(This kit is for research use only, not for clinical diagnosis!)

EnAb™ Sulfo-NHS-Biotin Labeling Kit

Catalog Number: RE80002q

Sizes: 40 μg/200 μg/2 mg

Please read the instructions carefully before use. If you have any questions, please contact us via the following methods:

Web: https://www.enkilife.com/

E- mail: order@enkilife.com techsupport@enkilife.com

Tel: 0086-27-87002838

Product Introduction

The EnkiLife Biotin Labeling Kit offers a selection of biotin types commonly used in laboratories. Each kit includes all necessary reagents for labeling, designed specifically for proteins and antibodies with primary amino groups. The components of this kit are intended for labeling antibodies or proteins of comparable size to antibodies, by default.

Features

- **Fast:** Labeling takes only about 30 minutes.
- **Rich in variety:** We provide a variety of biotin types to meet various application requirements.
- **Outstanding effect:** targeted optimization of labeling buffer, labeling site is far away from antigen binding site, and labeled antibody has high homogeneity.
- **Convenient:** Each biotin reagent has been optimized and designed with the corresponding antibody amount, and has been activated and can be used directly, without the need for tedious calculations. The batches are stable and the best results can be achieved by following the steps.

Biotin Information

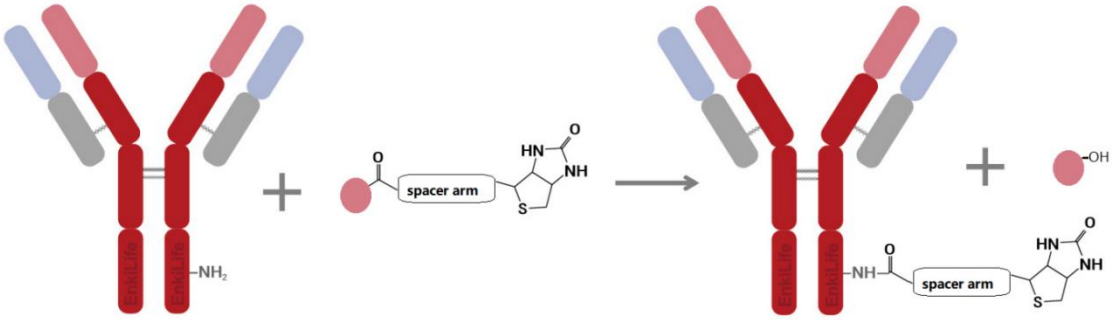
Biotin Type	Spacer arm length	Solubility	Key Features	Catalog Number
Sulfo-NHS-Biotin	13.5 Å	Water Solubility	Membrane impermeable, short arms, sulfonic acid group, high solubility in aqueous solution	RE80002q
Sulfo-NHS-LC-Biotin	22.4 Å	Water Solubility	Membrane impermeable, medium arm length, sulfonic acid group, high solubility in aqueous solution	RE80002s
Sulfo-NHS-LC-LC-Biotin	30.5 Å	Water Solubility	Membrane impermeable, enhanced arm length, sulfonic acid group, high solubility in aqueous solution	RE80002u
Sulfo-NHS-SS-Biotin	24.3 Å	Water Solubility	Membrane impermeable, selective cutting possible	RE80002v
NHS-PEG4-Biotin	29.0 Å	Water Solubility	PEGylation to prevent protein aggregation	RE80002w

Selection of labeling kit

- When the molecular weight of the final labeled product needs to be as small as possible, choose the Short-Arm Biotin Labeling Kit.
- When follow-up applications require the use of reducing agents to cleave the biotin tag, choose the Sulfo-NHS-SS-Biotin Labeling Kit.
- When you need to maximize protein solubility and prevent protein aggregation, choose the NHS-PEG4-Biotin labeling kit.
- When you want the final binding steric hindrance to be smaller, choose the long-arm biotin labeling kit and select the appropriate arm length based on the binding characteristics.

Labeling principle

Within a certain pH range, biotin reacts specifically with primary amino groups to form stable amide bonds, thereby achieving coupling with proteins.



