INSTRUCTIONS

DyLight® Long Stokes Shift Dyes

82491 82492 82493 82495

2448.0

Number	Description
82492	**DyLight 485-LS NHS Ester,** 1mg
82493	**DyLight 510-LS NHS Ester,** 1mg
82491	**DyLight 515-LS NHS Ester,** 1mg
82495	**DyLight 521-LS NHS Ester,** 1mg

Storage: Upon receipt store at -20°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific DyLight Long Stokes Shift Dyes are amine-reactive fluorescent dyes that produce long Stokes (LS) shifts of 80-145nm in excitation/emission wavelengths (see Table 1). The DyLight LS Dyes provide multicolor detection using a single excitation source in applications including flow cytometry, conjugation, DNA-sequencing, microscopy and fluorescence in situ hybridization (FISH).

These dyes contain *N*-hydroxysuccinimide (NHS) esters, the most commonly used reactive group for labeling proteins. NHS esters react with primary amines on proteins and other molecules, forming a stable, covalent amide bond and releasing the NHS group.

Table 1. Properties of the Thermo Scientific DyLight LS Dyes using a 488nm excitation laser line.

DyLight Dye	Ex/Em*	ε†	MW (g/mol)	Emission Laser Line
485-LS	485/559	50,000	600	Yellow
510-LS	509/590	50,000	652	Orange
515-LS	515/650	50,000	728	Red
521-LS	523/668	50,000	728	Far Red

*Excitation and emission maxima in nanometers

†Molar extinction coefficient ($M^{-1}cm^{-1}$)

Important Product Information

- NHS ester-activated fluorophores are moisture-sensitive. Store product in the original pouch at -20°C. Avoid moisture condensation onto the product by equilibrating the vial to room temperature before opening. Prepare the labeling reagents immediately before use. Do not store NHS-ester reagents in aqueous solutions.
- Low concentrations of sodium azide (≤ 3mM or 0.02%) or thimerosal (≤ 0.02mM or 0.01%) will not significantly interfere with protein labeling; however, 20-50% glycerol will reduce labeling efficiency.
- To remove excess non-reacted dye, use a dialysis membrane with a molecular-weight cutoff ≥10K or the Thermo Scientific Pierce Dye Removal Columns (Product No. 22858).

Procedure for Protein Labeling

Note: The following protocol is an example labeling application; specific applications require optimization.

A. Protein Preparation

The optimal labeling buffer is 0.05M sodium borate buffer at pH 8.5 (e.g., Thermo Scientific BupH Borate Buffer Packs, Product No. 28384). Buffers containing primary amines (e.g., Tris or glycine) will interfere because they react with the NHS-ester moiety. Dissolve protein directly in the labeling buffer. For each labeling reaction, use 100µL to 1mL of purified protein sample at 1-10mg/mL. After reconstitution, proceed to the **Calculations for Labeling** Section. If the protein is already in a buffer, perform a buffer exchange into the labeling buffer by dialysis or gel filtration.

Note: The following buffers may be substituted for borate buffer: 0.1M sodium phosphate, 0.15M NaCl at pH 7.2-7.5 (e.g., BupH™ Phosphate Buffered Saline Packs, Product No. 28372) **OR** 0.1M sodium carbonate at pH 8.3-9.0.

B. DyLight Dye Preparation

Equilibrate vial to room temperature before opening to avoid moisture condensation onto the reagent. Dissolve reagent in DMF at 10mg/mL. The reagent may also be dissolved at 1mg/mL to make pipetting small amounts more accurate; however, adjust for the concentration change when calculating the reagent amount added to the labeling reaction.

C. Calculations for Labeling

The amount of fluorescent-labeling reagent to use for each reaction depends on the amount of protein to be labeled and the specific fluorophore being used. Generally, the more concentrated the protein, the more efficient the reaction.

1. Calculate the amount (mg) of DyLight LS NHS-Ester Dye to be added to the labeling reaction:

$$\frac{\text{amount of protein (mg)}}{\text{MW of protein}} \times 10 \times \text{MW of dye} = \_\_\_\_\_ \text{ mg of dye}$$

- 10 = Molar-fold excess of the NHS ester dye to protein

2. Calculate microliters of dye solution to add to the reaction:

$$\text{mg of dye (calculation \#1)} \times \frac{100\,\mu\text{L}}{1\,\text{mg}} = \_\_\_\_\_ \,\mu\text{L NHS - ester dye solution at 10 mg/mL}$$

- 100µL = Solvent volume in which the 1mg of NHS-ester dye is dissolved

Example Calculation: For 1mL of a 2mg/mL solution of IgG (150,000 MW), 9.7µL of DyLight 515- LS NHS Ester (10mg/mL) will be used.

$$\frac{2\text{ mg IgG}}{150{,}000\text{ MW}} \times 10 \times 727.75 = 0.097\text{ mg of DyLight 515 - LS NHS Ester}$$

$$0.097\text{ mg of DyLight 515 - LS NHS Ester} \times \frac{100\,\mu\text{L}}{1\,\text{mg}} = 9.7\,\mu\text{L of DyLight 515 - LS NHS Ester}$$

D. Labeling Reaction

1. Equilibrate dyes to room temperature before opening vials.
2. Add 100µL of DMF to the dye. Pipette up and down or vortex until it is completely dissolved.

