INSTRUCTIONS

DyLight® NHS Ester Quenchers

2447.1

Number	Description
84512	**DyLight 425Q NHS Ester,** 1mg
84513	**DyLight 504Q NHS Ester,** 1mg
84508	**DyLight 543Q NHS Ester,** 1mg
84509	**DyLight 641Q NHS Ester,** 1mg
84514	**DyLight 662Q NHS Ester,** 1mg
84510	**DyLight 683Q NHS Ester,** 1mg
84515	**DyLight 696Q NHS Ester,** 1mg
84511	**DyLight 766Q NHS Ester,** 1mg

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific DyLight NHS Ester Quenchers are amine-reactive non-fluorescent dyes that use fluorescent resonance energy transfer (FRET) to quench fluorescent signals resulting from ultraviolet, visible and infrared light-excited fluorescent dyes. These dyes can be efficiently paired with many DyLight Dyes and other common fluorescent dyes including fluorescein, rhodamine, cyanine and Texas Red®Dye.

Table 1. Physical Properties of the Thermo Scientific DyLight NHS Ester Quenchers.

DyLight Dye	Absorption*	ε†	MW (g/mol)	Compatible Dyes	Laser / Filter Set
425Q	425	24,500	881	Dyes emitting in the 400-475nm range (e.g., DyLight 405)	Spectral line of the blue diode laser
504Q	504	39,000	811	Dyes emitting in the 475-525nm range (e.g., DyLight 488)	Green (526) laser
543Q	543	48,000	886	Dyes emitting in the 500-575nm range (e.g., DyLight 488, DyLight 550, DyLight 556-R1, DyLight 590-R2, DyLight 633-B3, DyLight 651-B1, Cy®3, Alexa Fluor® 555, rhodamine)	Green (532) laser
641Q	641	90,000	843	Dyes emitting in the 600-660nm range (e.g., DyLight 550, DyLight 556-R1, DyLight 590-R2, DyLight 594, DyLight 633, DyLight 633-B3, DyLight 651-B1, DyLight 650, Alexa Fluor 647, Cy5, Texas Red)	Red (633) laser
662Q	662	140,000	868	Dyes emitting in the 575-650nm range (e.g., DyLight 515-LS, DyLight 550, DyLight 594, DyLight 633, DyLight 650)	Red (633) laser

Continued on next page

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Continued from previous page

683Q	683	80,000	900	Dyes emitting in the 660-680nm range (e.g., DyLight 633, DyLight 633-B2, DyLight 650, DyLight 655-B3, DyLight 655-B4, DyLight 680)	Laser- and filter-based instruments emitting in the 700-900nm region of the spectrum
696Q	696	49,050	906	Dyes emitting in the 625-715nm range (e.g., DyLight 650, DyLight 680, DyLight 680-4xPEG)	Laser- and filter-based instruments emitting in the 700-900nm region of the spectrum
766Q	766	180,000	894	Dyes emitting in the 750-775nm range (e.g., DyLight 755, DyLight 800, Alexa Fluor 750)	Laser- and filter-based instruments emitting in the 700-900nm region of the spectrum

*Absorption maxima in nanometers
†Molar extinction coefficient ($M^{-1}cm^{-1}$)

Important Product Information

- NHS ester-activated fluorescent quenchers 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 DyLight Quenchers, use a dialysis membrane with a molecular-weight cutoff ≥10K or the Pierce Dye Removal Columns (Product No. 22858).

Procedure for Protein Labeling

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

A. Protein Preparation

The optimal labeling buffer is 0.05M sodium borate buffer at pH 8.5 (Thermo Scientific BupH Borate Buffer Packs, Product No. 28384). Buffers that contain primary amines (e.g., Tris or glycine) will interfere with the labeling efficiency since 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 Quencher 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 dye being used (Table 2). Generally, the more concentrated the protein, the more efficient the reaction.

- Step 1: Calculate the amount (mg) of DyLight NHS Ester Quencher 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

- Step 2: Calculate microliters of dye solution to add to the reaction:

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

  - 100µL = Solvent volume in which the 1mg of DyLight NHS Ester Quencher is dissolved.

Example Calculation:

For 1mL of a 2mg/mL solution of IgG (150,000 MW), 11.92µL of DyLight 766Q NHS Ester (10mg/mL) will be used.

$$\frac{2\text{ mg IgG}}{150{,}000} \times 10 \times 894.02 = 0.1192\text{ mg of DyLight 766Q NHS Ester}$$

$$0.1192\text{ mg of DyLight 766Q NHS Ester} \times \frac{100\,\mu\text{L}}{1\,\text{mg}} = 11.92\,\mu\text{L of DyLight 766Q NHS Ester}$$

D. Labeling Reaction

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

   Note: Allow the dye to dissolve for 5-10 minutes and 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 dye from the protein by a dialysis membrane with a molecular-weight cutoff ≥10K or Pierce Dye Removal Columns.

   Note: The non-reacted dye must be completely 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.
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.

Note: For labeling peptides or oligonucleotides:

- Perform labeling by adding the dye-labeling step to the synthesis.
- Remove free (unreacted) quencher using high-pressure liquid chromatography (HPLC).

E. Calculate the Degree of Labeling

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

Table 2. Physical Properties of the Thermo Scientific DyLight NHS Ester Quenchers.

DyLight Dye	A_{max}*	$\varepsilon^{\dagger}$	CF‡ (in PBS)
425Q	425	24,500	0.387
504Q	504	39,000	0.277
543Q	543	48,000	0.252
641Q	641	90,000	0.126
662Q	662	140,000	0.187
683Q	683	80,000	0.117
686Q	696	49,050	0.670
766Q	766	180,000	0.181

* 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 (A280/Amax)

- Step1: 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)

- Step 2: 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 766Q Quencher conjugated to antibodies:

- Dilution factor = 10
- A_{280} = 0.287
- A_{max} at 766Qnm = 0.878

$$\text{Protein concentration (M)} = \frac{[0.287 - (0.878 \times 0.181)]}{210{,}000} \times 10 = 0.0000061 \text{ M}$$

$$\text{Moles dye per mole protein} = \frac{0.878 \times 10}{150{,}000 \times 0.0000061} = 9.6$$

Troubleshooting

Problem	Cause	Solution
Unsuccessful application using the quencher-labeled molecule	Molecule was not labeled	If the molecule is protein, determine that the protein is labeled by calculating the A_{max}:A_{280} ratio; determine this ratio after thoroughly removing unconjugated dye **Note:** For dye-labeled antibodies the A_{max}:A_{280} ratio should be > 1
	Quencher-labeled molecule did not FRET with the fluorophore-labeled molecule	Use a quencher-fluorophore pair with efficient FRET; refer to Table 1
	No interaction between the dye and quencher-labeled molecules	Ensure the molecules interact before and after labeling
Protein was not labeled	Conjugation buffer contained primary amines (e.g., Tris or glycine) that interfered with the reaction	Use a primary amine-free conjugation buffer (e.g., borate, carbonate or PBS)
	NHS ester hydrolyzed and was 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

Cy is a trademark of GE Healthcare Limited UK.

Alexa Fluor and Texas Red are trademarks of Life Technologies, Inc.