INSTRUCTIONS

# DyLight Antibody Labeling Kits

1897.13

| Number | Description |
|---|---|
| **62275** | **DyLight 350 Antibody Labeling Kit** |
| **53020** | **DyLight 405 Antibody Labeling Kit** |
| **53024** | **DyLight 488 Antibody Labeling Kit** |
| **84530** | **DyLight 550 Antibody Labeling Kit** |
| **53044** | **DyLight 594 Antibody Labeling Kit** |
| **53046** | **DyLight 633 Antibody Labeling Kit** |
| **84535** | **DyLight 650 Antibody Labeling Kit** |
| **53056** | **DyLight 680 Antibody Labeling Kit** |
| **84538** | **DyLight 755 Antibody Labeling Kit** |
| **53062** | **DyLight 800 Antibody Labeling Kit** |

**Note:** Each kit contains sufficient reagents to label and purify 3 × 1mg (2mg/mL) of IgG or similar amounts of other proteins.

**Kit Contents:**

**DyLight NHS Ester,** 3 × 50µg vials (except 350 and 594 Kits, which contain 3 × 65µg vials)
**Borate Buffer (0.67M),** 1mL
**Purification Resin,** 5mL
**Spin Columns,** 6 each
**Microcentrifuge Collection Tubes,** 12 each

**Storage:** Upon receipt, store the DyLight NHS Ester at -20°C. Store all other kit components at 4°C.

## Introduction

Each Thermo Scientific™ DyLight™ Antibody Labeling Kit contains all the necessary components for three labeling reactions of any protein with a molecular weight of 50-150K and subsequent excess dye removal. The DyLight Dyes included in these kits are activated with *N*-hydroxysuccinimide (NHS) esters, which is the most commonly used reactive group for labeling proteins. NHS esters react with primary amines, forming stable, covalent amide bonds and releasing the NHS groups. The DyLight Dyes have absorption spectra ranging from 350nm to 770nm (Table 1). These reagents fluoresce in 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 water solubility of the DyLight Reagents allows conjugations using a high dye-to-protein ratio without precipitation.

The purification resin and spin columns eliminate equilibration steps and the need to collect and monitor gravity-flow fractions. This system enables efficient removal of excess dye and, therefore, accurate determination of the dye-to-protein ratio and exceptional protein recovery.

**Table 1. Properties of the Thermo Scientific 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 |
| **755** | 754 / 776 | 220,000 | 1092 | Alexa Fluor 750 |
| **800** | 770 / 794 | 270,000 | 1050 | IRDye 800 |

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

## Important Product Information

- NHS ester-activated dyes are moisture-sensitive. Prepare the DyLight Labeling Reagent immediately before use and discard any unused reconstituted reagent. Do not store reconstituted labeling reagent.
- 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.
- 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.

## Additional Materials Required

- Variable-speed centrifuge
- Phosphate-buffered saline (PBS; for measuring the dye-to-protein ratio)

## Procedure for Labeling Proteins with DyLight Dyes

### A. Protein Preparation

**Note:** When labeling with the DyLight 594 Dye, prepare the protein in phosphate-buffered saline.

**Note:** If Borate Buffer precipitates during storage, solubilize it by warming at 37-50°C and vigorously vortexing the vial.

1. The optimal labeling buffer is 50mM sodium borate, pH 8.5 (please see note above). For best results use 1mg of protein at ~2mg/mL. Prepare the protein as follows:
   - **Proteins Lyophilized in PBS:** Just before use, prepare the labeling buffer by diluting the Borate Buffer (0.67M) to 0.05M in PBS or ultrapure water. Prepare only enough labeling buffer required for the reaction (for example, to prepare 1mL, add 75µL of Borate Buffer (0.67M) to 925µL of ultrapure water or PBS). Reconstitute 1mg of protein with 0.5mL of labeling buffer.
   - **Proteins in PBS Solution:** Add 40µL of the Borate Buffer (0.67M) to 0.5mL of 2mg/mL protein in PBS. If the protein is > 2mg/mL, adjust the concentration to 2mg/mL with labeling buffer (for example, 0.05M sodium borate – see the above bullet point: Proteins Lyophilized in PBS).
   - **Proteins in Other Buffers:** Protein must not be in a buffer containing ammonium ions or primary amines (for example, Tris or glycine). If necessary, replace buffer with 50mM sodium borate (Product No. 28384), pH 8.5 by dialysis or buffer exchange.

