LumiDye™ AF Dye NHS Esters

Storage -20℃. Protect from light. Desiccated.
Specifications 100μg/1mg/5mg.

Information of LumiDye™ AF Dyes

Cat.No.	Dyes	Ex/Em (nm)	εdye ($M^{-1}cm^{-1}$)	CF280	MW (g/mol)	Target DOL
A1507078	LumiDye™ AF350 NHS Ester	343/441	19,000	0.19	410	4-9
A1507080	LumiDye™ AF405 NHS Ester	401/422	34,000	0.7	1028	4-9
A1507081	LumiDye™ AF488 NHS Ester	490/525	76,000	0.1	1081	4-9
A1507082	LumiDye™ AF532 NHS Ester	530/555	81,000	0.09	723	4-9
A1507083	LumiDye™ AF546 NHS Ester	554/570	112,000	0.12	1159.6	4-9
A1507084	LumiDye™ AF555 NHS Ester	553/568	150,000	0.08	1250	4-8
A1507085	LumiDye™ AF568 NHS Ester	578/602	88,000	0.46	791.82	4-9
A1507086	LumiDye™ AF594 NHS Ester	590/618	92,000	0.56	820	4-9
A1507087	LumiDye™ AF647 NHS Ester	650/668	239,000	0.03	1300	4-8
A1507088	LumiDye™ AF660 NHS Ester	663/691	132,000	0.1	1100	3-8
A1507089	LumiDye™ AF680 NHS Ester	681/704	184,000	0.05	1150	3-7
A1507090	LumiDye™ AF700 NHS Ester	696/719	192,000	0.07	1400	3-7
A1507091	LumiDye™ AF750 NHS Ester	752/776	240,000	0.04	1300	3-8

Introduction

The LumiDye™ AF Dyes are superior fluorophores with fluorescence emissions that span the visible spectrum and beyond. LumiDye™ AF conjugates exhibit brighter fluorescence and greater photostability than the conjugates of other spectrally similar fluorophores. These characteristics allow you to capture images that were previously unattainable with conventional fluorophores.

The LumiDye™ AF dyes are water-soluble and pH-insensitive within the range of 4－10,

providing better performance under various biological reaction conditions. The succinimidyl esters (commonly known as NHS esters) of AF dyes are available as standalone reagents, offering an efficient and convenient labeling solution that allows you to develop and optimize custom AF dye conjugates.

The LumiDye™ AF dyes can be selectively conjugated to primary amines (R-NH_2) on peptides, proteins, or amine-modified nucleic acids. Unlike other reactive groups, succinimidyl esters exhibit very low reactivity toward aromatic amines, alcohols, and phenols—including tyrosine and histidine. Succinimidyl esters are more suitable than other amine-reactive reagents (such as isothiocyanates) for linking fluorophores to amine-containing molecules, because the amide bond formed during the reaction is as stable as a peptide bond.

Guidelines For Use

1. Prepare the protein for Labeling

Dilute the protein with 0.1 M $NaHCO_3$ solution (pH 8.3) to a final protein concentration of ⩾2 mg/mL. Labeling efficiency may be higher when the protein concentration is above 5 mg/mL. Due to variations in buffer and protein purity, the optimal efficiency is determined by practical conditions. If the protein concentration is too low, it can be concentrated using an ultrafiltration device. If the product is already diluted in a phosphate buffer like PBS (without amine-containing compounds), approximately 1/10 volume of a 1 M $NaHCO_3$ stock solution can be added directly to this buffer to achieve a final $NaHCO_3$ concentration of 0.1 M.

2. Prepare the Dye Stock Solution

Bring the LumiDye™ AF Dye NHS Ester to room temperature. Dissolve the AF Dye in DMSO at 10 mg/mL. For a typical reaction, dissolve 1 mg of dye in 0.1 mL of DMSO. Dissolve the dye immediately before starting the reaction as reactive dyes are not very stable in solution. Briefly centrifuge to collect the solution at the bottom of the tube.

Note: The remaining dye stock solution should be stored at -20°C for future use. When prepared with anhydrous DMSO, the dye stock solution is stable for at least one month.

3. Labeling Reaction

(1) We recommend a protein concentration of ⩾2 mg/mL, with a dye-to-protein molar ratio ranging from 10:1 to 30:1. The amount of dye added can be adjusted as needed to achieve the optimal Degree of Labeling (DOL).

(2) Calculate the millimoles of AF Dye to add to the reaction for a 20-fold molar excess

$$\text{mmol AF Dye} = \frac{\text{mg/mL protein} \times \text{mL protein}}{\text{MW protein}} \times 20$$

(3) Calculate microliters of AF Dye to add to the reaction

$$\mu\text{L AF Dye} = \frac{\text{mmol AF Dye} \times \text{MW AF Dye}}{\text{mg/mL AF Dye}} \times 1000$$

(4) Mix the protein solution by stirring or vortexing. Gradually add the AF dye stock solution to achieve a dye-to-protein molar ratio ranging from 10:1 to 30:1.

(5) Incubate the reaction mixture at room temperature with stirring in the dark for 1 hour. For micro-scale labeling, incubation with shaking on a shaker for 1 hour is also acceptable.

4. Purify the Labeled Protein

Separating the conjugate from unreacted labeling reagent by dialysis or gel filtration media with an appropriate molecular weight cutoff.

Note: For small-scale labeling reactions, to avoid excessive dilution of the product, an ultrafiltration device can be used to remove unreacted free dye from the conjugate.

5. Determining the Degree of Labeling

(1) Use the absorption spectrophotometer to measure the absorbance of the protein–dye conjugate at 280 nm (A_{280}) and at the λmax for the dye (A_{max}) (with a 1cm path length).

(2) The following formula can be used to calculate the protein concentration

$$\text{protein concentration (mg/mL)} = \frac{(A_{280} - CF_{280} \times \text{Amax}) \times \text{MW protein}}{\varepsilon \text{ protein}}$$

where εprotein = the extinction coefficient of the protein at 280nm. Commonly the molar extinction coefficient of the IgG antibody at 280 nm is 210000 $M^{-1}cm^{-1}$.

(3) The following formula can be used to calculate the degree of labeling

$$\text{DOL} = \frac{\text{Amax} \times \text{MW protein}}{\text{protein concentration (mg/mL)} \times \varepsilon\text{AF dye}}$$

where MW = the molecular weight of the protein, εdye = the extinction coefficient of the AF dye at its absorbance maximum, and the protein concentration is in mg/mL.

6. Storing the Protein Conjugate

Add 0.05–0.2% Proclin 300 or 0.05% sodium azide, along with a protein stabilizer (such as 0.1% BSA), to the labeled protein. Store protected from light at 2–8°C for stable preservation up to six months. Alternatively, add an equal volume of glycerol and store at -20°C for stable preservation up to six months.