Product composition

Product composition	Content of components in different specifications			Storage temperature
	40 μg antibody	200 μg antibody	2 mg antibody	
Sulfo-NHS-Biotin	1 tube	5 tubes	5 tubes	-20 °C after opening, away from light
Ultrafiltration Tube * ,50K MWCO	1 set **	1 set **	1set **	RT
Labeling Buffer B	10 mL	10 mL	10 mL	2-8 °C
1× PBS (pH 7.4)	10 mL	10 mL	10 mL	2-8 °C
DMF	100 μL	100 μL	100 μL	2-8 °C, away from light
Labeled protein storage solution	200 μL	1 mL	5 mL	2-8°C
Recommended amount of labeled antibody	Each tube dye is for labeling 20-40 μg antibody.It is recommended to label 20 μg antibody.	Each tube dye is for labeling 20-40 μg antibody.It is recommended to label 20 μg antibody.	Each tube dye is for labeling 100-400 μg antibody.It is recommended to label 200 μg antibody.	

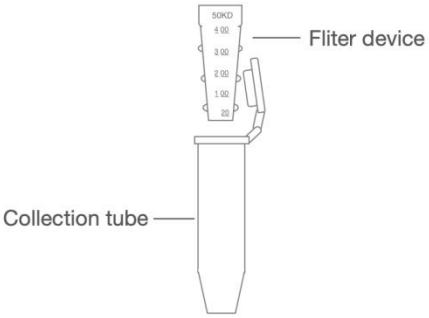
*Ultrafiltration tube instructions:

If the same biological molecule is labeled, the ultrafiltration tube membrane can be reused several times before it breaks.

If different biomolecules are labeled, different ultrafiltration tubes should be replaced to avoid cross contamination of biomolecules.

** If you need more of the 50K MWCO ultrafiltration tubes, please contact us for further supply.

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



Storage conditions

After receiving the kit, each component can be stored at the recommended temperature for one year, and the dissolved dye can be stored at -20°C or -80°C for one week.The entire kit should not be stored at 2-8 °C for more than 1 months.

