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

Elabscience® Biotin Labeling Kit

Catalog No: E-LK-B002C

Product size: 1 Reaction/3 Reactions/10 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 .

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Web: 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.

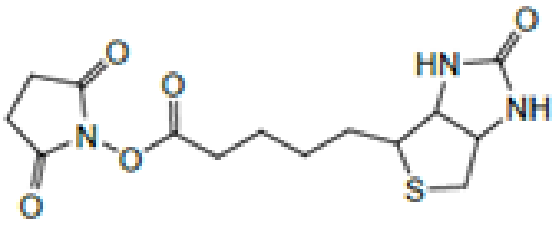
Introduction

Elabscience® Biotin Labeling Kit provides all the reagents required for labeling, which can label proteins containing primary amino-group (-NH_2) molecules simply and effectively.

Characteristic

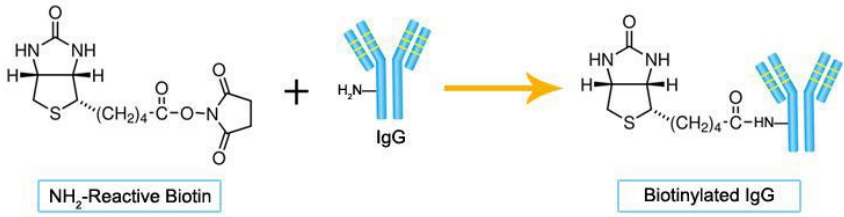
- ✓ **Fast**: The whole process takes only 90 min.
- ✓ **Convenient**: The NHS-Biotin has been activated and can be used directly. Filtration tube desalts without dialysis.
- ✓ **Flexible use**: It can be used for both micro-labeling and large-scale labeling, and can label 0.1-1 mg proteins each time.
- ✓ **Fat-soluble**: NHS-Biotin in this kit is fat-soluble. In some experiments, it is requirement for biotin-labeled proteins to enter the cell membrane for reaction. This labeling method is relatively effective.

Essential Information

Structural formula	
Molecular weight	341.38

Labeling Principle

Within a certain pH range, NHS-Biotin specifically reacts with primary amino groups (N-terminal and lysine residue side chains) to form a stable amide bond, so as to realize the coupling of NHS-Biotin with protein.

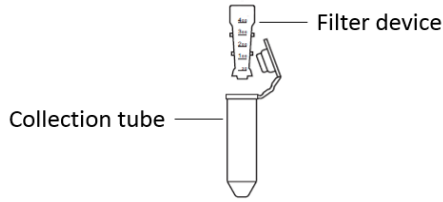


Components

Cat.	Products	1 Reaction	3 Reactions	10 Reactions	Storage
E-LK-B02L	NHS-Biotin (NHS ester)	0.1 mg×1	0.1 mg×3	0.1 mg×10	-20°C, shading light
E-LK-010	Labeling Buffer I	10 mL	20 mL	20 mL×2	2~8°C
E-LK-006	DMF	500 μL	500 μL	500 μL	2~8°C, shading light
E-LK-007	1×PBS(pH7.4)	10 mL	10 mL	10 mL×2	2~8°C
E-LK-008	1M Tris(pH 8.7)	500 μL	500 μL	500 μL×2	2~8°C
E-LK-001C	50 KD Filtration tube*	1 set**	3 set	10 set	RT

*The filtration tube is purchased from Millipore. Please refer to the appendix Ⅲ for usage.

**1 set 50 KD Filtration tube (0.5 mL) consisted of one filter device and two collection tubes.



Storage

The unopened kit can be stored at 2~8°C for 1 year, and the dissolved NHS-Biotin can be stored at -20°C or -80°C for 1 week.

Materials Not Supplied

1. Pipettor and tips (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL).
2. 37°C incubator.
3. Centrifuge (centrifugal force up to 12,000×g).

Calculation of the usage amount of NHS-Biotin:

The amount of NHS-Biotin used in each reaction depends on the mass, concentration and molecular weight of the protein to be labeled. For the protein above 100 KD, the recommended molecular ratio of NHS-Biotin and protein using this kit is 20:1.

