INSTRUCTIONS

DyLight® Specialty Dyes

2446.0

Number	Description
46609	**DyLight 415-Co1, NHS Ester,** 1mg
46611	**DyLight 530-R2, NHS Ester,** 1mg
46612	**DyLight 554-R0, NHS Ester,** 1mg
46614	**DyLight 554-R1, NHS Ester,** 1mg
46617	**DyLight 590-R2, NHS Ester,** 1mg
46624	**DyLight 610-B1, NHS Ester,** 1mg
46625	**DyLight 615-B1, NHS Ester,** 1mg
46631	**DyLight 633-B1, NHS Ester,** 1mg
46632	**DyLight 633-B2, NHS Ester,** 1mg
46633	**DyLight 633-B3, NHS Ester,** 1mg
46634	**DyLight 635-B2, NHS Ester,** 1mg
46635	**DyLight 655-B1, NHS Ester,** 1mg
46636	**DyLight 655-B2, NHS Ester,** 1mg
46637	**DyLight 655-B3, NHS Ester,** 1mg
46638	**DyLight 655-B4, NHS Ester,** 1mg
46646	**DyLight 675-B1, NHS Ester,** 1mg
46647	**DyLight 675-B2, NHS Ester,** 1mg
46648	**DyLight 675-B3, NHS Ester,** 1mg
46649	**DyLight 675-B4, NHS Ester,** 1mg
53003	**DyLight 679-C5, NHS Ester,** 1mg
53022	**DyLight 690-B1, NHS Ester,** 1mg
53023	**DyLight 690-B1, NHS Ester,** 1mg
53026	**DyLight 690-B2, NHS Ester,** 1mg
53028	**DyLight 700-B1, NHS Ester,** 1mg
53030	**DyLight 700-B2, NHS Ester,** 1mg
53032	**DyLight 730-B1, NHS Ester,** 1mg
53033	**DyLight 730-B2, NHS Ester,** 1mg
53036	**DyLight 730-B3, NHS Ester,** 1mg
53037	**DyLight 730-B4, NHS Ester,** 1mg
46623	**DyLight 747, NHS Ester,** 1mg
62283	**DyLight 747, Free Acid,** 1mg
53040	**DyLight 747-B2, NHS Ester,** 1mg
53041	**DyLight 747-B3, NHS Ester,** 1mg
53042	**DyLight 747-B4, NHS Ester,** 1mg

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53054	**DyLight 775-B2, NHS Ester,** 1mg
53055	**DyLight 775-B3, NHS Ester,** 1mg
85150	**DyLight 775-B4, NHS Ester,** 1mg
53064	**DyLight 780-B1, NHS Ester,** 1mg
53065	**DyLight 780-B2, NHS Ester,** 1mg
53066	**DyLight 780-B3, NHS Ester,** 1mg
53067	**DyLight 830-B2, NHS Ester,** 1mg

Storage: Upon receipt store at -20°C. Product shipped at ambient temperature. Store product in foil pouch with desiccant to protect from light and moisture.

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Introduction

The Thermo Scientific DyLight Specialty Dyes are fluorescent dyes with varying spectral characteristics, degrees of sulfonation, charges and hydrophobicities. These dyes are offered in their amine-reactive forms and are mainly derivatized with *N*-hydroxysuccinimide (NHS) esters, the simplest and most commonly used reactive group for labeling proteins. The large selection of near infrared (NIR) and infrared (IR) dyes may be used for *in vivo* and near-IR fluorescence (NIRF) applications.

Table 1. Properties of the Thermo Scientific DyLight Specialty Dyes.

