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

Fluor 488 Labeling Kit

Catalog No.: RE80011

Size: 0.5mg/1.25mg/2.5mg

Please read the instructions carefully before use. 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

EnkiLife fluorescent dyes are active fluorescent dyes. The series includes common fluorescent dyes from the ultraviolet, visible spectrum to the near-infrared spectrum, which are used to label biomolecules, especially proteins and antibodies. Innovative modifications to the core structure make EnkiLife dyes superior to other commercial dyes with many innovative and novel features, mainly manifested in higher labeling efficiency and stronger luminescence.

Fluor488 is a fluorescent dye with excitation and emission wavelengths of 494nm and 518nm respectively. It forms a more specific antibody-fluorescein conjugate with the antibody and has a lower background. It is an upgraded product of FITC, mainly characterized by better stability.

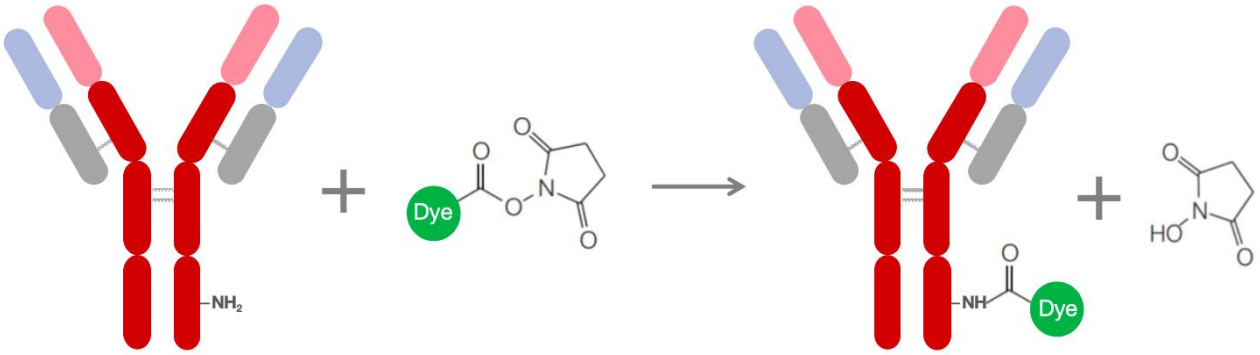
Product Features

• This kit is often used for direct antibody labeling, eliminating the need for secondary antibodies and the steps involved.

• Labeling can be easily completed in 60-90 minutes.

Labeling principle

This kit mainly uses the active bond of the fluorescein group to covalently bind to the free amino group of the biological molecule, and can be used to label antibodies and proteins.



Components

Components	Contents in different sizes		
	0.5 mg	1.25 mg	2.5 mg
Activated Fluor488 Dry Powder	Add 4 μl DMSO for dissolution	Add 10 μl DMSO for dissolution	Add 20 μl DMSO for dissolution
DMSO	40 μl	100 μl	200 μl
Labeling Buffer	10 ml	15 ml	30 ml
Storage Buffer	2.0 ml	2 ml*2	10 ml
Purification Ultrafiltration Tube	1 vial	1 vial	1 vial
Recommended Labeled Antibody Amount	0.1 - 0.5 mg	0.25 - 1.25 mg	0.5 - 2.5 mg

Storage

The kit can be stored at -20˚C for 6 months.

Calculation of Fluor488 usage for antibody labeling

The amount of dye used in each reaction depends on the quality, concentration and molecular weight of the protein to be labeled. This kit is for antibody labeling, and the optimal molecular ratio of Fluor488 to antibody is 23:1. (The molecular ratio of Fluor488 to antibody ranges from 8:1 to 23:1)

Example: To label 0.1 mg of protein (concentration of about 2 mg/mL), using a molecular ratio of Fluor488 to protein (IgG, 150 kDa) of 23:1, the molar concentration of Fluor488 is 7.7 mM. The calculation method for the amount of Fluor488 to be added is:

1. Calculate the required amount of substance n of Fluor488:

$$n_{\text{Fluor488}} = n_{\text{protein}} \times 23 = 0.1\ mg \div 150000\ mg/mmol \times 23$$

$$= 0.000015333\ mmol$$

2. Calculate the required volume V of Fluor488:

$$V_{\text{Fluor488}} = n_{\text{Fluor488}} \div C_{\text{Fluor488}} = 0.000015333\ mmol \div 7.7\ mM = 2\ \mu L$$