Example: Label 1 mg protein (concentration about 2 mg/mL), when the molecular ratio of NHS-Biotin and protein (150 KD) is 20:1, the molar concentration of NHS-Biotin is 10 mM (refer to the preparation of NHS-Biotin), the calculation of the amount of NHS-Biotin to be added is below:

1. Calculate the amount of substance required of NHS-Biotin:

$$n_{\mathrm{NHS-Biotin}} = \mathrm{n}_{\mathrm{protein}} \times 20 = \frac{1\ mg}{150000 mg/mmol} \times 20$$

$$= 0.00013333 mmol$$

2. Calculate the required volume of NHS-Biotin:

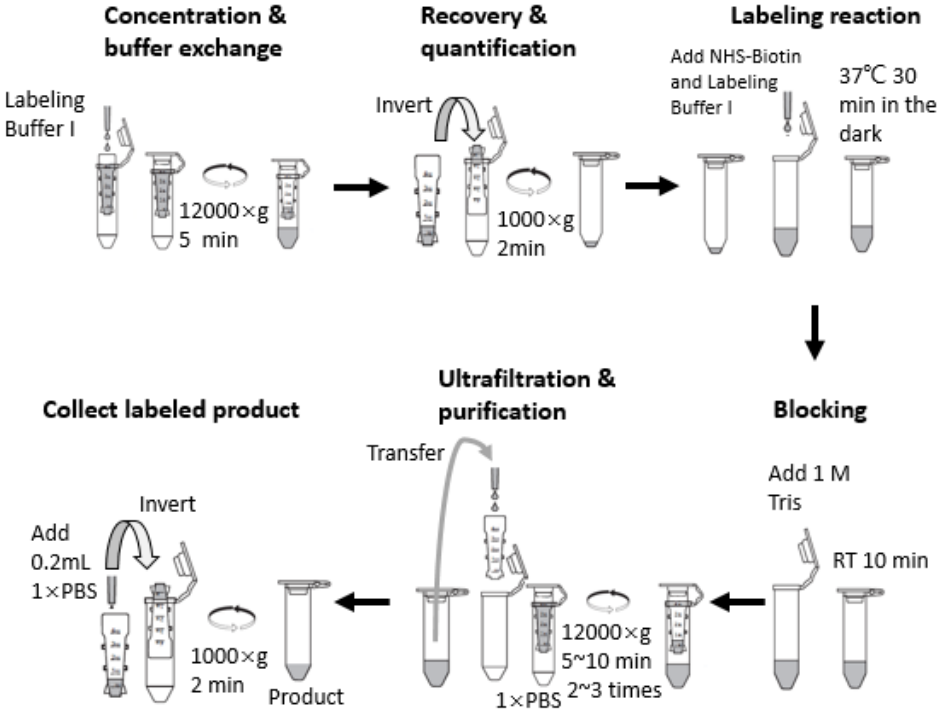
$$V_{\mathrm{NHS-Biotin}} = \frac{n_{\mathrm{NHS-Biotin}}}{C_{\mathrm{NHS-Biotin}}} = \frac{0.00013333\ mmol}{10\ mM} = 13.3\ \mu L$$

Experimental Operation

■ Experiment preparation

1. Read the instructions carefully.
2. Bring all reagents to room temperature for 20 min before use (note: the reagent components are temporarily unused are still in the refrigerator).
3. Infiltration of ultrafiltration tube: Add 500 μL of Labeling Buffer I into the dry filter device, stand at room temperature for 10 min, and discard Labeling Buffer I before adding the reagent to be labeled (the filter device should remain moist throughout the labeling process).
4. Preparation of NHS-Biotin: Dissolve 0.1 mg of NHS-Biotin NHS ester with 30 μL of DMF, and stand for 10 min until it is fully dissolved. At this time, the concentration of NHS-Biotin is 10 mM, and cover the tube for later use.

■ Labeling process



■ **Labeling procedure (This procedure is used to label 1 mg protein)**

1. **Concentration & buffer exchange:** Put the filter device in the collection tube, add 1 mg of protein to be labeled into the filter device, add Labeling Buffer I to the final volume of 0.5 mL, cover the filter tube, centrifuge at 12,000×g for 5 min, and discard the liquid in the collection tube.

   Note:

   a) The maximum volume of the filter device is 0.5 mL.

   b) If the volume of 1 mg protein is greater than 0.5 mL, please add it in several times and concentrate it by centrifugation and ultrafiltration.

   c) If the protein to be labeled contains free amino groups (Tris, amino acids or other interferents, repeat ultrafiltration with Labeling Buffer I to ensure that it is removed fully).

