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

Fluor 750 Labeling Kit

Catalog No.: RE80017

Size: 0.5mg/1.25mg/2.5mg

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, which include common fluorescent dyes from ultraviolet, visible spectrum to near-infrared spectrum. They are used for labeling biomolecules, especially proteins and antibodies. Innovations in the core structure make EnkiLife dyes superior to other commercial dyes with many innovative features, mainly characterized by higher labeling efficiency and stronger luminescence.

Fluor750 is a fluorescent dye with excitation and emission wavelengths of 749nm and 775nm, respectively. It forms more specific antibody-fluorophore conjugates with antibodies, with lower background.

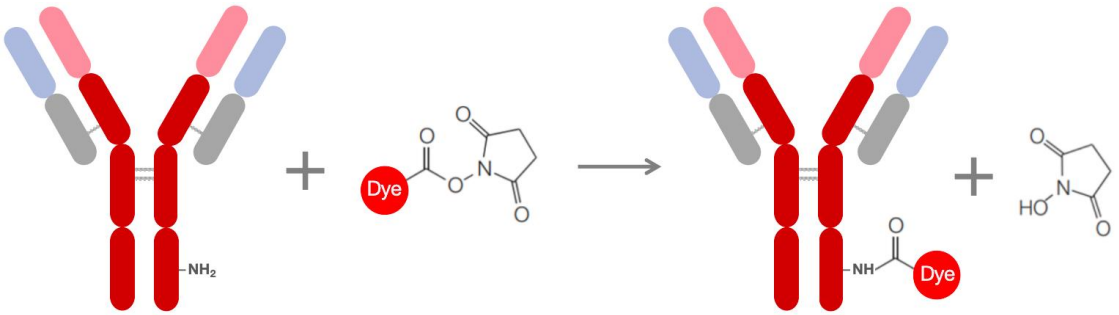
Product Features

•The kit is often used for direct labeling of primary antibodies, saving the use of secondary antibodies and their associated steps.

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

Labeling Principle

This kit mainly uses the active group of the fluorophore to covalently bind with free amino groups of biomolecules, which can be used to label antibodies and proteins.



Product Components

Component	Contents in different sizes:		
	0.5 mg	1.25 mg	2.5 mg
Activated Fluor750 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 for 6 months at -20˚C.

Calculation of Fluor750 Labeling Antibody Usage

The amount of dye used in each reaction depends on the mass, concentration, and molecular weight of the protein to be labeled. For antibody labeling with this kit, the optimal molar ratio of Fluor750 to antibody is 23:1. (The molar ratio range of Fluor750 to antibody is 8:1~23:1)

Example: To label 0.1 mg of protein (concentration approximately 2 mg/mL), using a molar ratio of Fluor750 to protein (IgG, 150 KD) of 23:1, the molar concentration of Fluor750 is 7.7 mM. The calculation method for the amount of Fluor750 to be added is as follows:

1. Calculate the amount of substance n of Fluor750:

   n_{Fluor750} =n $_{\text{protein}}$ ×23= 0.1 mg÷150000 $mg/mmol$ ×23

   =0.000015333 $mmol$

2. Calculate the volume V of Fluor750 needed:

   $V_{\text{Fluor750}} = n_{\text{Fluor750}} \div C_{\text{Fluor750}} = 0.000015333\ mmol \div 7.7\ mM = 2\ \mu\text{L}$

