LysoLight™ Green Antibody Labeling Kits

Catalog Numbers L36005 and L36006

Pub. No. MAN1000447 Rev. A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The LysoLight™ Green Antibody Labeling Kits provide a powerful tool for visualizing the lysosomal localization and degradation of antibodies and proteins. The labeling protocol involves a simple and rapid procedure, ensuring minimal hands-on time and reliable results.

The LysoLight™ Green Antibody Labeling Kits provide an efficient method for conjugating antibodies with LysoLight™ Green dye which is only active upon enzymatic cleavage by the lysosomal cathepsins. Outside of the lysosome, LysoLight™ Green dye has little to no observable background fluorescence, and only upon localization to the protease rich environment of the lysosome, LysoLight™ dye becomes activated and brightly fluoresce. This process allows specific and sensitive detection of internalized antibodies within lysosomes using fluorescence microscopy, flow cytometry, and high content live cell imaging platforms.

The LysoLight™ Green SDP esters contained in these kits readily react with antibody's amines to yield a covalently attached fluorogenic enzymatically activated probe. The spin columns included in the kits are used for purifying the labeled antibody from excess dye with yield of 70–95%. This user guide describes a general protocol for using the amine-reactive, SDP ester form of the LysoLight™ Green dye for labeling either 100 µg or 1 mg of IgG.

Contents and storage

The contents of each kit are sufficient for 3 labeling reactions of 100 µg or 1 mg of antibody.

Catalog numbers that appear as links open the web pages for those products.

Table 1 LysoLight™ Green Antibody Labeling Kits

Item	Cat. No. L36005 (3 × 100 µg)	Cat. No. L36006 (3 × 1 mg)	Storage[1]
LysoLight™ Green Amine Reactive Dye	3 vials (20 µg in each)	3 vials (100 µg in each)	• 2–8°C • Dessicate • Protect from light • Do not freeze
Dimethyl Sulfoxide (DMSO)	0.5 mL	0.5 mL	
1M Sodium Bicarbonate	84 mg	84 mg	
PBS Exchange Buffer	3 mL	10 mL	
Zeba™ Dye and Biotin Removal Columns[2]	3 × 0.5 mL	3 × 2 mL	
Wash Tubes–1.5 mL	3 each	—	Room temperature (18–25°C)
Collection Tubes–1.5 mL	3 each	—	
Wash and Collection Tubes	—	6 each	

[1] The product is stable for at least 6 months when stored as directed.
[2] The resin is supplied in a 0.1 N NaCl/0.05% sodium azide solution.

Table 2 Technical specifications

Product	Molecular weight	Ex/Em (nm)[1]	Molar extinction coefficient (ε dye)[2]	Correction factor at 280 nm[3]
LysoLight™ Green Amine Reactive Dye	~2800 g/mol	488 nm/525 nm	88,000 $cm^{-1}M^{-1}$	0.338

[1] Excitation/Emission maxima for the dye, in nm conjugated to an antibody.
[2] Extinction coefficient at λ_{496} in $cm^{-1}M^{-1}$.
[3] Absorbance max and extinction coefficients are solvent dependent. The values given are for PBS Exchange Buffer.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source[1]
Whole IgG Primary antibodies or any protein	Customer supplied or MLS
Suspension cells at 2 × 10^6 cells/mL in cell culture medium or adherent cells in a 96-well plate at 5,000 – 10,000 cells/well in cell culture medium	MLS
Cell culture medium	MLS
96-well plates	MLS
Instruments to analyze cells probed with LysoLight™ Green labeled IgG (Flow cytometer, fluorescence microscope, or high content analysis instrument)	MLS

[1] "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Guidelines for antibody preparation

- **IMPORTANT!** The purified antibody should be in a buffer that does not contain primary amines (for example, ammonium ions, Tris, glycine, ethanolamine, triethylamine, glutathione) or imidazole. All of these substances significantly inhibit protein labeling.
- Purify protein samples that contain carriers, such as BSA (for example, antibodies) or any partially-purified protein samples before labeling. The presence of low concentrations (<0.1% (w/v) of biocides, including sodium azide and thimerosal, will not significantly affect the labeling reaction.
- Use dialysis or small-scale gel filtration to remove low molecular weight components from the protein sample (desalting) before labeling. For dialysis, we recommend using the Slide-A-Lyzer™ Dialysis Cassettes (available from **thermofisher.com**). Zeba™ Dye and Biotin Removal Columns (Cat. Nos. A44296S or A44298) can also be used to re-equilibrate the antibody in an appropriate buffer before labeling.
- We recommend PBS, pH 7.2–7.5, as a prelabeling dialysis buffer. Alternatively, a 100 mM sodium bicarbonate buffer can also be used.

(Optional) Guidelines for determining degree of labeling (DOL)

- Several spectrophotometric methods are available for determining the DOL of LysoLight™ Green dye-labeled conjugates. Each are based on obtaining the concentration by measuring the absorbance of the antibody at 280 nm (A_{280}) and 496 nm (A_{496}).
- We recommend using a NanoDrop™ spectrophotometer to analyze the antibody conjugate spectrophotometrically. NanoDrop™ instruments (available from **thermofisher.com**) require only 1–2 µL of sample and are full-featured UV/Vis instruments.
- Excessive dilution of some antibodies with low intrinsic A_{280} may prevent you from deriving accurate A_{280} values for the samples. Use only a portion of the purified antibody conjugate, then dilute it only to the minimum volume necessary for the cuvettes and spectrophotometer to avoid readings below the optimal range for your instrument.