Emission Color	DyLight Dye	Ex/Em*	ε†	MW (g/mol)	Spectrally Similar Dyes	Laser / Filter Set
Blue	415-Co1	418/463	34,000	574	–	405 laser source
Green	530-R2	533/554	100,000	793	Alexa Fluor 532	488 laser source 532 flow-cytometry laser line Nd:YAG laser Argon ion laser
Yellow	554-R0	544/570	100,000	677	DyLight 550, Alexa Fluor® 546, Alexa Fluor 555, CF543, CF 555, Cy®3, TAMRA	546 laser source 532 flow-cytometry line 546 Mercury Arc Emission Cy3 filter set
	554-R1	548/574	100,000	813		
Orange	590-R2	591/598	120,000	863	DyLight 594, Alexa Fluor 594, Cy3.5, Texas Red®	
	610-B1	610/632	80,000	765	Alexa Fluor 610, CF620R	594 laser source 532 flow-cytometry line 561 diode laser Cy3.5 filter set

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Red	615-B1	623/643	200,000	676	Alexa Fluor 610, CF620R	Cy3.5 filter set
	633-B1	638/658	200,000	732	DyLight 633, Alexa Fluor 633, CF633	633 flow-cytometry line Red diode laser He-Ne laser
	633-B2	637/657	200,000	834		
	633-B3	636/658	200,000	950		
	635-B2	638/658	200,000	848		
Far Red	655-B1	656/676	220,000	794	DyLight 647, Alexa Fluor 647, CF647, Cy5	633 and 647 laser source 633 flow-cytometry line Red diode laser (633) He-Ne laser Cy5 filter set
	655-B2	655/677	220,000	886		
	655-B3	653/676	220,000	1002		
	655-B4	653/677	220,000	1102		
Near IR	675-B1	675/699	180,000	804	DyLight 680, Alexa Fluor 680, CF680, CF680R, Cy5.5	633 laser source Far-red diode laser LI-COR Odyssey®, Aerius® (700 channel) Other near-IR imaging systems Cy5.5 filter set
	675-B2	675/699	180,000	906		
	675-B3	674/698	180,000	1022		
	675-B4	674/694	180,000	1124		
	679-C5	679/697	200,000	1240		
	690-B1	691/709	140,000	732		
	690-B2	692/709	140,000	834		
	700-B1	707/728	140,000	766	Alexa Fluor 700, IRDye 700DX	633 laser source LI-COR Odyssey, Aerius (700 channel) Other near-IR imaging systems
	700-B2	709/730	140,000	868		
	730-B1	734/755	240,000	758	–	
	730-B2	736/755	240,000	860		
	730-B3	735/756	240,000	976		
	730-B4	733/755	240,000	1092		
	747	751/774	270,000	713 (acid) 810	DyLight 750, Alexa Fluor 750, CF750, Cy7, IRDye 750	633 laser source Xenon arc excitation LI-COR Odyssey, Aerius (700 and 800 channels) Other near IR imaging systems
	747-B2	752/772	270,000	912		
	747-B3	750/771	270,000	1028		
	747-B4	748/771	270,000	1130		
	775-B2	772/787	240,000	932	DyLight 800, CF770, IRDye 800	
	775-B3	770/788	240,000	1048		
	775-B4	767/787	240,000	1150		
	780-B1	783/799	170,000	758		
	780-B2	784/796	170,000	860		
	780-B3	784/794	170,000	976		
	830-B2	844/875	220,000	886	–	IR imaging systems

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 these 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

The following is an example labeling application; specific applications will require optimization.

A. Protein Preparation for Labeling

The optimal labeling buffer is 0.05M sodium borate buffer at pH 8.5 (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 amount (mg) of DyLight 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 NHS-ester dye solution to add to the reaction:

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

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

Example Calculation: 1mL of a 2mg/mL solution of IgG (150,000 MW) requires 14µL of DyLight 775-B3 NHS Ester (10mg/mL).

$$\frac{2\text{ mg IgG}}{150{,}000\text{ MW}} \times 10 \times 1048.14 = 0.14\text{ mg of DyLight 775 - B3 NHS Ester}$$

$$0.14\text{ mg of DyLight 775 - B3 NHS Ester} \times \frac{100\,\mu\text{L}}{1\,\text{mg}} = 14\,\mu\text{L of DyLight 775 - B3 NHS Ester}$$

D. Labeling Reaction

1. Equilibrate DyLight NHS Esters to room temperature before opening vials.
2. Add 100µL of DMF to the DyLight NHS Ester. 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 Pierce Dye Removal Columns.
6. Store labeled protein protected from light at 4°C for up to one month.

