

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

Catalog No: E-LK-B004

Product size: 3 Reactions

## **Elabscience<sup>®</sup> Long-arm Biotin Labeling Kit**

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 (info in the header of each page).

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 refer to specific expiry date from label outside of box.

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



## Product component and storage

The kit is stable for 1 year at 2-8 °C before opening.

The dissolved NHS-LC-LC-Biotin can be stored at 2-8 °C for 1 week.

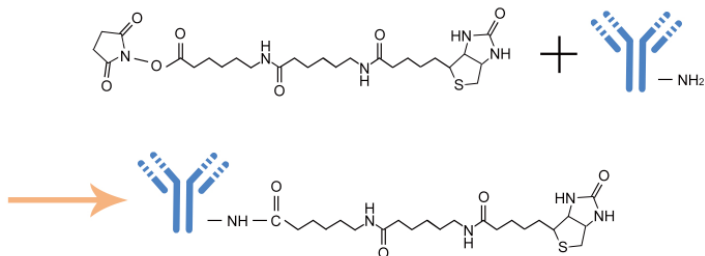
Component	3 Reactions
NHS-LC-LC-Biotin	0.17 mg ×3 vials
Labeling Buffer	10 mL ×1 vial
DMF	500 μL ×1 vial
Filtration tube	0.5 mL ×6

## Materials required but not included in this kit

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

## Principle of the assay

The 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 NHS-LC-LC-Biotin

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

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

$$\begin{aligned} \text{mL protein} \times \frac{2\text{mg protein}}{\text{mL protein}} \times \frac{1\text{mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol NHS-LC-LC-Biotin}}{\text{mmol protein}} \\ = \text{mmol NHS-LC-LC-Biotin} \end{aligned}$$

2. Calculate the microliters of 10mM NHS-LC-LC-Biotin to add to the reaction:

$$\begin{aligned} \text{mmol NHS - LC - LC - Biotin} \times \frac{1,000,000 \mu\text{L}}{\text{L}} \times \frac{\text{L}}{10 \text{ mmol}} \\ = \mu\text{L NHS - LC - LC - Biotin} \end{aligned}$$

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

$$\begin{aligned} 0.5\text{mL IgG} \times \frac{2\text{mg IgG}}{1\text{mL IgG}} \times \frac{1\text{mmol IgG}}{150,000\text{mg IgG}} \times \frac{20\text{mmol NHS - LC - LC - Biotin}}{1\text{mmol IgG}} \\ = 0.000133\text{mmol NHS - LC - LC - Biotin} \end{aligned}$$

$$\begin{aligned} 0.000133\text{mmol NHS - LC - LC - Biotin} \times \frac{1,000,000\mu\text{L}}{\text{L}} \times \frac{\text{L}}{10\text{mmol}} \\ = 13.3\mu\text{L NHS - LC - LC - Biotin Solution} \end{aligned}$$

### **Preparation before experiment:**

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

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

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

## **Precautions**

1. 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.
2. DMF should be preserved airtight in a dry place. Seal it with the parafilm immediately after use.
3. The Filtration tube provided in the 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.
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. 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 NHS-LC-LC-Biotin required according to the quantity of the antibodies.
6. This kit can be stored for 1 year before opening. Please use it within the expiration date.