INSTRUCTIONS

DyLight Microscale Antibody Labeling Kits

1898.12

Number	Description
62276	**DyLight 350 Microscale Antibody Labeling Kit**
53021	**DyLight 405 Microscale Antibody Labeling Kit**
53025	**DyLight 488 Microscale Antibody Labeling Kit**
84531	**DyLight 550 Microscale Antibody Labeling Kit**
53045	**DyLight 594 Microscale Antibody Labeling Kit**
53047	**DyLight 633 Microscale Antibody Labeling Kit**
84536	**DyLight 650 Microscale Antibody Labeling Kit**
53057	**DyLight 680 Microscale Antibody Labeling Kit**
84539	**DyLight 755 Microscale Antibody Labeling Kit**
53063	**DyLight 800 Microscale Antibody Labeling Kit**

Note: Each kit contains sufficient reagents to label and purify 5 × 100µg (1mg/mL) of IgG or similar amounts of other proteins.

Kit Contents:
DyLight NHS Ester, 5 × 15µg vials
Borate Buffer (0.67M), 1mL
Purification Resin, 5mL
Spin Columns, 5 each
Microcentrifuge Collection Tubes, 10 each

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

Introduction

Each Thermo Scientific™ DyLight™ Microscale Antibody Labeling Kit contains all the components necessary for five separate 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. The NHS ester reacts with primary amines, forming a stable, covalent amide bond and releasing the NHS group. The DyLight Dyes have absorption spectra ranging from 350nm to 770nm (Table 1). 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 water solubility of the DyLight Reagents allows a high dye-to-protein ratio without precipitation during conjugation.

The dye 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
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 Reagent immediately before use and discard any unused reconstituted reagent. Do not store reconstituted 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 700 nm and 800 nm 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 Microscale Antibody Labeling with DyLight Dyes

A. Antibody 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 0.05M sodium borate, pH 8.5 (see note above). For best results use 100µg of antibody at ~1mg/mL. Prepare the antibody as follows:
   - **Antibodies 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 200µL, add 15µL of Borate Buffer (0.67M) to 185µL ultrapure water or PBS]. Reconstitute 100µg of antibody with 100 µl of labeling buffer.
   - **Antibodies in PBS Solution:** To 100µL of 1mg/mL antibody in PBS, add 8µL of the Borate Buffer (0.67M). If the protein is > 1mg/mL, adjust the concentration to 1mg/mL with labeling buffer (for example, 0.05 M sodium borate; see the above bullet point: Antibodies Lyophilized in PBS).
   - **Antibodies in Other Buffers:** Antibodies in buffers containing ammonium ions or primary amines (for example, Tris or glycine) will interfere with the intended reaction. Replace these buffers with 0.05M 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.
2. Add 0.1mL of the prepared protein to the vial of DyLight Reagent, vortex gently and pipette up and down to mix.
3. Briefly centrifuge the vial to collect the sample in the bottom of the tube.
4. Incubate the reaction mixture for 60 minutes at room temperature protected from light.

C. Protein Purification

1. Place spin column in the supplied microcentrifuge collection tube.
2. Mix the Purification Resin to ensure uniform suspension and add **100µL** of the suspension into the spin column. Centrifuge for 1 minute at ~1000 × *g* to remove the storage solution. Discard the used collection tube and place the column into a new tube.
3. Add 100-108µL of the labeling reaction to the column and allow the sample to mix with the resin by briefly vortexing.
4. Centrifuge column for 1 minute at ~1000 × *g* to collect the purified protein. Discard the used column.
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:Protein Ratio Estimation

1. Dilute a small amount of the 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 $= \frac{A_{280}}{A_{max}}$ (see Table 2)

4. Calculate the degree of labeling as follows:

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

- ε_{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 nonreactive	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

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