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

EnAb™ Fluor488 Labeling Kit

Catalog Number: RE80011p

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

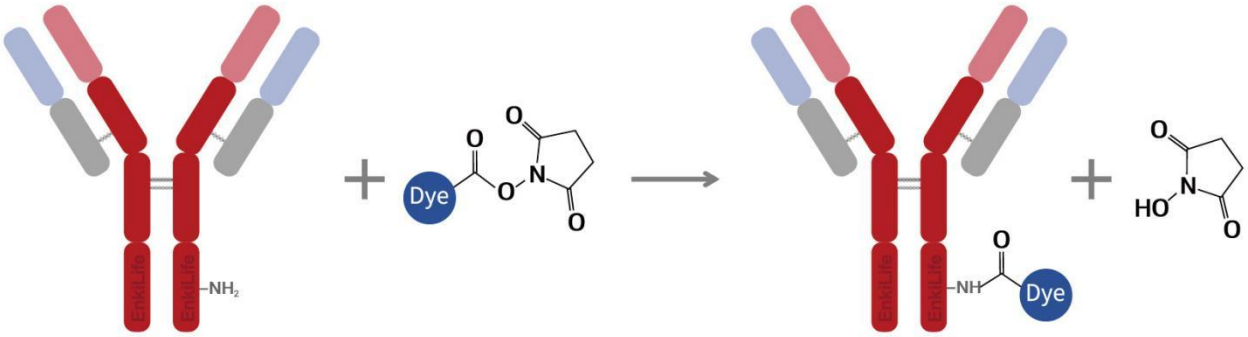
EnkiLife small molecule fluorescent dye labeling kit provides a variety of commonly used fluorescent dye types on the market, including the commonly used cyanine dye series, upgraded dyes of the cyanine dye series, and Fluor series dyes, etc., including a variety of common fluorescent dye types from ultraviolet spectrum, visible spectrum to near-infrared spectrum. All small molecule fluorescent dye labeling kits contain all the reagents required for labeling, which are used to label proteins and antibodies containing primary amino groups. The various dyes used in the EnkiLife small molecule fluorescent dye labeling kit have been optimized in core structure, with brighter fluorescence brightness, reduced non-specific binding to cells or tissues, and stable fluorescence properties, and are not prone to fluorescence bleaching or fluorescence quenching. The labeling process used in the kit is mature and reliable, and the labeling site has a certain selectivity. The labeled antibodies or proteins can meet a variety of scientific research needs, such as immunofluorescence,intracellular target labeling, animal fluorescence imaging, flow cytometry, WB, ELISA, etc.

Features

- **Fast:** Labeling time only takes approx. 30 minutes.
- **Simple:** Each dye reagent has been optimized and designed with the corresponding antibody amount, without the need for tedious calculations. The solid dye format is batch stable, and better results can be achieved by following the steps.
- **Outstanding labeling effect**: Optimized labeling buffer, relatively fixed dye labeling sites, enhanced homogeneity of labeled antibodies.
- **Dye characteristics:** Green fluorescent dyes, with a spectrum consistent with FITC, are resistant to fluorescence quenching, pH-insensitive, and particularly suitable for applications requiring long exposure times.

Labeling principle

Within a certain pH range, the fluorescent dye activation group specifically reacts with the primary amino group on the antibody protein to form a stable amide bond, thereby achieving coupling with the antibody protein.



Product composition

Product composition	Content of components in different specifications			Storage temperature
	40 μg antibody	200 μg antibody	2 mg antibody	
Fluor488	1 tube	5 tubes	5 tubes	-20 °C after opening, away from light
Ultrafiltration Tube* ,50K MWCO	1 set **	1 set **	1 set **	RT
Labeling Buffer S	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	1mL	1mL	5mL	2-8 °C
Recommended amount of labeled antibody	Each tube is labeled with 20-40 μg, and the best is 20 μg	Each tube is labeled with 20-40 μg, and the best is 20 μg	Each tube is labeled with 100-400 μg, and the best is 200 μg	

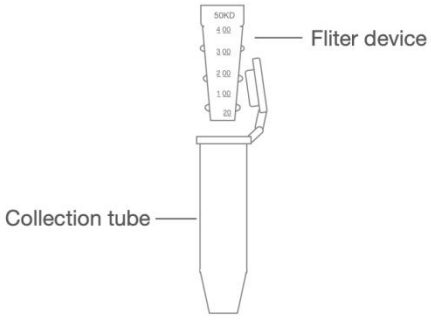
*Ultrafiltration tube instructions:

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

The unopened kit can be stored at 2-8°C for one year, and the dissolved dye can be stored at -20°C or -80°C for one week.

