INSTRUCTIONS

# DyLight 680-4xPEG Microscale Antibody Labeling Kit

## 53077

2505.1

| Number | Description |
|---|---|
| **53077** | **DyLight 680-4xPEG Microscale Antibody Labeling Kit,** each kit contains sufficient reagents to label and purify 5 × 100µg (1mg/mL) of IgG or similar amounts of other proteins |

**Kit Contents:**

**DyLight 680-4xPEG 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 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 Microscale Antibody Labeling Kit allows for fast, efficient labeling of antibodies. The kit contains all of the components necessary for five separate labeling reactions of 100µg 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 Dyes 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 Dyes make 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 Microscale Labeling of 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 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:
   - **Proteins Lyophilized in PBS:** Reconstitute 100µg of antibody with 100µL of labeling buffer. 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 200µL, add 15µL of Borate Buffer (0.67M) to 185µL of ultrapure water or PBS].
   - **Proteins in PBS Solution:** To 100µL of 1mg/mL protein 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 (e.g., 0.05 M sodium borate).
   - **Proteins in Other Buffers:** Proteins in buffers containing ammonium ions or primary amines (e.g., Tris or glycine) will interfere with the intended reaction. Replace these buffers with 0.05M 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 centrifuge the vial to collect the sample in the bottom of the tube.
2. Add 0.1mL of the prepared protein to the vial of DyLight Dye, gently vortex and pipette up and down to mix.
3. 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 collection tube.
3. Add 100-108µL of the labeling reaction to the spin column and mix the sample 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 1 month. Alternatively, store labeled protein in single-use volumes 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 680-4xPEG Dye.**

| DyLight Dye | $A_{max}$* | $\varepsilon^{\dagger}$ | CF$^{\ddagger}$ |
|---|---|---|---|
| **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
$^{\dagger}$Molar extinction coefficient ($M^{-1}$ $cm^{-1}$) at $A_{max}$
$^{\ddagger}$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 680-4xPEG Dye conjugated to antibodies:**

- Dilution factor = 20
- $A_{280}$ = 0.047
- $A_{max}$ at 684nm = 0.113

$$\text{Protein concentration (M)} = \frac{[0.047 - (0.113 \times 0.09)]}{210{,}000} \times 20 = 0.00000351\text{M}$$

$$\text{Moles dye per mole protein} = \frac{0.113 \times 20}{180{,}000 \times 0.00000351} = 3.6$$

## 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 |

## 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

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|---|---|
| **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** |



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