2. **Recovery & quantification:** Invert the filter device into the collection tube, centrifuge at 1000×g for 2 min, collect the protein in the collection tube, take out the filter device, add an appropriate amount of Labeling Buffer II into the collection tube, make sure that the protein concentration is about 2 mg/mL. At the same time, add 0.5 mL Labeling Buffer II into the filter device and put it on a pipe rack for later use.
3. **Labeling reaction:** Immediately add 13.3 μL of 10 mM Elab Fluor® 488 to the protein solution, gently blow and mix fully, sealed with a lid, and incubate at 37 ℃ for 30 min in the dark.
4. **Blocking:** Add 1 M Tris (pH 8.7) to stop the reaction at the ratio of 10 μL of 1 M Tris (pH 8.7) per 100 μg protein, mix fully and incubate at room temperature for 10 min.
5. **Ultrafiltration & purification:** Add an appropriate amount of 1×PBS into the above reaction solution to the final volume of 0.5 mL, gently mix and transfer the reaction solution to the filter device, make sure that the Labeling Buffer I in the filter device in step 2 should be discard (if the above reaction solution exceeds 0.5 mL, it can be transferred to the spin-dried filter device for several times after ultrafiltration), and cover the

cap after matching with the collection tube, and centrifuge for 5~10 min at the speed of 12,000×g. Discard the liquid in the collection tube, replenish 1×PBS to 500 μL in the filter device, and repeat the centrifugal ultrafiltration operation for 2~3 times.

6. **Collect labeled product:** Add 0.2 mL 1×PBS into the filter device and pipet gently. Invert the filter device in another collection tube and centrifuge at 1000×g for 2 min. Collect the solution in the collection tube, which is the protein labeled by NHS-Biotin.

■ **The storage and use of protein**

Add 0.05~0.2% Proclin 300 or 0.05% sodium azide and stabilizer protein (such as 0.1% BSA) to the labeled protein, the protein can be stored at 2~8°C in the dark for 6 months. Or add the same volume of glycerol, the protein can be stored at -20°C for 6 months.

Notes

1. Please select the appropriate kit according to the molecular weight of the protein to be labeled. The kit provides a 50 KD Filtration tube.
2. NHS-Biotin is susceptible to moisture hydrolysis failure, and should be stored at -20℃ or -80℃ with the desiccant. In order to prevent water vapor from condensing into the NHS-Biotin, it is necessary to equilibrate the NHS-Biotin to room before the experiment.
3. The kit can also be used to label other proteins containing free amino groups. The specific labeling ratio is determined according to the number of available amino groups in the marker or set different molar ratios for labeling.
4. The optimal molecular ratio of the NHS-Biotin and protein (150KD) recommended for labeling with this kit is 20:1, which can ensure that the NHS-Biotin can label the protein, but it cannot ensure the best experimental results. The optimal labeling ratio may vary according to the difference of protein, and the user can optimize according to the actual situation.

Related Products

Cat.No	Product
E-LK-R002	BSA Removal Kit
E-LK-B004C	Long-arm Biotin Labeling Kit
E-LK-B007C	Water-soluble Biotin Labeling Kit

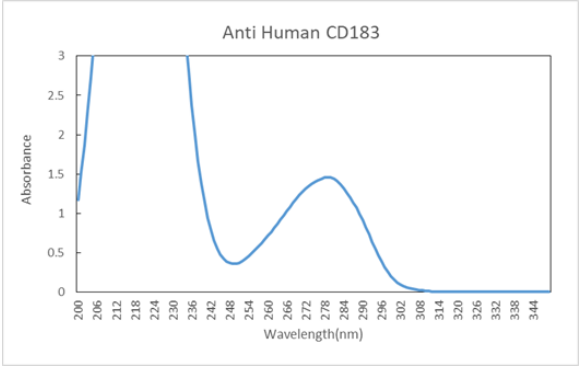
Declaration

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Although this labeling kit has verified that many different proteins have been labeled, it is still possible that the protein binding affinity is impaired or even lost during the NHS-Biotin-labeled protein process. Although this phenomenon is rare, the reason for this phenomenon is likely that the protein has one or more key lysine amino acid residues directly at the antigen binding site, and its binding function is impaired during the labeling process. In addition, some proteins have key lysine residues (not necessarily at the binding site), which are absolutely critical to maintaining the stability/solubility of the protein. Once modified, the protein is completely unavailable due to precipitation. In this rare case, this labeling kit is not fully responsible. We believe that our customers should be aware that the use of NHS-Biotin in this kit may in some cases impair the biological function of the modified protein.
4. Although this kit can also be used to label proteins, it should be recognized that changes in various properties of different proteins are quite different from proteins, such as protein solubility in different buffers, pH stability, temperature stability, protein purity, accessibility of labeling sites, and so on. Therefore this labeling kit does not guarantee the quality of labeling proteins other than IgG and IgM proteins.