## B. Protein Labeling

1. Tap the bottom of the DyLight Reagent vial against a hard surface to ensure the dye is in the bottom of the tube. Add 0.5mL of the prepared protein to the vial of DyLight Reagent, vortex gently and pipette up and down to mix.
2. Briefly centrifuge the vial to collect the sample in the bottom of the tube. Incubate the reaction mixture for 60 minutes at room temperature protected from light.

## C. Protein Purification

1. Place two spin columns in two microcentrifuge collection tubes.
2. Mix the Purification Resin to ensure uniform suspension and add 250µL of the suspension into both spin columns. Centrifuge for 1 minute at ~1000 × *g* to remove the storage solution. Discard the used collection tubes and place the columns in new collection tubes.
3. Add 250-270µL of the labeling reaction to each spin column and mix the sample with the resin by briefly vortexing.
4. Centrifuge columns for 1 minute at ~1000 × *g* to collect the purified proteins. Combine the samples from both columns (~0.5mL total). Discard the used columns.
5. Store the labeled protein protected from light at 4°C for up to one month. Alternatively, store labeled protein in single-use aliquots at -20°C. Avoid repeated freeze/thaw cycles. If the final concentration of conjugate is < 1mg/mL, add a stabilizing agent, such as bovine serum albumin at 1-10mg/mL.

## D. Dye-to-Protein Ratio Estimation

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

**Table 2. Properties of the Thermo Scientific 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 ($A_{280}/A_{max}$)

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 as follows:

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

- $\varepsilon_{dye}$ = dye (fluorophore) molar extinction coefficient (see Table 2)

**Example calculations for DyLight 550 Dye conjugated to antibodies:**

- Dilution factor = 10
- $A_{280}$ = 0.287
- $A_{max}$ at 557nm = 0.878

$$\text{Protein concentration (M)} = \frac{[0.287 - (0.878 \times 0.081)]}{210{,}000} \times 10 = 0.00001028\ \text{M}$$

$$\text{Moles dye per mole protein} = \frac{0.878 \times 10}{150{,}000 \times 0.00001028} = 5.7$$

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Protein was not labeled | Protein buffer contained amines that interfered with labeling | Perform buffer exchange via dialysis or other method into 50mM sodium borate |
| | The NHS ester is hydrolyzed and non-reactive | Prepare labeling reagent immediately before use – do not store reagent in aqueous solution |
| The downstream application was unsuccessful | Protein was not labeled | Determine if the protein was labeled by calculating the dye-to-protein ratio |
| Sample or buffer does not flow through resin | Centrifugation problem | Ensure that centrifuge is in proper working condition |
| Low yield | Improper centrifugation | Make sure to use the indicated centrifugation speed |
| | Unstable protein | Equilibrate the column with PBS or other suitable buffer before adding the labeled protein |
| Problems with image capture | Photostability | DyLight 350 Dye is a relatively dim dye and quickly photobleaches under microscope light |

## Visit our Website for Additional Information

- Tech Tip #43: Protein stability and storage
- Tech Tip #31: Calculate dye:protein (F/P) molar ratios

## Related Thermo Scientific Products

**28384** **BupH™ Borate Buffer Packs,** 40 packs, each pack yields 500mL

**28372** **BupH Phosphate Buffered Saline Packs,** 40 packs, each pack yields 500mL



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