

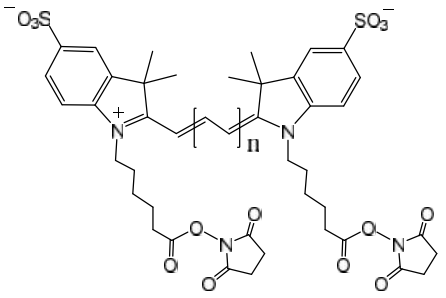
The NHS esters (also known as succinimidyl esters) of CyDye® are amine-reactive fluorescent dyes for conjugation to proteins, amine-modified oligonucleotides,or other amine-containing compounds. These succinimidyl esters provide an efficient and convenient way to selectively link the CyDye® to primary amines ($R\text{-}NH_2$) located on peptides, proteins, or amine-modified nucleic acids. The CyDye are supplied as monofunctional NHS esters and bifunctional NHS esters.



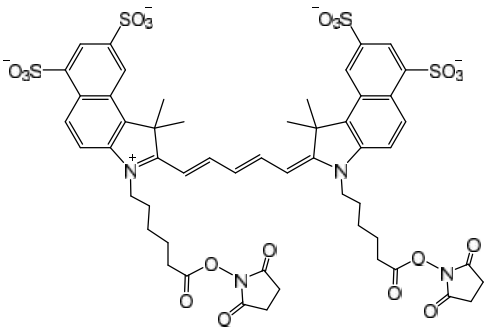
Where n = 1, 2, or 3 for Cy3, Cy5, or Cy7 NHS ester



Cy5.5 NHS ester



Where n = 1, 2, or 3 for Cy3, Cy5, or Cy7 Bis NHS ester



Cy5.5 Bis NHS ester

Experimental Protocols

Protein Labeling Protocol

Important: The following protocol is optimized for labeling 10 mg of an IgG antibody. You may scale this procedure up or down, maintaining the same molar ratios of reagents. The reactivity between different proteins and CyDye® NHS esters will vary greatly, so it's important to try three different molar ratios of the reactive reagent to protein to give the most satisfactory results for your specific protein.

1.1 Dissolve ~10 mg of the protein in 1 mL of 0.1 M sodium bicarbonate buffer. The protein concentration in the reaction should usually be 5-10 mg/mL. Concentrations lower than 2 mg/mL will greatly decrease the efficiency of the reaction.
Protein solutions must be free of any amine-containing substances such as Tris, glycine, ammonium ions, or stabilizing proteins such as bovine serum albumin. You can dialyze antibodies that have been previously dissolved in buffers containing amines against PBS, and you can obtain the desired pH for the reaction by adding 0.1 mL of 1 M sodium bicarbonate buffer for each mL of antibody solution. The presence of low concentrations of sodium azide (<3 mM) will not interfere with the conjugation reaction.

1.2 Dissolve the CyDye® NHS ester in DMF or DMSO at 10 mg/mL. For a typical reaction, dissolve 1 mg of CyDye® NHS ester in 100 µL of DMF or DMSO. Dissolve the dye immediately before starting the reaction as reactive compounds are not very stable in solution. Briefly sonicate or vortex.

1.3 While stirring or vortexing the protein solution (step 1.1), slowly add 50-100 µL of the CyDye® NHS ester solution (step 1.2).

1.4 Incubate the reaction for 1 hour at room temperature with continuous stirring.

1.5 Equilibrate a 10 × 300 mm gel filtration column (Sephadex® G-25, BioGel® P-30, or equivalent) with PBS.

1.6 Separate the conjugate on the gel filtration column.

1.7 Store the conjugates under the same conditions used for the parent protein. For storage in solution at 2-8°C, add sodium azide (2 mM final concentration) as a preservative. Since azide is an inhibitor of horseradish peroxidase (HRP), substitute thimerosal as a preservative for conjugates that are derived from HRP or those that will be used for experiments in which HRP is present.

Determining the Degree of Labeling

2.1 Measure the absorbance of the protein-dye conjugate at 280 nm (A_{280}) and at the λ_{max} for the dye (A_{max}). Dilute the protein-dye conjugate to approximately 0.1 mg/mL. Dilute only as much as you need to make the measurement. The λ_{max} values for CyDye® are given in the *Tabel 2*.

2.2 Determine the concentration of the protein in mg/mL.

$$[\text{protein}] = (A_{280} - CF_{280} \times A_{max})/1.4$$

Note: CF_{280} values for CyDye® are given in the *Tabel 2*.

2.3 Calculate the degree of labeling (DOS):

$$DOS = (A_{max} \times Mw)/([\text{protein}] \times \varepsilon_{dye})$$

where Mw = the molecular weight of the protein, ε_{dye} = the extinction coefficient of the dye at its absorbance maximum, and the protein concentration is in mg/mL.

Labeling Amine-Modified Oligonucleotides

Note: The following protocol is optimized for labeling 100 nmol of an 5'-amine-modified oligonucleotide, 18 to 28 bases in length. You may label slightly shorter or longer oligonucleotides using the same procedure; however, adjustments to the protocol may be necessary for significantly shorter or longer oligonucleotides. You may scale the reaction up or down as long as you do not change the concentration of each component. Following the labeling reaction, you may purify the conjugate from the reaction mixture using reverse-phase HPLC.

3.1 Dissolve ~100 nmol of amine-modified oligonucleotide with 225 µL of H_2O, then add 75 µL of 1 M sodium bicarbonate buffer, and 150 µL of acetonitrile.

3.2 Dissolve 1 mg of the CyDye® NHS ester in 30 µL DMSO.

Note: It is important that you prepare the CyDye® NHS ester freshly for each labeling reaction as reactive compounds are not stable in solution.

3.3 While stirring or vortexing the amine-modified oligonucleotide solution (step 3.1), slowly add 30 µL of the CyDye® NHS ester solution (step 3.2).

3.4 Incubate the reaction for 3 hour at room temperature with continuous stirring.

3.5 Add 1 mL of cold absolute ethanol to the reaction vial. Mix well and incubate at –20°C for 30 minutes.

3.6 Centrifuge the solution in a microcentrifuge at ~10,000 rpm for 5 minutes. Carefully remove the supernatant, rinse the pellet once or twice with cold 70% ethanol, and dry briefly.

3.7 Dissolve the pellet from the ethanol precipitation (step 3.6) in 100 µL of H_2O, and purify the labeled oligonucleotide by reverse-phase HPLC.

Table 2 Physical characteristics of the CyDye®

Dye	$^{a}_{max}$ (nm)	E_m (nm)	£ ($cm^{-1}M^{-1}$)	CF_{280}	CF_{260}
Cy3	550	570	150,000	0.08	0.07
Cy5	650	670	250,000	0.05	0.02
Cy5.5	675	694	240,000	0.04	0.02
Cy7	747	776	210,000	0.04	0.02