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

Biotin Labeling Kit

Catalog No.: RE80002

Size: 1 Reaction/3 Reactions/10 Reactions

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

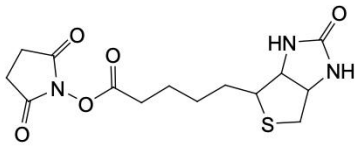
Product Introduction

This biotin labeling kit provides all the reagents required for biotin labeling, which is used to label proteins/antibodies or other macromolecules containing primary amino groups (NH2-).

Product Features

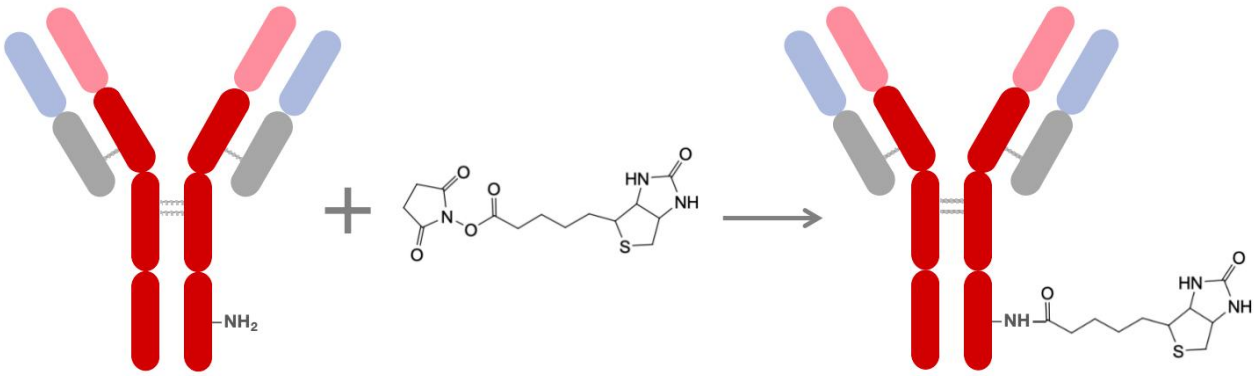
●Fast: The whole process takes only 90 minutes.
●Convenient: Biotin has been activated and can be used directly; ultrafiltration tube desalination, no dialysis required.
●Flexible use: It can be used for both trace labeling and large-scale labeling. Each ultrafiltration tube can label 0.1-1mg protein each time.
●Lipid soluble: The biotin in this kit is lipid soluble. In some experiments, the biotin-labeled protein needs to enter the cell membrane for reaction. This labeling method is relatively effective.

Basic information

Structural Formula	
Molecular Weight	341.38

Labeling Principle

Within a certain pH range, biotin reacts specifically with primary amino groups (N-terminus and lysine residue side chains) to form stable amide bonds, thereby achieving coupling with proteins.

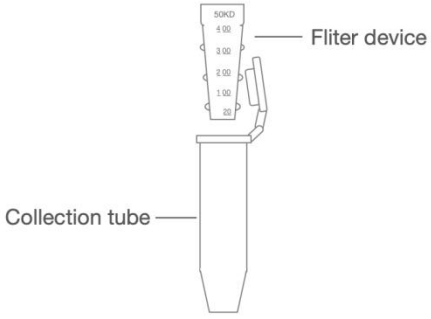


Components

Components	Contents in different sizes			Storage
	1 Reaction	3 Reactions	10 Reactions	
Biotin	0.1 mg×1	0.1 mg×3	0.1 mg×10	-20℃, away from light
50kDa ultrafiltration tube*	1 set**	3 set**	10 set**	RT
Labeling buffer	10 mL	20 mL	20 mL×10	2~8℃
1×PBS (pH 7.4)	10 mL	10 mL	10 mL×2	2~8℃
DMF	500 μL	500 μL	500 μL	2~8℃, away from light
Amount of labeled antibody	0.05 - 2 mg	0.05 - 6 mg	0.05 - 20 mg	

*50kDa ultrafiltration tubes are purchased from Millipore. Please refer to the appendix for instructions.

**1 set of 50 kDa ultrafiltration tubes (0.5 mL) includes 1 filter device and 2 collection tubes.



Storage

The unopened kit can be stored at 2~8℃ for one year, and the dissolved biotin can be stored at -20℃ or -80℃ for one week.

