INSTRUCTIONS

Thermo SCIENTIFIC

DyLight 680-4xPEG Antibody Labeling Kit

53076

2504.1

Number	Description
53076	**DyLight 680-4xPEG Antibody Labeling Kit,** each kit contains sufficient reagents to label and purify 3 × 1mg (2mg/mL) of IgG or similar amounts of other proteins

Kit Contents:

DyLight 680-4xPEG NHS Ester, 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 680-4xPEG NHS Ester at -20°C. Store all other kit components at 4°C. Kit is shipped with an ice pack.

Introduction

The Thermo Scientific™ DyLight™ 680-4xPEG Antibody Labeling Kit allows for fast, efficient labeling of antibodies. The kit contains all of the components necessary for three separate labeling reactions of 1mg of IgG or similar quantities of other proteins and subsequent excess dye removal. The dye purification resin and spin columns eliminate equilibration steps and the need to collect and monitor gravity-flow fractions. Furthermore, the kit enables efficient removal of excess dye and subsequent accurate determination of the dye-to-protein ratio and efficient protein recovery.

The Thermo Scientific DyLight 680-4xPEG NHS Ester Dye included in the kit is 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 680-4xPEG Dyes contain four polyethylene glycol (PEG) chains that are non-toxic, enhance fluorescence and reduce nonspecific binding of conjugates. Additionally, the PEG chains also improve solubility of the dye and labeled molecules in aqueous solution, and help in cell permeability. Conjugates made with DyLight 680-4xPEG Dye can be used as molecular probes for cellular imaging and other fluorescence detection methods. The DyLight 680-4xPEG Dye is a derivative of our high-performance DyLight 680 Dye with 684nm absorption and 706nm emission spectra (Table 1). The DyLight 680-4xPEG Dye is an excellent alternative to Alexa Fluor™ 680 Dye with superior fluorescence characteristics (e.g., brightness, high-fluorescence intensity, photostability, pH insensitivity and water solubility). The chemical properties of the DyLight 680-4xPEG Dye makes it especially useful in various biological, chemical and pharmaceutical settings.

Table 1. Properties of the Thermo Scientific PEGylated DyLight NHS-Ester Dyes.

DyLight Dye	Ex/Em*	ε†	MW (g/mol)	Spectrally Similar Dyes
680-4xPEG Dye	684/706	180,000	1729	DyLight 680 Dye, Alexa Fluor 680 Dye, Cy™ 5.5 Dye, CF680, CF680R, IR Dye 680

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

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

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.
- 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:
  - DyLight 680-4xPEG Dye: laser- and filter-based instruments (e.g., LI-COR Odyssey™ and Aerius™ Infrared Imaging Systems) that emit in the 700nm and 800nm region of the spectrum, respectively.

Additional Materials Required

- Variable-speed centrifuge
- Phosphate-buffered saline (PBS, Product No. 28372; for measuring the dye-to-protein ratio)

Procedure for Labeling Proteins with DyLight Dyes

A. Protein Preparation

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 buffer at pH 8.5. For best results use 1mg of protein at ~2mg/mL. Prepare the protein as follows:
   - **Proteins Lyophilized in PBS:** Immediately 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 (e.g., 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 (e.g., 0.05M sodium borate buffer).
   - **Proteins in Other Buffers:** Ensure protein is not in a buffer containing ammonium ions or primary amines (e.g., Tris or glycine). If necessary, replace buffer with 50mM sodium borate buffer (Product No. 28384), pH 8.5 by dialysis or buffer exchange. Alternative buffer: 0.2M carbonate-bicarbonate buffer, pH 9.4.

B. Protein Labeling

1. Briefly vortex the DyLight Dye to ensure the dye is in the bottom of the tube. Add 0.5mL of the prepared protein to the vial of DyLight Dye, 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 1 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 PEGylated DyLight Dyes.

DyLight Dye	A_{max}*	ε†	CF‡
680-4xPEG Dye	684	180,000	0.09

* 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:

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

- ε_{dye} = dye (fluorophore) molar extinction coefficient (see Table 2)

Example calculations for DyLight 680-4xPEG Dye conjugated to antibodies:

- Dilution factor = 20
- $A_{280} = 0.158$
- A_{max} at 684nm = 0.192

$$\text{Protein concentration (M)} = \frac{[0.158 - (0.192 \times 0.09)]}{210{,}000} \times 20 = 0.0000134 \text{ M}$$

$$\text{Moles dye per mole protein} = \frac{0.192 \times 20}{180{,}000 \times 0.0000134} = 1.6$$

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

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Additional Information Available on Our Website

- 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
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs
22858	**Dye Removal Columns**
46646-53067	**DyLight Near Infrared Specialty Dyes**
62278	**DyLight 755 NHS Ester**
62279	**DyLight 755 NHS Ester**
84538	**DyLight 755 Antibody Labeling Kit**
84539	**DyLight 755 Microscale Antibody Labeling Kit**
62298	**DyLight 755 Maleimide**
46421	**DyLight 800 NHS Ester**
46422	**DyLight 800 NHS Ester**
53062	**DyLight 800 Antibody Labeling Kit**
53063	**DyLight 800 Microscale Antibody Labeling Kit**
46621	**DyLight 800 Maleimide**
46645	**Pierce Immunostain Enhancer,** 2mL
46644	**Pierce Immunostain Enhancer,** 20mL
62247	**DAPI Nuclear Counterstain**
62248	**DAPI Solution**
62249	**Hoechst 3342 Solution**
62254	**DRAQ5™ Fluorescent Probe**
20036	**Bioconjugate Techniques, 2nd Edition**

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