Troubleshooting

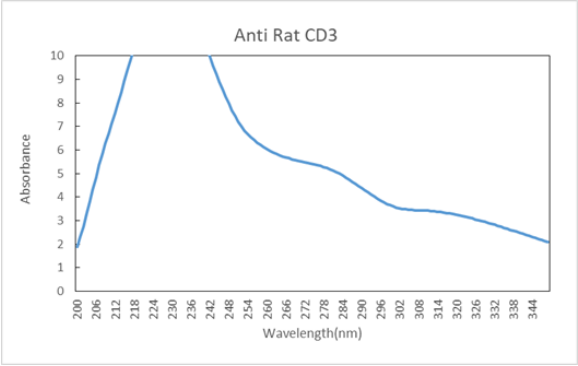
Symptoms	Causes	Comments
The proteins are not labeled with NHS-Biotin at all.	Improper operation, such as incomplete mixing of NHS-Biotin and protein, excessive ammonium ion or amino component in protein, or other improper operation.	Set a positive control.
	Improper preservation of NHS-Biotin.	Before labeling, the NHS-Biotin should not be mixed with water. When taking the NHS-Biotin, it should be kept at room temperature for about 5~10 min before unsealing.
	Improper use of ultrafiltration tube.	After ultrafiltration, there is less liquid in the filter device. Do not use the pipette to directly absorb the sample in the filter device. The sample should be centrifuged in an inverted state.
	Due to the difference of centrifuges, the rotation speed is too high during ultrafiltration.	Centrifuge speed is 12,000×g, not 12,000rpm
	Leakage of ultrafiltration tube.	Overload of filter to cause leakage.
Low recovery of protein	Protein aggregated and precipitated during the labeling process.	Add 1M Tris to terminate the reaction in time.
	Excessive protein concentration during ultrafiltration.	Do not load too much protein in filter device, such as more than 1 mg of protein.
	The protein cannot be completely dissolved in the labeling buffer.	Choose other labeling kits.

Appendix Ⅰ: Normal absorbance curve of protein concentration (for reference only)



Description: 1 mg/mL Mouse anti human CD183, the protein type was Mouse IgG1, PBS (pH 7.2, no preservatives), measured by the Nano 100 spectrophotometer, the concentration curve was normal, and A280=1.454, in line with the labeled concentration.

Appendix Ⅱ: Abnormal absorbance curve of protein concentration (for reference only)



Description: 0.5 mg/mL Mouse anti rat CD3, protein type Mouse IgG3, containing protein stabilizer and sodium azide (≤ 0.09%), measured by Nano-100 spectrophotometer, the concentration curve was abnormal, A280=5.195, which does not meet the labeled concentration.

Appendix Ⅲ: Protein retention and concentrate recovery (from Millipore product manual)

(Cite from the User Guide of Millipore: https://www.emdmillipore.com/US/en/product/Amicon-Ultra-0.5-Centrifugal-Filter-Unit,MM_NF-UFC500324#documentation)

For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. Merck Millipore Ltd. (Millipore) recommends using a membrane with a NMWL at least two times smaller than the molecular weight of the protein solute that one intends to concentrate. Refer to the table below.

Marker/Concentration	Molecular Weight	Device NMWL	% Retention	Spin Time (min)
α -Chymotrypsinogen (1 mg/mL)	25,000	3K	＞95	30
Cytochrome C (0.25 mg/mL)	12,400		＞95	30
Vitamin B-12 (0.2 mg/mL)	1,350		＞42	30
α -Chymotrypsinogen (1 mg/mL)	25,000	10K	＞95	15
Cytochrome C (0.25 mg/mL)	12,400		＞95	15
Vitamin B-12 (0.2 mg/mL)	1,350		＞23	15
BSA (1 mg/mL)	67,000	30K	＞95	10
Ovalbumin (1 mg/mL)	45,000		＞95	10
Cytochrome C (0.25 mg/mL)	12,400		＜35	10
BSA (1 mg/mL)	67,000	50K	＞95	10
Ovalbumin (1 mg/mL)	45,000		~40	10
Cytochrome C (0.25 mg/mL)	12,400		＜20	10
Thyroglobulin (0.5 mg/mL)	677,000	100K	＞95	10
IgG (1 mg/mL)	156,000		＞95	10
Ovalbumin (1 mg/mL)	45,000		＜30	10

Spin Conditions: 40° fixed angle rotor, 14,000×g, room temperature, 500 µL starting volume, n=12.