Calculation of Biotin usage for antibody labeling

The amount of biotin reagent used in each reaction depends on the amount and concentration of the protein to be labeled. For example, our experimental data analysis shows that labeling 2mg/ml of antibody (IgG, 150kDa) with a biotin to antibody molecular ratio of 20:1 can achieve the best effect; labeling of other proteins can refer to this ratio based on actual conditions.

Example: When labeling 1 mg of protein (concentration: about 2 mg/mL), using a biotin to

protein (150 kDa) molecular ratio of 20:1, the molar concentration of biotin is 10 mM, and the calculation method of the amount of biotin to be added is:

1.Calculate the required amount of substance n of Biotin:

$$n_{Biotin} = n_{Antibody/Protein} \times 20 = \frac{1\ mg}{150000\ mg/mmol} \times 20 = 0.00013333\ mmol$$

2.Calculate the required volume V of Biotin:

$$n_{Biotin} = \frac{n_{Biotion}}{C_{Biotion}} = \frac{0.00013333\ mmol}{10\ mM} = 13.3\ \mu L$$

Operation process

· Experimental preparation

1. Read the instruction manual carefully.

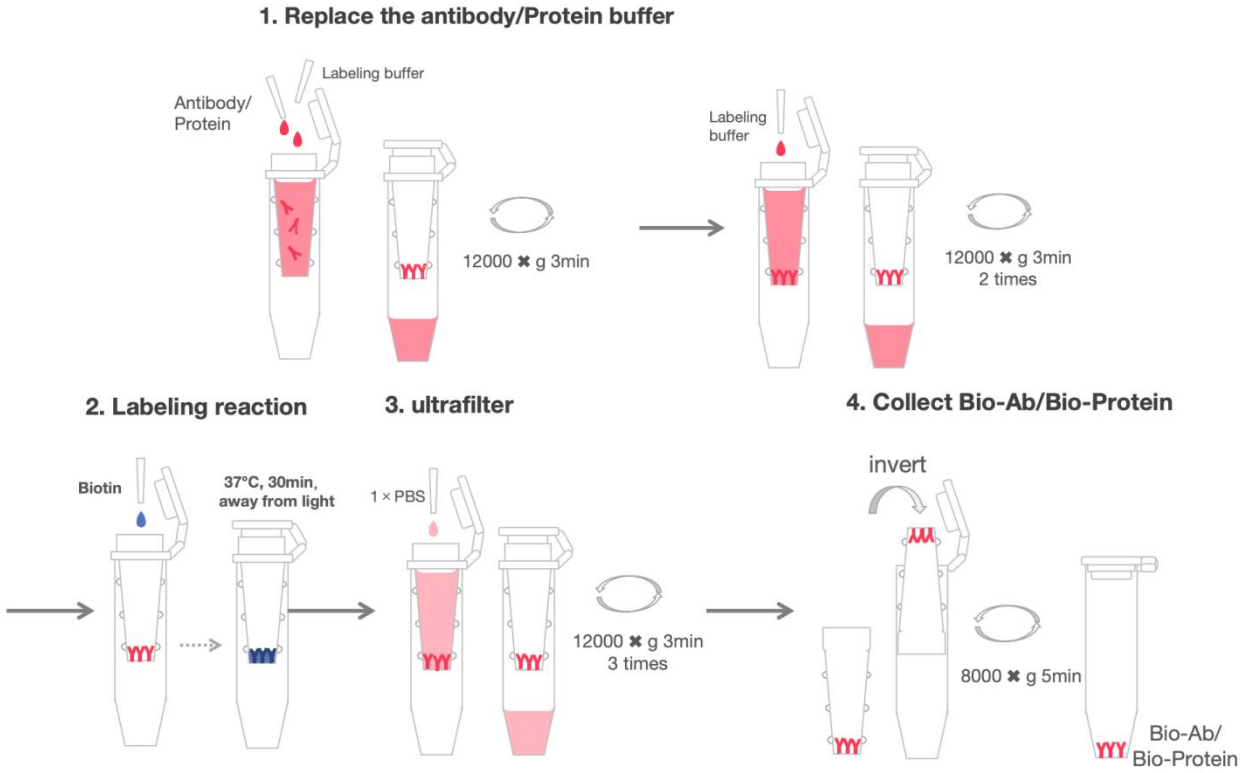
2. Calculate the amount of biotin required according to the formula for calculating the amount of biotin labeling.

3. Take out the kit from the refrigerator 20 minutes in advance and allow the components of the kit to equilibrate to room temperature. (Note: Unnecessary reagent components continue to be placed in the refrigerator).

