Sulfo-NHS-LC-Biotinylation Kit

B1491943

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 60 minutes, with minimal hands-on time. The kit includes all necessary reagents for antibody labeling, including Sulfo-NHS-LC-Biotin, buffer, and purification 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 (-NH2) in pH 7-9 buffers to form stable amide bonds (Fig 1). Spin columns included in the kit are used for purifying the biotinylated antibody from excess biotin reagent with yields of 70–95%. The kit contains sufficient reagents for 10 biotinylation reactions of 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.

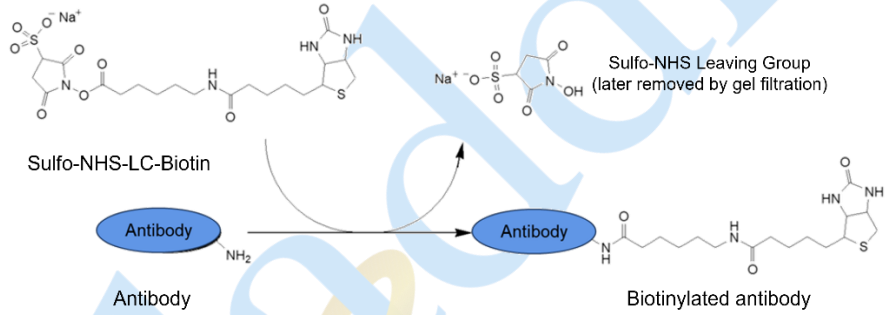

Fig. 1 Sulfo-NHS-LC-Biotinylation Kit (B1491943) Labeling Principle Schematic

Kit Contents

B1491943	Component	10 reactions	Storage	Quantity Per reaction
B1491943A	Sulfo-NHS-LC-Biotin	10 vials	-20℃. Store in the dark.	1 vial for labeling 100 µg of antibody
B1491943B	$NaHCO_3$	100 mg	RT.	Prepare according to instructions
B1491943C	Spin Desalting Column	10 EA	2-8℃. Do not freeze.	1 EA for 1 reaction
B1491943D	Collection Tubes	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).

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. Concentrate the antibody to ≥1 mg/mL.
4. Do not reuse the purification resin.
5. Before opening the vials, warm all components to room temperature.

Instructions for Use

1. 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 deionized 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.

2. Label the antibody

(1) If the antibody to be labeled has a concentration of ≥1 mg/mL and is in an appropriate buffer, dilute it to 1 mg/mL, and add a 10% volume of 1 M sodium bicarbonate buffer. If the antibody is a powder lyophilized from an appropriate buffer, prepare a 1 mg/mL solution of the antibody by adding an appropriate amount of 0.1 M sodium bicarbonate buffer to the antibody. Prepare 0.1 M sodium bicarbonate buffer by diluting the 1 M solution 10-fold with deionized water.

(2) Transfer 100 µL of the antibody solution to the vial of biotin reagent. Cap the vial and gently invert it a few times to fully dissolve the biotin reagent.

Note: To visually ensure that the biotin reagent has fully dissolved, peel the label off the vial of biotin reagent.

(3) Incubate the solution for 60 minutes at room temperature in the dark. The reaction can be carried out on a shaker or mixer, recommended speed for flipping up and down is 25rpm. If a mixing instrument is not used during the reaction process, the reaction solution should be mixed upside down every 10 minutes.

Note: During the incubation period, proceed to steps 3 below, to prepare a spin column for the purification of the labeled protein.

3. Prepare the spin column

(1) Loosen the cap on a spin column, twist the tab off of the bottom, then place the column into a collection tube.

(2) Centrifuge the column-tube assembly at 1,000 × g for 2 minutes to remove the storage solution.

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 flowthrough, then place the column back into the collection tube.

(4) Add 500 µL of PBS Buffer, then centrifuge the column-tube assembly at 1,000 × g for 2 minutes to equilibrate the column. Repeat this step (3) 3 times, discarding the buffer from the collection tube each time.

4. Purification with Desalting Column

(1) Transfer the equilibrated column into a new collection tube.

(2) Carefully pipette the entire reaction mixture onto the center of the column.

(3) Centrifuge the column-tube assembly at 1,000 × g for 2 minutes. The purified antibody conjugate is in the collection tube.

5. Determine the antibody concentration (Optional)

Determination of antibody concentration by measuring absorption at 280 nm.

6. Storage

Add 0.05–0.2% Proclin 300 or 0.05% sodium azide, along with a protein stabilizer (such as 0.1% BSA), to the labeled protein. Store protected from light at 2–8°C for stable preservation up to six months. Alternatively, add an equal volume of glycerol and store at -20°C for stable preservation up to six months.