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- Expressversand

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**(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)**

## **Water-soluble Long-arm Biotin Labeling Kit**

**Catalog No:** E-LK-B008

3 Reactions

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

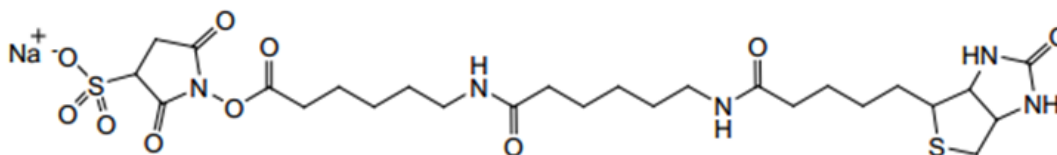
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Introduction

The water-soluble Sulfo-NHS-LC-LC-Biotin Labeling Kit of Elabscience offering a collection of reagents required for biotin labeling, enables simple and efficient biotin labeling of antibodies, proteins and any other primary amine-containing macromolecules. Long-arm reduces the steric hindrance when several biotinylated molecules combine with the same avidin molecular compound, effectively optimizes the labeling and detection results. The biotin in the kit is sufficient and has been activated for direct use. And the reagents are enough for approximately 3 labeling reactions each containing 0.1-2 mg of antibody or other protein. Each kit includes 6 Filtration tubes for desalination of antibody labeling without the need for dialysis. The whole procedure is simple and can be completed in 90min in proficient operation.

## Information of the Water-soluble Long-arm Biotin

### Chemical structure:



**Molecular Weight:** 670

**Molecular formula:** C<sub>26</sub>H<sub>40</sub>O<sub>10</sub>N<sub>5</sub>S<sub>2</sub>Na

**Spacer arm length:** 30.5 Å

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## Product Features

1. **All-inclusive:** This kit provides all the reagents required for biotin labeling.
2. **Quick:** The whole procedure takes only 90 min.
3. **Convenient:** Desalination can be achieved with Filtration tube, dialysis or gel filtration is not necessary.
4. **Flexible:** The procedure can be easily adapted to both micro and large scales, with 0.1-2 mg of protein labeled each time.
5. **Water-soluble:** The Sulfo-NHS-LC-LC-Biotin reagent in the kit is water soluble, enabling reactions to be performed in the absence of organic solvents.
6. **Perfect results:** This kit has been optimized to determine the optimum labeling ratio of biotin to antibody, lowering the possibility of protein inactivation resulted from insufficient labeling or excess biotin labeling.
7. **Cell labeling:** The Sulfo-NHS-LC-LC-Biotin can label the proteins exposed on the surface of cell, without permeating the cell membrane.

## Product component

Component	Amount
Sulfo-NHS-LC-LC-Biotin	0.2 mg ×3 vials
Labeling Buffer	10 mL×1 vial
Ultrapure water	500 μL×1 vial
Filtration tube	0.5 mL×6

### Materials required but not included in this kit

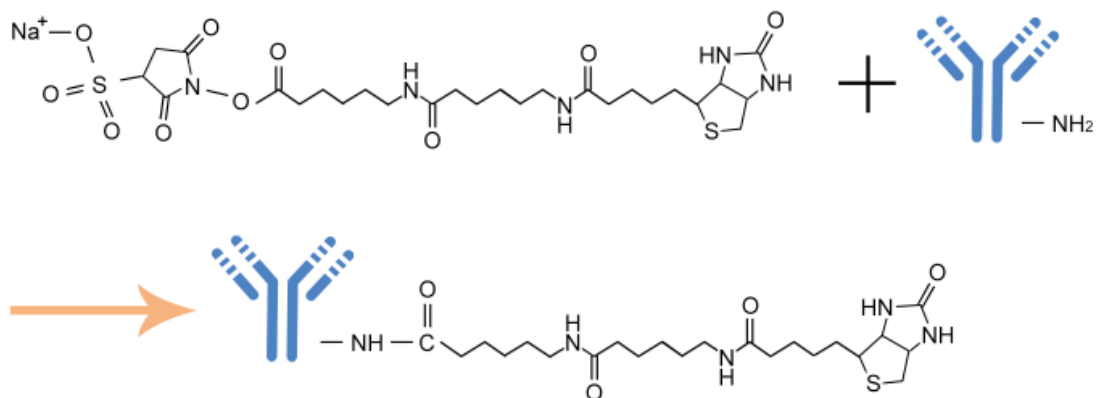
1. Adjustable high-precision transferpeltor (10 μL, 50 μL, 200 μL, 1000 μL).
2. Incubator (37 °C).
3. Centrifuge (the centrifugal speed can be up to 12,000×g).