Operation process

Experimental preparation

1. Read the instructions carefully.

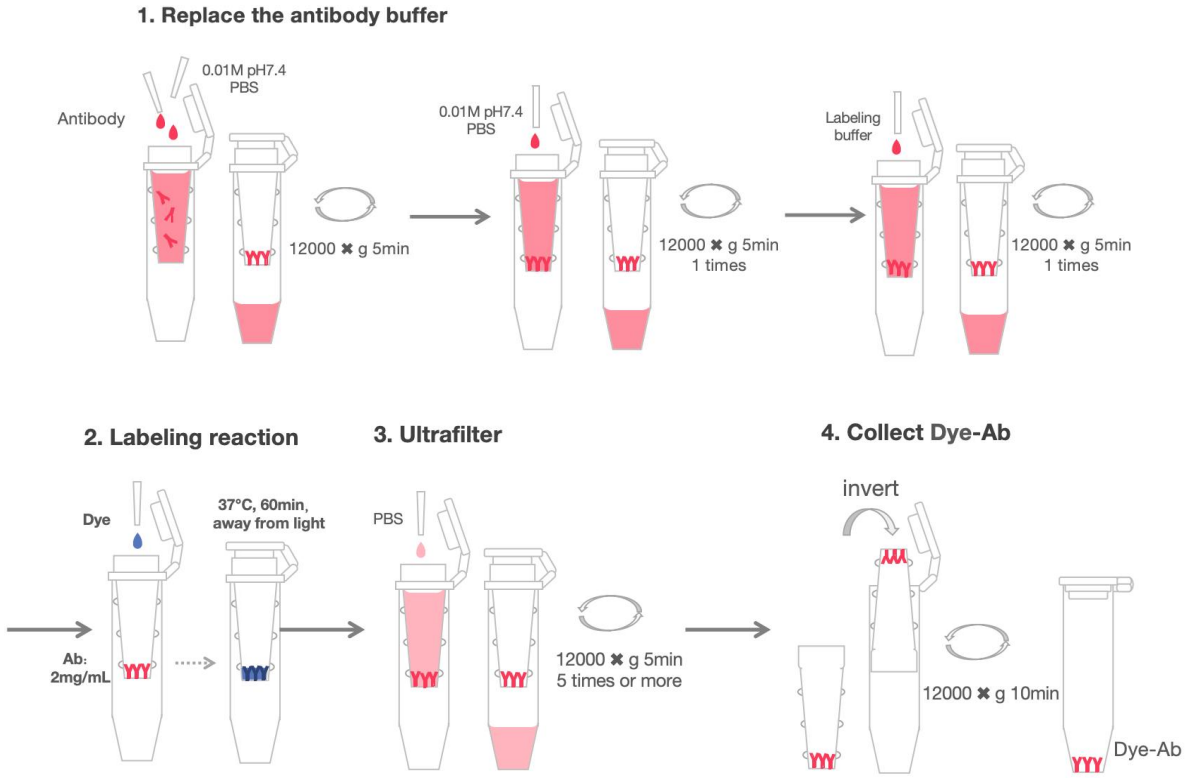
2. Prepare reagents and consumables: Take out the reagents from the refrigerator 20 min in advance and equilibrate to room temperature (Note: Reagent components that are not used temporarily should continue to be placed in the refrigerator).

3. Ultrafiltration tube infiltration: Add the labeling buffer to the dry ultrafiltration tube filter, let it stand at room temperature for 10 minutes before use, and then discard the labeling buffer before adding the material to be labeled (the ultrafiltration tube filter should remain moist throughout the entire labeling process).

4. Dissolve and activate Fluor488 dry powder (assuming a product specification of 0.5 mg): Dissolve the Fluor488 dry powder with 4 μL of DMSO, let it stand for 10 minutes to ensure complete dissolution. At this point, the concentration of Fluor488 is 7.7 mM. Cap the tube and set it aside for later use.

Labeling process

(Taking the antibody solution replaced with labeling buffer as an example)



Labeling steps

(Taking the labeling of 100 μg of antibody at a concentration of 2 mg/mL as an example)

1. Replace the antibody buffer: Replace the antibody solution to be labeled with labeling buffer, and then add labeling buffer to make the antibody concentration reach 2 mg/mL.

2. Labeling reaction: Take 2uL of 7.7 mM Fluor488 solution and add it to the antibody in the above step, mix gently, cover with a lid and seal, and react at 37℃ in the dark for 1 hour.

3. Ultrafiltration: Add an appropriate amount of PBS (approximately 450uL) to the above reaction solution, mix gently, and centrifuge at 4°C at 12,000×g for 5 minutes. After centrifugation, remove the tube core and discard the solution in the outer tube, reinsert the tube core into the outer tube, and add an appropriate amount of PBS (approximately 450 μL) to the tube core again, then centrifuge at 4°C at 12,000×g for 5 minutes. Repeat the ultrafiltration centrifugation process 4 times.

4. **Collect Dye-Ab:** Gently mix the solution inside the ultrafiltration tube core and blow the inner wall of the tube core to ensure thorough mixing, then transfer the solution to a clean, light-protected centrifuge tube.

