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LiFluor™ 405 Blue Fluorescent Dye (NHS ester)

Cat. #: C0099 Size: 1 µmol

Introduction

LiFluor™ 405 Blue Fluorescent Dye is a blue fluorescent dye spectrally similar to Pacific Blue dye. However, compared to Pacific Blue dye, LiFluor™ 405 blue fluorescent dye is more bright, and more photostable. It can be used as a replacement for Pacific Blue, BD Horizon V450, eFluor 450.

The NHS ester (or succinimidyl ester) is the most popular amine reactive group for labeling with the primary amines ($R\text{-}NH_2$) of proteins, amine-modified oligonucleotides, and other amine-containing molecules. The resulting LiFluor™ conjugate will exhibit brighter fluorescence and greater photostability than the conjugates of other spectrally similar fluorophores.

Package Information

Component	C0099
LiFluor™ 405 Blue Fluorescent Dye (NHS ester)	1 µmol

Storage

Store at -20°C

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 LiFluor™ 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 LiFluor™ NHS ester in DMF or DMSO to make 10 mM concentration.** For a typical reaction, dissolve 1 µmol of LiFluor™ 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 LiFluor™ 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.** 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 LiFluor™ dyes are given in the Tabel 1.

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 LiFluor™ dyes are given in the Tabel 1.

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 LiFluor™ NHS ester in 30 µl DMSO.**

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

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3.3 While stirring or vortexing the amine-modified oligonucleotide solution (step 3.1), slowly add 30 µl of the LiFluor™ 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 1. Physical characteristics of the LiFluor™ dyes

Dye	λ_{max} (nm)	E_m (nm)	Em ($cm^{-1}M^{-1}$)	CF_{280}	CF_{260}
LiFluor™ 350	350	440	19,000	0.19	0.24
LiFluor™ 405 blue	405	450	38,500	0.22	ND
LiFluor™ 405 green	412	500	28,500	0.23	ND
LiFluor™ 488	495	520	70,000	0.10	0.29
LiFluor™ 546	555	575	104,500	0.12	0.29
LiFluor™ 555	553	565	150,000	0.07	0.21
LiFluor™ 568	578	602	91,000	0.45	0.43
LiFluor™ 594	590	615	90,000	0.55	0.42
LiFluor™ 610	610	625	100,000	0.45	0.40
LiFluor™ 635	630	650	130,000	0.48	0.39
LiFluor™ 647	650	666	250,000	0.03	0.00
LiFluor™ 680	680	700	235,000	0.04	0.00
LiFluor™ 750	755	770	240,000	0.04	0.00