   Note: Allow the dye to completely dissolve for 5-10 minutes and then vortex again.
3. Transfer the protein solution to be labeled to a reaction tube.
4. Add the calculated amount of reagent to the reaction tube containing the protein. Mix well and incubate at room temperature for 1 hour, protected from light.
5. Remove non-reacted reagent from the protein by dialysis or with Pierce® Dye Removal Columns.

6. Store labeled protein protected from light at 4°C for up to one month.

   Note: For long-term storage, add bovine serum albumin (5-10mg/mL) and sodium azide (0.01-0.03% final concentration) to the conjugate and store the labeled protein in single-use volumes at -20°C. Exact storage conditions may vary for different proteins and should be determined empirically.

E. Calculate the Degree of Labeling

1. Remove excess dye reagent from the sample using a dialysis membrane with a molecular-weight cutoff ≥ 10K or the Pierce Dye Removal Columns.

   Note: For optimal results and accurate determination of the dye-to-protein ratio, remove all non-reacted dye. Remove excess dye by dialyzing for ~4 hours using three dialysis buffer changes. Gel filtration (e.g., desalting columns) is not as effective as dialysis and, therefore, is not recommended.

2. Dilute a small amount of labeled, purified protein in PBS. Using a 1cm path length cuvette, measure the absorbance at 280nm (A_{280}) and the A_{max} of the specific dye (Table 2).

Table 2. Properties of the Thermo Scientific DyLight LS Dyes.

DyLight Dye	A_{max}*	ε†	CF§
485-LS	485	50,000	0.137 (in EtOH)
510-LS	509	50,000	0.174 (in EtOH)
515-LS	519	50,000	0.142 (in EtOH) 0.105 (in PBS)
521-LS	526	50,000	0.184 (in EtOH) 0.155 (in PBS)

*Excitation wavelength – note that upon protein conjugation, the absorption maximum shifts to the right of the spectra
†Molar extinction coefficient ($M^{-1}cm^{-1}$) at A_{max}
§Correction factor (A_{280}/A_{max}) in ethanol (EtOH) or phosphate-buffered saline (PBS)

3. Calculate protein concentration as follows:

$$\text{Protein concentration (M)} = \frac{[A_{280} - (A_{max} \times CF)]}{\varepsilon_{protein}} \times \text{dilution factor}$$

- $\varepsilon_{protein}$ = protein molar extinction coefficient (e.g., the molar extinction coefficient of IgG is ~210,000 M^{-1} cm^{-1})
- CF = Correction factor $= \frac{A_{280} \text{ of the dye}}{A_{max} \text{ of the dye}}$ (see Table 2)

4. Calculate the degree of labeling:

$$\text{Moles of dye per mole of protein} = \frac{A_{max} \text{ of the labeled protein} \times \text{dilution factor}}{\varepsilon_{dye} \times \text{protein concentration (M)}}$$

- ε_{dye} = see Table 2

Example calculations for DyLight 515-LS Dye conjugated to antibodies in PBS:

Dilution factor = 10
A_{280} = 0.287
A_{max} at 519nm = 0.878

Protein concentration calculation:

$$\text{Protein concentration (M)} = \frac{[0.287 - (0.878 \times 0.105)]}{210{,}000} \times 10 = 0.0000093\ \text{M}$$

Mole Dye-to-mole protein calculation:

$$\text{Moles of dye per mole of protein} = \frac{0.878 \times 10}{150{,}000 \times 0.0000093} = 6.3$$

Troubleshooting

Problem	Cause	Solution
Dye-labeled protein application is unsuccessful	The protein was not labeled	Before troubleshooting, determine if the protein is labeled by calculating the A_{max}:A_{280} ratio; determine this ratio after thorough desalting or dialysis **Note:** For dye-labeled antibodies the A_{max}:A_{280} ratio should be > 1.
Protein is not labeled	Conjugation Buffer contained primary amines (e.g., Tris or glycine) that interfered with the reaction	Use a conjugation buffer (e.g., borate, carbonate, PBS) free of primary amines
	The NHS ester hydrolyzed, becoming non-reactive	Prepare labeling reagent immediately before use – do not store NHS-ester reagents in aqueous solutions

Additional Information

Visit our website for additional information including the following items:

- Tech Tip #43: Protein stability and storage
- Tech Tip #3: Determine reactivity of NHS-ester biotinylation and crosslinking reagents
- Tech Tip #30: Modify and label oligonucleotide 5´ phosphate groups

Related Thermo Scientific Products

22858 **Pierce Dye Removal Columns**

28384 **BupH Borate Buffer Packs,** 40/pkg

28341 **20X Borate Buffer,** 500mL

28372 **BupH Phosphate Buffered Saline Packs,** 40/pkg

28348 **20X Phosphate Buffered Saline (PBS),** 500mL

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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