INSTRUCTIONS

# DyLight® Amine-Reactive Dyes

2032.11

| Number | Description |
|---|---|
| 46427 | **DyLight 350 NHS Ester,** 5 × 65µg |
| 46401 | **DyLight 405 NHS Ester,** 5 × 50µg |
| 46403 | **DyLight 488 NHS Ester,** 5 × 50µg |
| 62263 | **DyLight 550 NHS Ester,** 5 × 50µg |
| 46413 | **DyLight 594 NHS Ester,** 5 × 65µg |
| 46417 | **DyLight 633 NHS Ester,** 5 × 50µg |
| 62266 | **DyLight 650 NHS Ester,** 5 × 50µg |
| 46419 | **DyLight 680 NHS Ester,** 5 × 50µg |
| 62279 | **DyLight 755 NHS Ester,** 5 × 50µg |
| 46422 | **DyLight 800 NHS Ester,** 5 × 50µg |

**Note:** Each vial is sufficient to label 0.25-1mg of protein (> 45kDa).

**Storage:** Upon receipt store DyLight NHS Esters at -20°C. Products are shipped with an ice pack. Store all dyes in the foil pouch with desiccant to protect from light and moisture.

## Introduction

The Thermo Scientific DyLight Amine-Reactive Dyes have absorption spectra ranging from 350nm to 770nm (Table 1) and are packaged in a convenient amount suitable for one labeling reaction. These reagents fluoresce over a broad pH range, are more intense than Alexa Fluor® or Cy® Dyes in many applications and match the output wavelengths of common fluorescence instrumentation. Additionally, the single-use packaging and water solubility of the DyLight Reagents allows protein samples to be added directly to the reagent vial for high dye-to-protein ratio conjugations without precipitation.

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

**Table 1.** Properties of the DyLight NHS-Ester Dyes.

| DyLight Dye | Ex/Em* | ε† | MW (g/mol) | Spectrally Similar Dyes |
|---|---|---|---|---|
| **350** | 353 / 432 | 15,000 | 874 | Alexa Fluor 350, AMCA |
| **405** | 400 / 420 | 30,000 | 793 | Alexa Fluor 405 |
| **488** | 493 / 518 | 70,000 | 1011 | Alexa Fluor 488, Cy2 |
| **550** | 562 / 576 | 150,000 | 1040 | Alexa Fluor 555, Cy3 |
| **594** | 593 / 618 | 80,000 | 1078 | Alexa Fluor 594, Texas Red |
| **633** | 638 / 658 | 170,000 | 1066 | Alexa Fluor 633 |
| **650** | 652 / 672 | 250,000 | 1066 | Alexa Fluor 647, Cy5 |
| **680** | 682 / 715 | 140,000 | 950 | Alexa Fluor 680, Cy5.5 |
| **755** | 755 / 776 | 220,000 | 1092 | Alexa Fluor 750 |
| **800** | 770 / 794 | 270,000 | 1050 | IRDye 800 |

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

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## 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 prepared in aqueous solutions.
- Use the following fluorescent imagers:
  - 350 dye: UV argon-ion laser at 351-363nm
  - 405 dye: Spectral line of the blue diode laser
  - 488 dye: Green (526) laser
  - 550 and 594 dyes: Green (532) laser
  - 633 and 650 dyes: Red (633) laser
  - 680, 755 and 800 dyes: laser- and filter-based instruments that emit in the 700nm and 800nm region of the spectrum, respectively; these dyes are well-suited for the 700 and 800 channels of the LI-COR Odyssey® and the LI-COR Aerius™ Infrared Imaging Systems.
- 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 Dye, use a dialysis membrane with a molecular-weight cutoff ≥ 10K.

## Procedure for Protein Labeling

The following is an example application for the DyLight Amine-Reactive Dyes. Specific applications will require optimization.

