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Expressway to Discovery

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Andy Fluor™ NHS Esters (Succinimidyl Esters)

Table 1. Products and Storage

Cat. No.	Product Name	Ex/Em (nm)	Unit	Storage	Stability
L001	Andy Fluor 350 NHS Ester	350/440	1 µmol	-20 °C	The product is stable for at least six month when stored as directed.
L002	Andy Fluor 405M NHS Ester	405/450	1 µmol	-20 °C	
L003	Andy Fluor 430 NHS Ester	430/540	1 µmol	-20 °C	
L004	Andy Fluor 488 NHS Ester	495/520	1 µmol	-20 °C	
L005	Andy Fluor 555 NHS Ester	553/565	1 µmol	-20 °C	
L006	Andy Fluor 568 NHS Ester	578/602	1 µmol	-20 °C	
L007	Andy Fluor 594 NHS Ester	590/615	1 µmol	-20 °C	
L008	Andy Fluor 610 NHS Ester	610/625	1 µmol	-20 °C	
L009	Andy Fluor 635 NHS Ester	630/650	1 µmol	-20 °C	
L010	Andy Fluor 647 NHS Ester	650/666	1 µmol	-20 °C	
L011	Andy Fluor 680 NHS Ester	680/700	1 µmol	-20 °C	
L012	Andy Fluor 750 NHS Ester	755/770	1 µmol	-20 °C	

Introduction

Andy Fluor™ dyes are a series of superior fluorophores spanning the visible and near-infrared (IR) spectrum for labeling biomolecules, especially proteins and nucleic acids. Andy Fluor™ conjugates exhibit brighter fluorescence and greater photostability than the conjugates of conventional fluorophores. Andy Fluor™ dyes are also highly water soluble and pH insensitive from pH 4–10, to aid researchers who are working in biological environments.

The NHS esters (also known as succinimidyl esters) of Andy Fluor™ dyes 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 superior Andy Fluor™ dyes to primary amines ($R\text{-}NH_2$) located on peptides, proteins, or amine-modified nucleic acids. Unlike other reactive moieties, succinimidyl esters demonstrate very low reactivity with aromatic amines, alcohols, and phenols, including tyrosine and histidine. Succinimidyl esters are preferred over other amine-reactive reagents, such as isothiocyanates, for attaching fluorophores to amine-containing molecules, because the amide bonds formed in the reaction are as stable as peptide bonds.

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 Andy Fluor™ 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 Andy Fluor™ NHS ester in DMF or DMSO to make 10 mM concentration. For a typical reaction, dissolve 1 µmol of Andy Fluor™ 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 Andy Fluor™ 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 Andy Fluor™ dyes 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 Andy Fluor™ dyes 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 µmol of the Andy Fluor™ NHS ester in 30 µL DMSO.

Note: It is important that you prepare the Andy Fluor™ 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 Andy Fluor™ 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 Andy Fluor™ dyes

Dye	$^{a}_{max}$ (nm)	E_m (nm)	£ ($cm^{-1}M^{-1}$)	CF_{280}	CF_{260}
Andy Fluor™ 350	350	440	19,000	0.19	0.24
Andy Fluor™ 405M	405	450	42,000	0.22	ND
Andy Fluor™430	430	540	15,500	0.27	ND
Andy Fluor™ 488	495	520	70,000	0.10	0.29
Andy Fluor™ 555	553	565	150,000	0.07	0.07
Andy Fluor™ 568	578	602	91,000	0.45	0.43
Andy Fluor™ 594	590	615	90,000	0.55	0.42
Andy Fluor™ 610	610	625	100,000	0.45	0.40
Andy Fluor™ 635	630	650	130,000	0.48	0.39
Andy Fluor™ 647	650	666	250,000	0.03	0.00
Andy Fluor™ 680	680	700	235,000	0.04	0.00
Andy Fluor™ 750	755	770	240,000	0.04	0.00