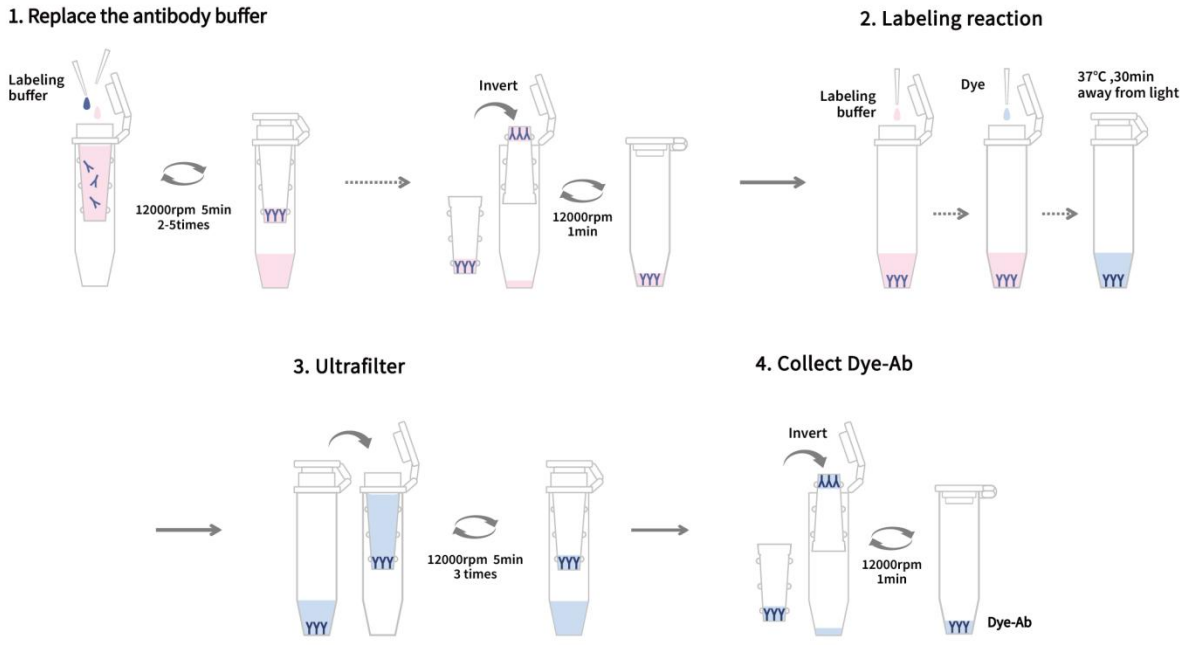
Operation process

Preparation before the experiment

1. Read the instruction manual carefully.
2. Remove the kit from the refrigerator 20 minutes in advance to allow all components of the kit to equilibrate to room temperature. (Note: Components of the kit that are 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 Fluor488: Dissolve each dye that labels 20 µg of antibody in 1µL DMF , OR each dye that labels 200 µg of antibody in 10 µL DMF. Vortex to mix or pipette to completely dissolve, 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 the 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 Fluor488 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 for 30 minutes in a constant temperature incubator.

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. Repeat this step 3 to 5 times until the filtrate in the collection tube is almost transparent.

4. Add 200μL 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 Fluor488 labeled antibody.

Note: If you need to calculate the DOL of the labeling effect, please refer to the appendix instructions and perform it before adding the labeled protein storage solution.

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 cartridge of the ultrafiltration tube, and add labeling buffer up to the maximum volume of the filter cartridge. Centrifuge at 14000 × g for 3 to 5 minutes and discard the filtrate. This step can be repeated 2 times. After the last ultrafiltration, approximately 30 to 40 μL of the antibody solution should be obtained. Invert the filter cartridge into the collection tube and centrifuge at 14000 × g for 1 minute to collect the antibody solution. Add 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 Fluor488 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 for 30 minutes in a constant temperature incubator..

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

4. Take an appropriate amount of 1 × PBS (e.g. 50 μL) into the ultrafiltration tube and shake 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 Fluor488 labeled antibody.

Note: If you need to calculate the DOL of the labeling effect, please refer to the appendix instructions and perform it before adding the labeled protein preservation solution.

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 ; repeat this step 2 times , and after the last ultrafiltration , obtain 20 to 30 μL. Invert the filter column of the ultrafiltration tube into the collection tube and centrifuge at 14000 × g for 1 minute to collect 20 to 30 μL of antibody solution. Add an appropriate amount of 1× PBS to the filter column to keep it moist.
2. Dissolve Fluor488,Add 1 μL of the solution to the 20-30 μL antibody solution and mix thoroughly by gently pipetting. Cover the lid and incubate in the dark at 37°C for 30 minutes in a constant temperature incubator.
3. Discard the buffer in the filter column, transfer the labeling mixture into the filter column again, add 1× PBS to a total volume of 500 μL, and centrifuge at 14000 × g for 3 to 5 minutes. Discard the filtrate. Repeat this step 3 to 5 times until the filtrate in the collection tube is almost transparent.
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 Fluor488 labeled antibody.
Note: If you need to calculate the DOL of the labeling effect, please refer to the appendix and perform it before adding the labeled protein storage solution.
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. The dye is easily affected by moisture and becomes ineffective due to hydrolysis. Before the experiment, move it to room temperature for equilibrium before opening it.
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 50K MWCO ultrafiltration tube 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, a certain volume can be added 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.

Appendix 2 DOL calculation explanation for labeling effect

1. Dilute a small amount of purified conjugate into PBS or other suitable buffer, and measure the absorbance at 280nm wavelength (A_{280}) and the maximum absorbance of the corresponding dye (A_{dye}) in a cuvette with a 1cm optical path. If you use a NanoDrop or microplate, which may provide a shorter optical path, please refer to the instrument instructions for calculation and modification. For example, if the optical path measured by a microplate is 0.5mm, and A280 = 0.1 is measured, then convert it to 1cm (=10mm) optical path A280 = 0.1*10/0.5=2

2. Calculation of labeled antibody concentration

Antibody concentration (mg/mL) = (A_{280} -CF280$_{dye}$ × A_{dye}) × 150000 × dilution factor / 203000

Antibody concentration (mol/L) = (A_{280} -CF280$_{dye}$ × A_{dye}) × dilution factor / 203000

3. Degree of Antibody Labeling (DOL) Calculation

DOL=(A_{dye} × 203000)/(E_{dye} × (A_{280} -CF280$_{dye}$ × A_{dye}))

Appendix 3 Technical parameters of each small molecule fluorescent dye

Dye Name	Labeling Kit Catalog Number	Ex max/Em max	E_{dye}(L· mol^{-1}· cm^{-1})	CF280$_{dye}$
Fluor488	RE80011p	490nm/513nm	71000	0.11