### A. Protein Preparation

The optimal labeling buffer is 0.05M sodium borate buffer at pH 8.5 (Product No. 28384). When labeling with DyLight 594 NHS-Ester, prepare the protein in phosphate-buffered saline to avoid precipitation. Buffers that contain 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 100-500µL of purified protein sample at 1-2.5mg/mL. 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., Thermo Scientific BupH Phosphate Buffered Saline Packs, Product No. 28372) or 0.1M sodium carbonate at pH 8.3-9.0.

### B. Labeling Reaction

**Note:** The DyLight NHS-Ester reagents are moisture-sensitive. Store the reagent in the original container at -20°C with desiccant.

1. Transfer the protein solution to the vial containing the dye. Mix well by vortexing up-and-down several times and incubate at room temperature for 1 hour.
2. Remove excess dye reagent from the sample using the Thermo Scientific Dye Removal Columns (Product No. 22858) or a dialysis membrane with a molecular-weight cutoff ≥ 10K.

   **Note:** The non-reacted dye must be completely removed for optimal results and accurate determination of the dye-to-protein ratio. For best results, use the Dye Removal Columns or dialyze for ~4 hours using three dialysis buffer changes. Gel filtration, such as with desalting columns, is not typically as effective as dialysis.
3. Store labeled protein protected from light at 4°C for up to one month. Alternatively, store labeled protein in single-use aliquots at -20°C.

### C. Calculate the Degree of Labeling

1. 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 (Table 2).

**Table 2.** Properties of the DyLight Dyes.

| DyLight Dye | $A_{max}$* | ε† | CF‡ |
|---|---|---|---|
| **350** | 353 | 15,000 | 0.144 |
| **405** | 405 | 30,000 | 0.564 |
| **488** | 493 | 70,000 | 0.147 |
| **550** | 557 | 150,000 | 0.081 |
| **594** | 595 | 80,000 | 0.585 |
| **633** | 627 | 170,000 | 0.110 |
| **650** | 655 | 250,000 | 0.037 |
| **680** | 684 | 140,000 | 0.128 |
| **755** | 755 | 220,000 | 0.030 |
| **800** | 777 | 270,000 | 0.045 |

* 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 ($A_{280}/A_{max}$)

2. 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 $= \frac{A_{280} \text{ of the fluor}}{A_{max} \text{ of the fluor}}$ (see Table 2)

3. Calculate the degree of labeling:

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

- $\varepsilon_{dye}$ = See Table 2

**Example calculations for DyLight 550 NHS Ester-conjugated antibody:**

- Dilution factor = 40
- $A_{280}$ = 0.177
- $A_{max}$ at 557nm = 0.411

$$\text{Protein concentration (M)} = \frac{[0.177 - (0.411 \times 0.081)]}{210{,}000} \times 40 = 0.00002737 \text{ M}$$

$$\text{Moles dye per mole protein} = \frac{0.177 \times 40}{150{,}000 \times 0.00002737} = 2.9$$

## Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| The application in which the dye-labeled protein was used was 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<br>**Note:** For dye-labeled antibodies the $A_{max}$:$A_{280}$ ratio should be > 1. |
| The protein was 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 such as borate, carbonate or PBS |
| | The NHS ester has hydrolyzed and is 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

| | |
|---|---|
| **46426** | **DyLight 350 NHS Ester,** 1mg |
| **46400** | **DyLight 405 NHS Ester,** 1mg |
| **46402** | **DyLight 488 NHS Ester,** 1mg |
| **62262** | **DyLight 550 NHS Ester,** 1mg |
| **46412** | **DyLight 594 NHS Ester,** 1mg |
| **46414** | **DyLight 633 NHS Ester,** 1mg |
| **62265** | **DyLight 650 NHS Ester,** 1mg |
| **46418** | **DyLight 680 NHS Ester,** 1mg |
| **62278** | **DyLight 755 NHS Ester,** 1mg |
| **46421** | **DyLight 800 NHS Ester,** 1mg |
| **69576** | **Slide-A-Lyzer® MINI Dialysis Unit Kit,** for 10-100µL samples, 10 units plus float |
| **66382** | **Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO,** for 0.5-3mL samples, 10 units, buoys and syringes |
| **66807** | **Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO,** for 3-12mL samples, 10 units, buoys and syringes |



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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