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Lieferung & Zahlungsart

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- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elabscience®

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

Long-arm Biotin Labeling Kit

Catalog No: E-LK-B004

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
Website: www.elabscience.com

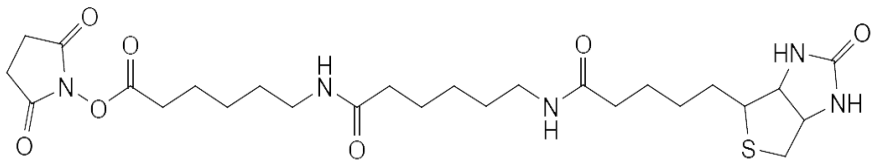
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Introduction

The Long-arm Biotin Labeling Kit of Elabscience offering a collection of reagents required for biotin labeling is designed to label antibody with amidogen (NH2-). 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 this kit is sufficient and has been activated for direct use. The reagents are enough for approximately 3 labeling reactions each containing 0.1-2mg 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 with proficient operation.

Information of the Long-arm Biotin

Chemical structure:



Molecular Weight: 568

Molecular formula: $C_{26}H_{41}N_5O_7S$

Spacer arm length: 30.5 Å

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. **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.

Kit component

Component	Specification
NH2-Reactive Biotin	0.17 mg ×3 vials
Labeling Buffer	10 mL×1 vial
DMF	500 μL×1 vial
Filtration tube	0.5 mL×6

Materials required but not included in this kit

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

Storage

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

Principle

The NH2-Reactive 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 NH2-Reactive Biotin

The volume of NH2-Reactive 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 NH2-Reactive Biotin to make the ratio of Biotin to antibody is 20:1 when labeling 2 mg/mL antibody:

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

2. Calculate the microliter of 10 mM NH2-Reactive Biotin to add to the reaction:

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

Example: About 13.3 μL of 10 mM NH2-Reactive Biotin solution is to be added for 0.5 mL of 2 mg/mL IgG (150,000 MW) solution.

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

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

Preparation before experiment

1. Read the manual carefully.
2. Calculate the volume of NH2-Reactive Biotin to be added.
3. Bring the kit to room temperature for 20 min before experiment (**Note**: The unused NH2-Reactive Biotin should be stored in the refrigerator).
4. Dissolve NH2-Reactive Biotin: add 30 μL of DMF to the vial of NH2-Reactive Biotin, let it stand for 10 min until it dissolved fully. The final concentration of Biotin is 10 mM

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. Add 13.3 μL of NH2-Reactive 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℃.
3. Centrifuge at 12,000×g for 10 min.
4. 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.
5. 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.
6. Collect the solution in the centrifugal tube, namely antibody labeled by Biotin

Precautions

1. This kit can be also used to label antigen, HRP and polypeptides with amidogen (NH_2-). The labeling ratio depends on the amount of amidogen.
2. DMF should be preserved airtight in a dry place. Seal it with the parafilm immediately after use.
3. In the Step 5 above, Labeling Buffer is used to collect the labeled protein. You can also use other buffer or protective agents as you like.
4. This kit can be stored for 1 year before opening. Please use it within the expiration date.
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 2 above, for other quality antibodies, the final concentration of antibody should be controlled to 2 mg/mL strictly, then calculate the volume of NH2-Reactive Biotin required according to the quantity of the antibodies.