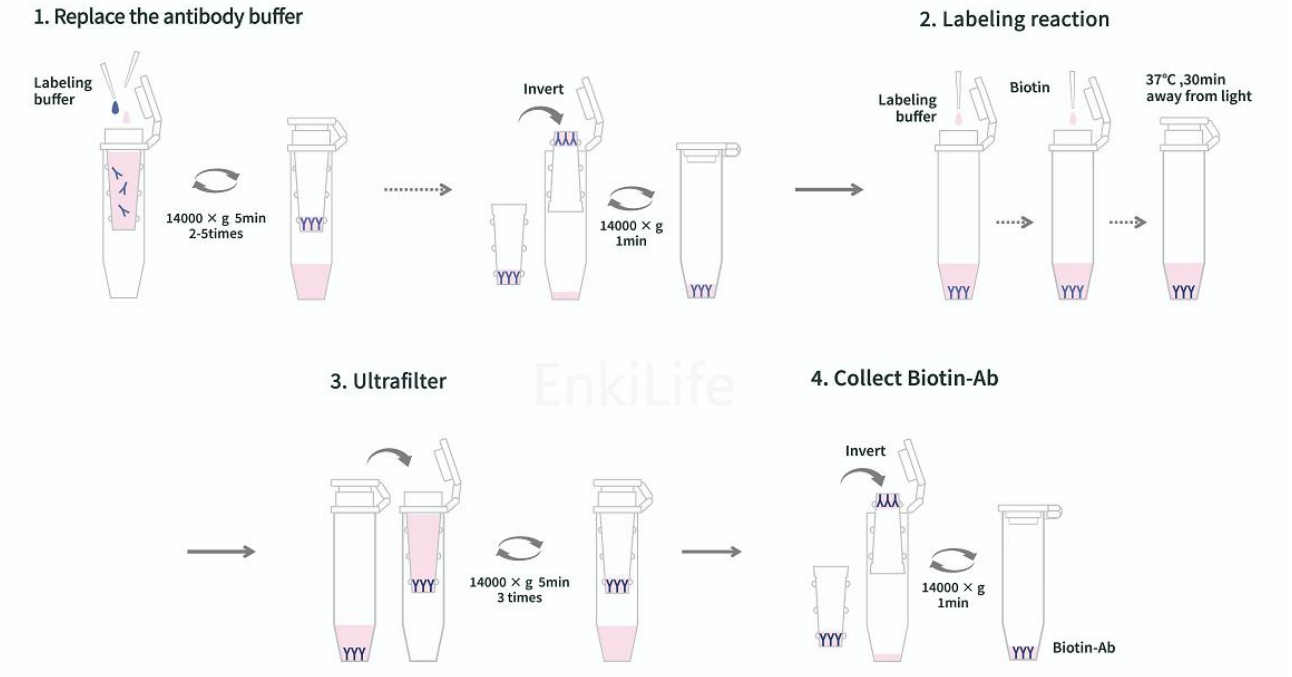
Operation process

Preparation before the experiment

1. Read the instruction manual carefully.
2. Take the kit from the refrigerator 20 minutes in advance to allow the components to equilibrate to room temperature. (Note: Components not needed should remain in the refrigerator.)
3. Ultrafiltration tube infiltration: Add 500 µL labeling buffer, place at room temperature for 10 minutes for later use, and discard the labeling buffer before adding the labeled substance (the ultrafiltration tube filter should be kept moist during the entire labeling process).
4. Dissolve biotin: Dissolve the biotin for each tube that labels 20-40 µg of antibody with 1 µL DMF , and dissolve the biotin for each tube that labels 100-400 µg of antibody with 10µL DMF. Vortex to mix or pipette to completely dissolve it and set aside.
5. Antibody preparation: Please ensure that the antibody sample meets the standards in the following table

Antibody Components	Kit Labeling Compatibility
Sodium azide <0.1%, glycerol <50%, Tris <50 mM, glycine <50 mM, Proclin <0.5%, EDTA <10 mM, trehalose <5%, sucrose <5%, or other small molecule additives	Yes, perform ultrafiltration concentration and buffer exchange steps for the antibody sample, which is Step 1 of the labeling procedure.
Antibodies in ascites, serum, and cell culture supernatant	No, perform the purification step before using this kit
Contains BSA	No, use a BSA removal kit (such as RE80028: BSA Removal Kit) to purify the antibody before using this kit

Labeling Process



Labeling steps (applicable to labeling 500 μg-2 mg antibody)

1. **Ultrafiltration concentration and buffer exchange of antibody samples** : Take 500 μg-2 mg of the antibody to be labeled into the ultrafiltration tube filter , and add the labeling buffer that does not exceed the maximum volume of the ultrafiltration tube filter , 14000 × g centrifuge for 3 to 5 minutes, discard the filtrate ; repeat this step 2 times , and after the last ultrafiltration , obtain 50 to 100 μL . Invert the ultrafiltration tube filter into the collection tube and centrifuge at 14000 × g for 3 to 5 minutes. Centrifuge at 14000 × g for 1 minute, collect the antibody solution, add labeling buffer to a total volume of 450 μL, and add an appropriate amount of 1× PBS to the filter element to keep it moist.

2. Mix the 5 tubes of dissolved Sulfo-NHS-Biotin together, a total of 50 μL, and add all to the above 450 μL antibody solution (the total volume is about 500 μL at this time), and gently blow to mix. Cover the lid and incubate in the dark at 37°C in a constant temperature incubator for 30 minutes.

3. Discard the buffer in the filter cartridge, transfer 500 μL of the labeled mixture to the filter cartridge, and Centrifuge at 4 °C for 3 to 5 minutes , discard the filtrate, and replace with 1 × PBS buffer and repeat this step 3 to 5 times.

4. Add 200 μL 1 × PBS to the ultrafiltration tube and pipette gently. Invert the ultrafiltration tube filter into another collection tube and centrifuge at 14000 × g for 1 minute. The solution obtained in the collection tube is Sulfo-NHS-Biotin labeled antibody.

5. (Optional) Add an appropriate amount of labeled protein storage solution to the labeled antibody and store at 4°C or store at -20°C after aliquoting . (you can also store it in low-absorption tubes without adding protein preservation solution), avoid repeated freezing and thawing, and can be stably stored for more than 6 months.

Labeling step (applicable to labeling 50 μg-200 μg antibody)

1. **Ultrafiltration concentration and buffer exchange of antibody samples** : Place 50 μg to 200 μg of the antibody to be labeled into the filter of the ultrafiltration tube, and add the labeling buffer not exceeding the maximum volume of the ultrafiltration tube filter. Centrifuge at 14000 × g for 3 to 5 minutes and discard the filtrate. This step can be repeated 2 times. After the last ultrafiltration, about 30 to 40 μL will be obtained. Invert the ultrafiltration

tube filter into the collection tube and centrifuge at 14000 × g for 1 minute to collect the antibody solution. Add the labeling buffer to bring the total volume to 45 μL. Meanwhile, add an appropriate amount of 1×PBS to the filter to keep it moist.

2. Mix the 5 μL of dissolved Sulfo-NHS-Biotin together and add all of them to the 45 μL of antibody solution (the total volume is about 50 μL at this time), and gently pipette to mix. Cover the lid and incubate in the dark at 37°C in a constant temperature incubator for 30 minutes.