### Storage

This kit can be stored at 2-8 °C for 1 year before opening.

### Principle

The Sulfo-NHS-LC-LC-Biotin reacts with the primary amine (N-terminal and the side chain of lysine residue) specifically, forming stable amide bond.



### Calculation on the amount of Sulfo-NHS-LC-LC-Biotin

The volume of Sulfo-NHS-LC-LC-Biotin used in each reaction depends on the amount and concentration of the protein to be labeled. With optimization, we determine that the optimum molar ratio of the Biotin to protein is 20:1 when labeling 2 mg/mL of protein sample (IgG, 150KD).

1. Calculate the millimole of Sulfo-NHS-LC-LC-Biotin to make the ratio of Biotin to antibody is 20:1 when labeling 2 mg/mL antibody:

$$mL \text{ protein} \times \frac{2 \text{ mg protein}}{mL \text{ protein}} \times \frac{1 \text{ mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol Biotin}}{1 \text{ mmol protein}} = \text{mmol Biotin}$$

2. Calculate the microliter of 10 mM Sulfo-NHS-Biotin to add to the reaction:

$$\text{mmol Biotin} \times \frac{1,000,000 \mu\text{L}}{L} \times \frac{L}{10 \text{ mmol}} = \mu\text{L Biotin}$$

**Example:** About 13.3  $\mu\text{L}$  of 10 mM Sulfo-NHS-LC-LC-Biotin solution is to be added for 0.5 mL of 2 mg/mL IgG (150,000 MW) solution.

$$0.5 \text{ mL IgG} \times \frac{2 \text{ mg IgG}}{1 \text{ mL IgG}} \times \frac{1 \text{ mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol Biotin}}{1 \text{ mmol IgG}} = 0.000133 \text{ mmol Biotin}$$

$$0.000133 \text{ mmol Biotin} \times \frac{1,000,000 \mu\text{L}}{L} \times \frac{L}{10 \text{ mmol}} = 13.3 \mu\text{L Biotin Solution}$$

## Preparation before experiment

1. Read the manual carefully.
2. Calculate the volume of Sulfo-NHS-LC-LC-Biotin to be added.
3. Bring the kit to room temperature for 20 min before experiment (**Note:** The unused Sulfo-NHS-LC-LC-Biotin should be stored in the refrigerator).

## Assay procedure (we label 1 mg of protein in this assay):

1. Add 1 mg of protein sample and corresponding volume of Labeling Buffer to a Filtration tube to make the total volume is 0.5 mL. Centrifuge at 12,000 ×g for 10 min.  
**Note:** ①The maximum volume of Filtration is 0.5 mL. ②The protein sample can be treated with centrifugal ultrafiltration first when at low concentration.
2. Dissolve Sulfo-NHS-LC-LC-Biotin: add 30 μL of ultrapure water to the vial of Sulfo-NHS-LC-LC-Biotin, let it stand for 10 min until it dissolved fully. The final concentration of Biotin is 10 mM
3. Add 13.3 μL of Sulfo-NHS-LC-LC-Biotin and appropriate volume of Labeling Buffer to the Filtration tube, making the final concentration of the protein solution is 2 mg/mL. Mix it thoroughly with a pipette and incubate the tube for 30 min at 37 °C.
4. Centrifuge at 12,000×g for 10 min.
5. Add appropriate volume of Labeling Buffer to the Filtration tube to make the total volume is 0.5 mL. Mix it thoroughly with a pipette and centrifuge at 12,000×g for 10 min. Repeat this step once again.
6. Add 0.2 mL of Labeling Buffer to the Filtration tube and mix it thoroughly with a pipette. Invert the filtration tube and put it into another centrifugal tube. Centrifuge at 6,000×g for 10 min.
7. Collect the solution in the centrifugal tube, namely antibody labeled by Biotin

## **Precautions**

1. Sulfo-NHS-Biotin is moisture-sensitive. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.
2. This kit can be also used to label antigen, HRP and polypeptides with amidogen ( $\text{NH}_2$ -). The labeling ratio depends on the amount of amidogen.
3. In the Step 2 above, dissolve the Sulfo-NHS-LC-LC-Biotin immediately before use to avoid hydrolyzing and becoming non-reactive.
4. In the Step 6 above, Labeling Buffer is used to collect the labeled protein. You can also use other buffer or protective agents as you like.
5. The Filtration tube provided in this kit has a molecular weight cutoff (MWCO) of 10KD. So please be careful of the molecular weight of the antigen or polypeptide to be labeled.
6. In the Step 3 above, for other quality antibodies, the final concentration of antibody should be controlled to 2 mg/mL strictly, then calculate the volume of Sulfo-NHS-LC-LC-Biotin required according to the quantity of the antibodies.