Perform the labeling procedure

1 Prepare the reagents

- Prepare a 1 M Sodium Bicarbonate solution—Add 1 mL of deionized water to the vial of Sodium Bicarbonate. Vortex or pipet up and down until the reagent is fully dissolved.

  The Sodium Bicarbonate solution will have a pH of ~8.3 and can be stored at 2–8°C for up to two weeks, or frozen for long-term storage.

- Resuspend LysoLight™ Green dye in supplied DMSO, vortex and spin down to ensure all dye is resuspended and at the bottom of the reaction vessel.
  - For **100 µg kit**—Resuspend 20 µg of dye in 10 µL of DMSO.
  - For **1 mg kit**—Resuspend 100 µg of dye in 50 µL of DMSO.

IMPORTANT! Allow dye to come to room temperature before opening vial to avoid hydrolysis of reactive dye.

2 Label the antibody

1. Prepare the antibody to be labeled in supplied PBS Exchange Buffer or a pH neutral buffer.
   - For **100 µg kit**—Prepare 80 µL of antibody at a concentration of 1.25 mg/mL.
   - For **1 mg kit**—Prepare 400 µL of antibody at a concentration of 2.5 mg/mL.

   Note: Do not use any amine containing buffers.

2. Combine the following components (in the order indicated) in an appropriately-sized container.

   Table 3 Labeling reaction

Component	Volume	
	100-µg scale	1-mg scale
Antibody (diluted with PBS Exchange Buffer or a pH neutral buffer)	80 µL	400 µL
1 M Sodium bicarbonate solution	10 µL	50 µL
LysoLight™ Green dye (diluted with DMSO)	10 µL	50 µL
Final volume	**100 µL**	**500 µL**

3. Mix well to ensure complete dissolution of the dye and antibody by pipetting up and down several times. If any liquid splashes up the side of the container, spin briefly to return the entire reaction volume to the bottom.

4. Vortex, then incubate the reaction mixture for 4 hours at 25°C.

3 Prepare the spin column

Zeba™ Dye and Biotin Removal Columns contain a ready to use resin that is designed for rapid removal of LysoLight™ Green dye with exceptional antibody recovery. Removal of free dye after a labeling reaction is essential for the accurate determination of dye to antibody ratios.

IMPORTANT! For optimal antibody recovery and dye removal, ensure that the appropriate amount of sample and buffer conditions are used.

1. 10 minutes before the end of the 4 hour incubation, loosen the cap on a spin column, twist the tab off of the bottom of the column, then place into a wash tube.

   Note: For 1-mg kits, the wash tubes and collection tubes are the same. Designate three as wash tubes (lid can be discarded) and three as collection tubes (lid can be saved). For 100-µg kits, the wash tubes do not have caps.

3 *(continued)*

2. Centrifuge the column tube assembly at 1,000 × *g* for 2 minutes to remove the storage buffer and pack the column.

   Note: When using a fixed angle rotor, place a mark on the side of the column that faces away from the rotor center. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

3. Discard the flow-through, then set the column back into the wash vial.

4. Add the appropriate volume of PBS Exchange Buffer, then centrifuge the column-tube assembly at 1,000 × *g* for 2 minutes to equilibrate the column.

   - **For 100-µg kits—**Add 400 µL of PBS Exchange Buffer
   - **For 1-mg kits—**Add 1 mL of PBS Exchange Buffer

5. Discard the flow-through.

4 **Process the sample**

1. Transfer the packed and equilibrated column into a fresh collection tube.

   Note: The collection tubes that are provided in 100-µg kits have caps.

2. Carefully drip the entire reaction mixture onto the center of the column.

3. Centrifuge the column-tube assembly at 1,000 × *g* for 2 minutes. Discard the column.

   The purified antibody conjugate is in the collection tube.

(Optional) Record the volume of purified antibody conjugate for yield determination.

(Optional) Determine the protein yield and DOL

Labeling efficiency may require optimization to achieve the desired results of the conjugate in your application. You can determine the relative efficiency of a labeling reaction by measuring the absorbance of the protein at 280 nm and the absorbance of the dye at its excitation maximum.

1. Measure OD_{280} and OD_{496} using a NanoDrop™ or your preferred spectrophotometer.

2. Determine DOL and protein yield using the extinction coefficients and correction factors given in Table 2.

   Note: Alternatively, calculate the DOL using the **Degree of Labeling Calculator for Antibody Labeling** (go to **thermofisher.com/us/en/home/life-science/antibodies/antibody-labeling/dol-calculator**).

Optimization

Observation	Possible cause	Recommended action
Overall yield is insufficient due to precipitation	It is possible that precipitation occured due to over-labeling.	The conjugation conditions described in this user guide are a general starting point, however, dye to antibody ratio may need to be optimized. It is recommended to either reduce the amount of dye used or reduce the conjugation time.
DOL value is insufficient	It is possible that the removal of free dye in the antibody conjugate solution was inefficient.	The antibody conjugate can be purified through a second round of purification using Zeba™ Dye and Biotin Removal Columns (A44296) to remove residual dye.
	It is possible that there was an inefficient incubation.	To increase the DOL, it is recommended to increase incubation time up to 24 hours or temperature up to 37°C.

Related products

Cat. No.	Product name	Amount
L36007	LysoLight™ Green Amine Reactive Dye	3 × 100 µg
L36008	LysoLight™ Green Amine Reactive Dye	1 × 500 µg
A44296S	Zeba™ Dye and Biotin Removal Columns, 0.5 mL	5 columns
A44298	Zeba™ Dye and Biotin Removal Columns, 2 mL	5 columns

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A	3 September 2024	New document for LysoLight™ Green Antibody Labeling Kits.