Storage of labeled antibodies

Volume adjustment: Based on experimental requirements, adjust to the appropriate concentration by adding an appropriate amount of BSA, glycerol, and preservatives, etc., aliquot and store at -20°C in the dark; alternatively, mix the labeled product with the Storage buffer that comes with the kit in a volume ratio of 1:1, then aliquot and store.

Storage: The labeled product containing preservatives can be stored stably for 1 month at 4°C in the dark, or can be stored stably for 6 months at -20°C.

Notes

A. It is recommended that the dissolved Fluor488 be used up once and not saved for next use.

B. **Ultrafiltration tube specification selection:** The ultrafiltration tube provided with this kit has a default molecular weight cut-off (MWCO) of 30k, which is suitable for labeling antibodies. If you need to label substances with different molecular weights, it is recommended to select an ultrafiltration tube specification based on the principle that the molecular weight of the substance to be labeled is more than twice the MWCO of the ultrafiltration tube, and contact us before placing an order.

C. **Selection of the molecular ratio of Fluor488 to antibody:** The molecular ratio of Fluor488 to antibody recommended by this kit (23:1) is for reference only. Experimenters may explore and adjust the ratio according to actual needs. The recommended range of the molecular ratio of Fluor488 to antibody is 8:1~23:1.

D. **Applicability of the kit:** This kit can also be used to label other proteins containing free amino groups. The specific labeling ratio is determined based on the number of available amino groups in the substance to be labeled or by setting different molar ratios for labeling.

E. **Requirements for antibodies to be labeled:** The optimal reaction concentration for antibody labeling is 2 mg/mL. If the concentration is lower, it needs to be concentrated to 2 mg/mL before the experiment.

F. **Requirements for reaction buffer:** The reaction environment for the substance to be labeled should meet the following requirements. If your antibody buffer meets these requirements, you can proceed directly with the labeling. If not, please use the labeling buffer or 0.01M pH 7.4 PBS to replace the solution (dialysis, ultrafiltration, etc.).

pH	6.5-8.0
No free amino groups	MES, PBS, HEPES
Chelator (e.g., EDTA)	×
Glycerol	< 5%
Bovine Serum Albumin	×
Glycine	×
Amino component	×
Protective protein like BSA, etc.	×

Frequently Asked Questions and Solutions

Q: If the concentration of the molecule to be labeled still does not reach 2 mg/ml after concentration and further concentration results in precipitation, what should I do?
A: When labeling, try to reach this concentration as much as possible. If it is really not achievable, appropriately increase the amount of activated fluorophore added. The optimal labeling effect can be determined by testing with a gradient increase in the amount of fluorophore used.
Q: Is the optimal molar ratio of the molecule to be labeled to the fluorophore limited to between 1:8 and 1:23?
A: This needs to be determined based on the properties of different biomolecules, more accurately, it is related to the number of amino groups on the surface of the biomolecule. The optimal labeling ratio can be determined based on gradient dosage testing.
Q: How to choose the ultrafiltration tube model in the labeling kit?
A: Generally speaking, it is best if the molecular weight of the biomolecule you intend to label is more than twice the molecular weight cut-off (MWCO) of the ultrafiltration tube. For example, when labeling antibodies with a molecular weight of 150 kDa, you can choose ultrafiltration tubes with a MWCO of less than 75 kDa. The smaller the MWCO, the slower the ultrafiltration process will be. If the molecular weight is too small, it is recommended to use a more precise purification method after labeling, such as HPLC purification for biomolecules with a molecular weight of 10 kDa.
Q：Low labeling efficiency.
A：There are several reasons: 1. The buffer contains trace amounts of ammonium components that react with the dye and reduce labeling efficiency. If the protein is already dissolved in an amino-containing buffer (such as Tris or amino acetic acid), dialyze with PBS before labeling. 2. Low protein content (≤1 mg/mL) will affect labeling efficiency. 3. The role of adding sodium bicarbonate in the labeling steps is to raise the pH of the reaction mixture to about 8, because the labeling reaction efficiency is highest in a weakly alkaline environment. If the buffer range of the protein solution is at a low pH, even adding sodium bicarbonate cannot adjust the pH to the optimal level. Either increase the amount of sodium bicarbonate or change the buffer to PBS, or dialyze with 0.1 M sodium bicarbonate, etc. 4. Studies have shown that raising the pH to 9.0-9.4 significantly improves labeling efficiency and speed (only 10 minutes are needed). 5. Different antibodies have different reaction rates with the fluorophore, and the degree of biological activity retained after dye labeling is also different. Therefore, standard steps do not always yield the best labeling results. To increase the labeling rate, you can re-label the same sample or reduce the amount of protein and increase the amount of dye for re-labeling. Some researchers have improved the situation by incubating at room temperature for 1 hour and then overnight at 4℃.