4. Ultrafiltration tube infiltration: Add 500μL labeling buffer to the dry ultrafiltration tube filter element, leave it at room temperature for 10 minutes, and discard the labeling buffer before adding the labeled substance (the ultrafiltration tube filter element should be kept moist during the entire labeling process).

5. Dissolve biotin: Dissolve 0.1 mg biotin with 30μL DMF, let it stand for 10 minutes, and wait for it to be fully dissolved. At this time, the concentration of biotin is 10 mM. Cover the tube and set aside.

· Labeling procedures



· Labeling steps

(Taking the labeling of 1mg of antibody as an example)

1. Take 1 mg of the antibody to be labeled in an ultrafiltration tube, and add a labeling buffer that does not exceed the maximum volume of the ultrafiltration tube, and centrifuge at 12,000 x g for 3 minutes; this step can be repeated 3 times; after the last ultrafiltration, add an appropriate amount of labeling buffer to adjust the antibody concentration to about 2 mg/mL.

2. Add 13.3μL of 10 mM biotin to the above ultrafiltration tube and gently blow to mix. Incubate in a 37℃ constant temperature incubator in the dark for 30 minutes.

3. Centrifuge at 12,000 x g for 10 minutes.

4. Add an appropriate amount of 1×PBS to the above ultrafiltration tube, and gently blow to mix, centrifuge at 12,000 x g for 3 minutes, and repeat this step 3 times.

5. Collect the solution in the ultrafiltration tube (i.e., biotin-labeled antibody), add 0.05-0.2% Proclin 300 or 0.05% sodium azide and a protein stabilizer (such as 0.1% BSA) to the labeled protein, and store it at 2-8°C in the dark. It can be stored stably for half a

year. Alternatively, add an equal volume of glycerol and store it at -20°C. It can be stored stably for half a year.

Notes

1. Please select the appropriate kit according to the molecular weight of the protein to be labeled. This kit provides a 50 kDa ultrafiltration tube.
2. Biotin is easily hydrolyzed by moisture and becomes ineffective. It should be stored at -20°C or -80°C together with a desiccant. To prevent water vapor from condensing into biotin, move it to room temperature for equilibrium before the experiment.
3. The dissolved biotin is best used up at one time. If it is not used up, it can be sealed and placed in a refrigerator at -20°C. It can be used within one month, but the labeling efficiency will be reduced. The biotin cosolvent needs to be sealed and stored immediately after use to prevent moisture absorption.
4. This kit can also label other proteins containing free amino groups. The specific labeling ratio is determined according to the number of available amino groups in the labeled substance or different molar ratios are set for labeling.

The optimal molecular ratio of biotin and protein (150kDa) recommended for labeling in this kit is 20:1, which can ensure that biotin can be labeled with protein, but it cannot ensure the best experimental results. The optimal labeling ratio may vary depending on the difference in protein. Users can optimize according to actual conditions.

Statement

1. This product is for Research Use Only.
2. Please pay attention to safety precautions and follow the laboratory reagent operation specifications.
3. Although this labeling kit has been verified to label many different proteins, it is still possible that the protein binding affinity is damaged or even lost during the biotin labeling

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 in the antigen binding site, and its binding function is impaired during the labeling process. In addition, some proteins have key lysine residues (not necessarily in the binding site) that are absolutely critical to maintaining protein stability/solubility. Once modified, the protein is completely unusable due to precipitation. This labeling kit does not carry full responsibility for this rare situation. We believe that our customers should be aware that the use of biotin in this kit may impair the biological function of the modified protein in some cases.

4. Although this kit can also be used to label proteins, it should be recognized that the various properties of different proteins vary greatly, such as the solubility of proteins in different buffers, pH stability, temperature stability, protein purity, accessibility of labeling sites, etc., which are quite different from proteins. Therefore, this labeling kit does not provide quality assurance for labeling proteins other than IgG and IgM proteins.

Appendix

Protein Retention and Concentration Recovery (Refer to Millipore Product Manual)

(Excerpt from Millipore User Guide:

https://www.emdmillipore.com/US/en/product/Amicon-Ultra-0.5mL-Centrifugal-Filters-for-DNA-and-Protein-Purification-and-Concentration,MM_NF-C82301?bd=1#overview)

For most applications, molecular weight is a convenient parameter for evaluating retention characteristics. Merck Millipore Ltd. (Millipore) recommends using a membrane with an NMWL at least two times smaller than the molecular weight of the protein solute to be concentrated. Please refer to the table below.

Marker/Concentration	Molecular Weigh	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.