Sulfo-NHS-LC-Biotinylation Kit

B1491967

Storage For detailed storage information, please refer to the kit contents.

Introduction

The Sulfo-NHS-LC-Biotinylation Kit is designed for efficiency and reliability, delivering ready-to-use labeled antibody in just 90 minutes, with minimal hands-on time. The kit includes all necessary reagents for antibody labeling, including Sulfo-NHS-LC-Biotin reagent, buffers, purification resin, and empty spin columns.

The Sulfo-NHS-LC-Biotin reagent included in the kit is an intermediate-length, water-soluble biotinylation reagent for labeling antibodies, proteins, and other molecules that have primary amines. NHS-activated biotins react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds (Fig 1).

The included empty spin columns and purification resin can be quickly assembled into 3 mL centrifugal purification columns for the rapid purification of biotinylated antibodies from excess biotin reagent. The 3 mL purification column is optimally suited for processing reaction mixtures with a volume of approximately 0.4–0.7 mL, achieving a recovery rate of 70–95%.

The reagents supplied in this kit are sufficient for performing 10 biotinylation reactions, each capable of labeling up to 1.5 mg of antibody. For smaller amounts of protein and/or smaller reaction volumes, we recommend you use Sulfo-NHS-LC-Biotinylation Kit (B1491943), which is designed to label 100 μg of antibody.

The biotinylated antibody can be detected in ELISA, Western Blot, IF/ICC or Flow Cytometry application using avidin or streptavidin probes. Aladdin offers a series of high-performance streptavidin conjugates designed for detecting the performance of biotinylated antibodies.

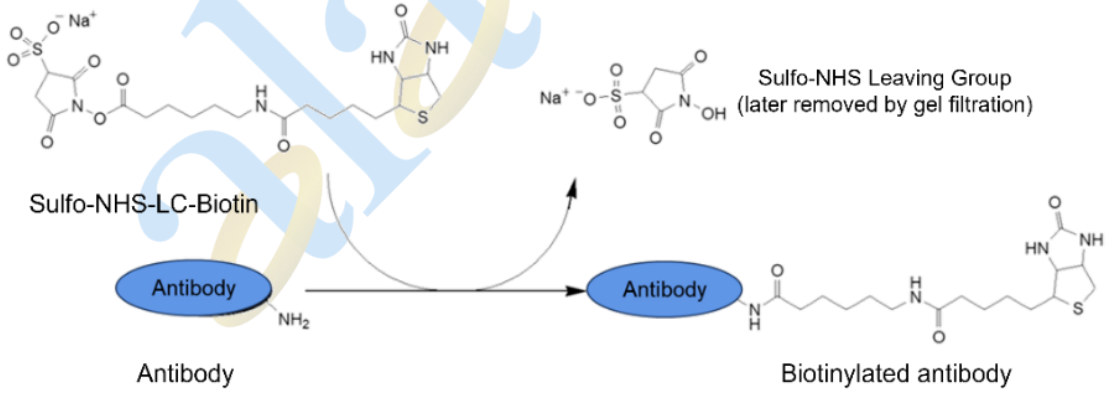

Fig. 1 Sulfo-NHS-LC-Biotinylation Kit (B1491967) Labeling Principle Schematic

Kit Contents

B1491967	Component	10 reactions	Storage	Quantity Per reaction
B1491967A	Sulfo-NHS-LC-Biotin	10 vials	-20℃. Store in the dark.	1 vial for labeling up to 1.5mg of antibody
B1491967B	$NaHCO_3$	100 mg	RT.	Prepare according to instructions
B1491967C	Purification Resin	30 mL	2-8℃. Do not freeze.	3 mL for 1 reaction
B1491943D	Empty Spin Column	10 EA	RT.	1 EA for 1 reaction

Required materials not supplied

1. Microcentrifuge capable of 1,000 × g.
2. Desired antibody for labeling (free of BSA or any carrier protein).
3. PBS buffer (pH 7.2-7.4).
4. 2mL tubes.

Matters needing attention

1. 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.
2. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
3. An antibody concentration of 1–2 mg/mL is optimal. Concentrations below 1 mg/mL may reduce labeling efficiency, while excessively high concentrations may result in too small a volume of antibody, which is unfavorable for purification recovery.
4. The volume of the antibody to be labeled should be controlled between 0.4 mL and 0.7 mL to ensure optimal removal efficiency using the purification column.
5. The Sulfo-NHS-LC-Biotin reagent is moisture-sensitive. To prevent condensation and moisture absorption, allow the vial to equilibrate to room temperature before opening.
6. Strictly follow the operating procedure and dissolve the biotin reagent immediately before use. Its NHS-ester group is highly susceptible to hydrolysis and inactivation. Therefore, dissolve an appropriate amount of biotin reagent based on the number of antibodies to be labeled, and do not prepare stock solutions for storage. Any dissolved but unused reagent should be discarded. Prepared biotin reagent should be used within 10 minutes.
7. The purification resin included in this kit is recommended for single use only.