   Note: For long-term storage, we recommend adding 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.

   Note: The non-reacted dye must be removed for optimal results and accurate determination of the dye-to-protein ratio. For best results, remove excess non-reacted dye by dialyzing for ~4 hours using three dialysis buffer changes.
2. Dilute a small amount of labeled, purified protein in PBS. Using a 1cm path length cuvette and measure the absorbance at 280nm and the A_{max} of the specific dye (Table 2).

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

DyLight Dye	A_{max}*	ε†	CF‡
415-Co1	418	34,000	0.140 (EtOH)
530-R2	533	100,000	0.125 (EtOH) 0.148 (PBS)
554-R0	544	100,000	0.233 (EtOH)
554-R1	548	100,000	0.180 (EtOH)
590-R2	581	120,000	0.143 (EtOH)
610-B1	610	80,000	0.210 (EtOH)
615-B1	623	200,000	0.058 (EtOH)
633-B1	638	200,000	0.092 (EtOH)
633-B2	637	200,000	0.312 (EtOH) 0.104 (PBS)
633-B3	636	200,000	0.108 (EtOH)
635-B2	638	200,000	0.088 (EtOH)
655-B1	656	220,000	0.058 (EtOH)
655-B2	655	220,000	0.052 (EtOH)
655-B3	653	220,000	0.060 (EtOH)
655-B4	653	220,000	0.067 (EtOH) 0.076 (PBS)

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675-B1	675	180,000	0.080 (EtOH)
675-B2	675	180,000	0.078 (EtOH)
675-B3	674	180,000	0.087 (EtOH)
675-B4	674	180,000	0.100 (EtOH)
679-C5	679	200,000	0.110 (EtOH) 0.115 (PBS)
690-B1	691	140,000	0.099 (EtOH)
690-B2	692	140,000	0.089 (EtOH) 0.135 (PBS)
700-B1	707	140,000	0.103 (EtOH)
700-B2	709	140,000	0.127 (EtOH)
730-B1	734	240,000	0.047 (EtOH)
730-B2	736	240,000	0.052 (EtOH)
730-B3	735	240,000	0.064 (EtOH) 0.138 (PBS)
730-B4	733	240,000	0.045 (EtOH) 0.069 (PBS)
747	751	270,000	0.030 (EtOH)
747-B2	752	240,000	0.029 (EtOH)
747-B3	750	270,000	0.028 (EtOH)
747-B4	748	270,000	0.035 (EtOH) 0.047 (PBS)
775-B2	772	240,000	0.074 (EtOH) 0.196 (PBS)
775-B3	770	240,000	0.070 (EtOH) 0.096 (PBS)
775-B4	767	240,000	0.081 (EtOH) 0.118 (PBS)
780-B1	783	170,000	0.079 (EtOH)
780-B2	784	170,000	0.075 (EtOH)
780-B3	785	170,000	0.096 (EtOH) 0.145 (PBS)
830-B2	844	240,000	0.061 (EtOH)

* Excitation wavelength in nanometers – 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 3)

4. Calculate the degree of labeling:

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

- ε_{dye} = See Table 2

Example calculations for DyLight 775-B3 NHS Ester conjugated to antibody:

- Dilution factor = 10
- A_{280} = 0.287
- A_{max} at 770nm = 0.878

$$\text{Protein concentration (M)} = \frac{[0.287 - (0.878 \times 0.096)]}{210{,}000} \times 10 = 0.0000096 \text{ M}$$

$$\text{Moles dye per mole protein} = \frac{0.878 \times 10}{150{,}000 \times 0.00000096} = 6.1$$

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 free of primary amines (e.g., borate, carbonate or PBS
	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**
28341	**20X Borate Buffer,** 500mL
28348	**20X Phosphate Buffered Saline (PBS),** 500mL
28372	**BupH Phosphate Buffered Saline Packs,** 40/pkg
28384	**BupH Borate Buffer Packs,** 40/pkg

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