3. Discard the buffer in the filter, transfer 50 μL of the labeling mixture into the filter, add 1×PBS to a total volume of 500μL, and centrifuge at 14000 × g for 3 to 5 minutes. Discard the filtrate. Repeat this step 3 to 5 times.

4. Take an appropriate amount of 1 × PBS (e.g. 50 μL) into the ultrafiltration tube and shake gently. Place the ultrafiltration tube filter inverted in another collection tube and centrifuge at 14000 × g for 1 minute. The solution obtained in the collection tube is Sulfo-NHS-Biotin labeled antibody.

5. (Optional) Add an appropriate amount of labeled protein storage solution to the labeled antibody and store at 4°C or store at -20°C after aliquoting . (you can also store it in low-absorption tubes without adding protein preservation solution), avoid repeated freezing and thawing, and can be stably stored for more than 6 months.

Labeling step (applicable to labeling 20 μg-40 μg antibody)

1. **Ultrafiltration concentration and buffer exchange of antibody samples** : Take 20 μg-40 μg of the antibody to be labeled in the ultrafiltration tube filter , and add the labeling buffer that does not exceed the maximum volume of the ultrafiltration tube filter , 14000 × g centrifuge for 3 to 5 minutes, discard the filtrate ; The step can be repeated 2 times. After the last ultrafiltration, 20 to 30 μL will be obtained. Invert the ultrafiltration tube filter into the collection tube and centrifuge at 14000 × g for 1 minute to collect the 20 to 30 μL antibody solution. Add an appropriate amount of 1×PBS to the filter to keep it moist.

2. Dissolve Sulfo-NHS-Biotin ,Add 1 μL of the solution to the 20-30 μL antibody solution and mix thoroughly by gently pipetting. Incubate in the dark at 37°C in a constant temperature incubator for 30 minutes.

3. Discard the buffer in the filter cartridge, transfer the labeled mixture back into the filter cartridge, add 1×PBS to a total volume of 500 μL, and centrifuge at 14000 × g for 3 to 5 minutes. Discard the filtrate and repeat this step 3 to 5 times.

4. Place the ultrafiltration tube cartridge upside down in another collection tube and centrifuge at 14000 × g for 1 minute. The solution obtained in the collection tube is Sulfo-NHS-Biotin labeled antibody.

5. (Optional) Add an appropriate amount of labeled protein storage solution to the labeled antibody and store at 4°C or store at -20°C after aliquoting . (you can also store it in low-absorption tubes without adding protein preservation solution), avoid repeated freezing and thawing, and can be stably stored for more than 6 months.

Precautions

1. This labeling kit is designed for antibodies (150 KDa) labeling. If you need to label other proteins, please contact us to select relevant reagents and consumables.

2. Biotin is easily hydrolyzed by moisture and becomes ineffective. Before the experiment, move it to room temperature for equilibrium before opening.

3. The dye and antibody dosages designed in this kit are relatively fixed, based on the empirical ratio obtained by labeling multiple antibodies, which can ensure good results. Some antibodies or proteins may have large differences in special structure or quantity. Users can choose a kit with appropriate specifications to increase or decrease the dye dosage to optimize the labeling ratio according to actual conditions.

Statement

1. This product is for scientific research use by professionals only.

2. Please pay attention to safety precautions and follow the laboratory reagent operation specifications.
3. This kit can also be used to label other proteins besides IgG antibodies, but it should be recognized that the various properties of different proteins vary greatly from IgG, such as protein solubility in different buffers, pH stability, temperature stability, protein purity, accessibility of labeling sites, etc. Therefore, this labeling kit does not provide quality assurance for labeling proteins other than IgG.

Appendix 1 Ultrafiltration tube instructions

1. The 50 kDa MWCO ultrafiltration tubes equipped with this kit has a maximum filter capacity of 500 µL and is marked with scales. If the user is using it for the first time, he can add a certain volume of marked buffer in advance, check the correspondence between the scale and volume, and increase or decrease the ultrafiltration centrifugation time.
2. Ultrafiltration centrifugation time is related to the characteristics of the protein sample or conjugate. The centrifugation concentration time of some antibodies needs to be adjusted according to actual conditions. For example, when ultrafiltration concentrates 20 µg of antibody to 20-30 µL, the centrifugation time can be appropriately extended to make the antibody concentration as close to 1mg/mL as possible.
3. The ultrafiltration membrane is the key component of the ultrafiltration tube. When blowing and mixing the protein solution, be careful not to touch the ultrafiltration membrane.