Instructions for Use

1. Calculations

The extent of biotin labeling depends on the size and distribution of amino groups on the protein

and the amount of reagent used per protein concentration. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Using a 20-fold molar excess of biotin reagent to label antibody (IgG) at 1-10 mg/mL is sufficient for most labeling requirements. Adjust the molar ratio of Sulfo-NHS-LC-Biotin to protein to obtain the level of incorporation desired.

(1) Calculate millimoles of Sulfo-NHS-LC-Biotin to add to the reaction for a 20-fold molar excess:

$$\text{mmol Biotin} = \frac{\text{mg/mL protein} \times \text{mL protein}}{\text{MW protein}} \times 20$$

(2) Calculate microliters of 10mM Sulfo-NHS-LC-Biotin (prepared in Step 3.2) to add to the reaction:

$$\mu\text{L Biotin} = \frac{\text{mmol Biotin} \times 557\text{mg/mmol}}{5.5\ \text{mg/mL}} \times 1000$$

- Molecular weight of Sulfo-NHS-LC-Biotin is 557.
- Adding 90 µL of ultrapure water to one vial of Sulfo-NHS-LC-Biotin reagent yields a solution with a concentration of 5.5 mg/mL and a molarity of 10 mM.

(3) Example: For 0.5mL of a 2mg/mL IgG (150,000 MW) solution, 13.5 µL of 10mM Sulfo-NHS-LC-Biotin will be added.

$$\text{mmol Biotin} = \frac{2\text{mg/mL IgG} \times 0.5\text{mL IgG}}{150000} \times 20 = 0.000133\ \text{mmol Biotin}$$

$$\mu\text{L Biotin} = \frac{\text{mmol Biotin} \times 557\text{mg/mmol}}{5.5\ \text{mg/mL}} \times 1000 = 13.5\ \mu\text{L Biotin}$$

2. Prepare the reagents

(1) Prepare a 1 M sodium bicarbonate solution: Prepare an appropriate amount of 1M $NaHCO_3$ solution based on the sample volume. For example, weigh 42mg of $NaHCO_3$ and add 0.5mL of ultrapure water to obtain a 1M $NaHCO_3$ solution. $NaHCO_3$ can maintain the pH of the labeling reaction system between 7-9, thereby improving labeling efficiency.

Note: The $NaHCO_3$ solution must be prepared fresh before each use.

3. Label the antibody

(1) Dissolve 1 mg protein in 0.4-0.7mL of phosphate-buffered saline (PBS) according to the calculation made in step 1. If the antibody to be labeled has a concentration of ≥1 mg/mL and is in an appropriate buffer, add a 10% volume of 1 M sodium bicarbonate buffer. Prepare 0.1 M sodium bicarbonate buffer by diluting the 1 M solution 10-fold with ultrapure water.

(2) Immediately before use, prepare 10mM Sulfo-NHS-LC-Biotin by dissolving one vial of Sulfo-NHS-LC-Biotin reagent in 90µL ultrapure water.

(3) Add the appropriate volume of Sulfo-NHS-LC-Biotin solution (see calculations in step 1) to the protein solution.

(4) Incubate reaction on ice for two hours or at room temperature for 30-60 minutes.

Note: There is no harm in reacting longer than the specified time other than the possibility of ordinary protein degradation or microbial growth.

(5) During the incubation period, proceed to steps 4 below, to prepare a spin column for the purification of the labeled protein.

4. Purification with Purification Column

(1) Place an empty spin column in a 15mL collection tube.

(2) Suspend the purification resin by repeated inversion of the reagent bottle. Then add 3.0 mL of the suspension into the column and allow the resin to settle. Allow the column buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Centrifuge the column at 1,000 × g for 2 minutes, discard the storage buffer and return column to the same collection tube.

Note: The purification resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

(3) Equilibrate the column by adding 2mL of PBS to the top of the resin bed and centrifuging at 1,000 × g for 2 minutes. Discard the flowthrough and repeat this step a total of 3 times.

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.

(4) Place column into a new 15mL collection tube and apply the protein sample directly onto the center of the resin bed. Allow sample to absorb into the resin.

Note: For samples < 400µL, add 100µL ultrapure water on top of the absorbed sample to increase protein recovery.

(5) Centrifuge the column at 1,000 × g for 2 minutes. The collected flowthrough solution is the purified protein sample. Store the protein solution in appropriate conditions.

5. Determine the protein concentration (Optional)

Determination of antibody concentration by measuring absorption at 280 nm.

6. Storage

Store the labeled antibody at 4°C. If the final concentration of purified antibody conjugate is less than 1 mg/mL, add bovine serum albumin (BSA) or other stabilizing protein to a concentration of 1-10 mg/mL. The conjugate should be stable at 4°C for several months. For long-term storage, divide the solution into single-use aliquots and freeze at -20°C. Avoid repeated freeze-thaw cycles.