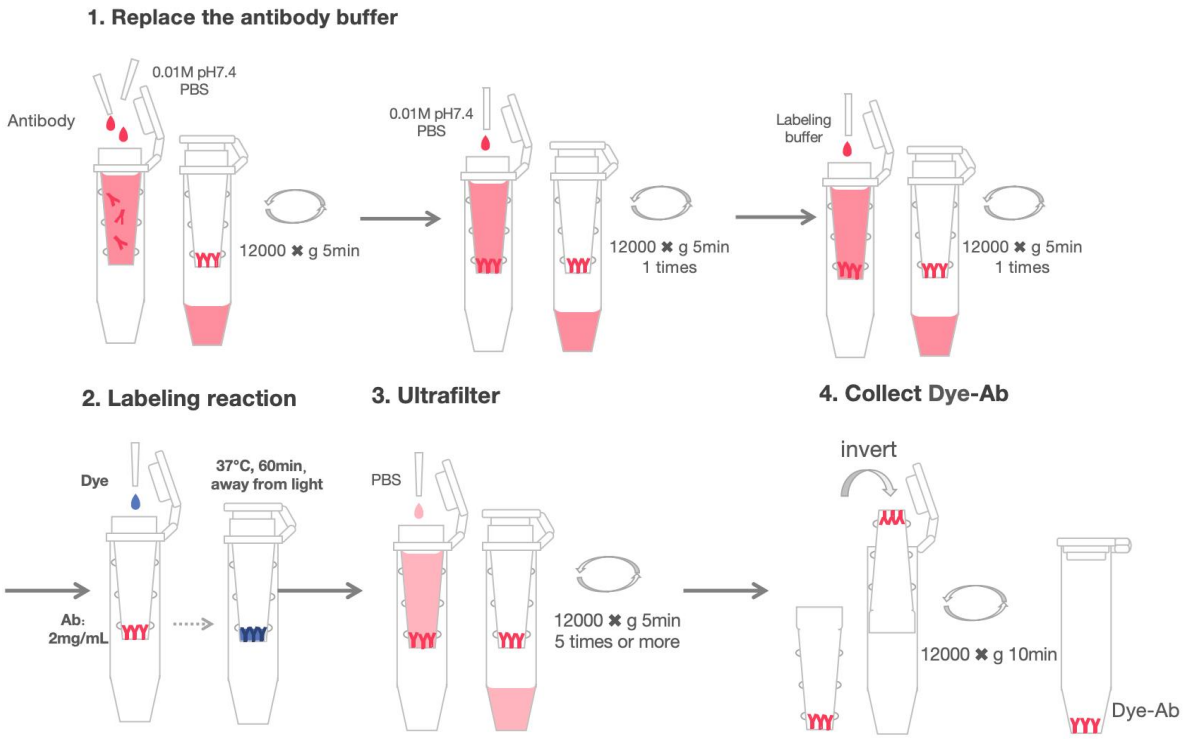
Operation Process

Preparation before the experiment

1. Read the instructions carefully.
2. Prepare reagents and consumables: Take the kit out of the refrigerator 20 minutes in advance and let it equilibrate to room temperature (note: keep the unused reagent components in the refrigerator).
3. Soak the ultrafiltration tube: Add labeling buffer to the dry ultrafiltration tube filter, let it sit at room temperature for 10 minutes, and discard the labeling buffer before adding the substance to be labeled (the ultrafiltration tube filter should remain moist throughout the labeling process).
4. Dissolve the activated Fluor750 dry powder (for example, with a product specification of 0.5 mg): Dissolve the Fluor750 dry powder with 4 μL DMSO, let it stand for 10 minutes until fully dissolved, at this point, the concentration of Fluor750 is 7.7 mM, cap the tube and set aside.

Labeling Process

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



Labeling Steps

(Taking the labeling of 100 μg antibody as an example, 2 mg/ml)

1. Replace antibody buffer: Replace the buffer of the antibody to be labeled with the labeling buffer, then add labeling buffer to adjust the antibody concentration to 2 mg/mL.
2. Labeling reaction: Take 2 μL of 7.7 mM Fluor750 solution and add it to the antibody from the above step, gently mix, seal with a cap, and react at 37℃ in the dark for 1 hour.
3. Ultrafiltration: Add an appropriate amount of PBS (about 450 μL) to the above reaction solution, gently mix, and centrifuge at 4℃ for 5 minutes at 12000 rpm.

After centrifugation, remove the tube core and discard the solution in the outer tube, reinsert the tube core, and add an appropriate amount of PBS (about 450 μL) to the tube core, centrifuge at 4℃ for 5 minutes at 12000 rpm. Repeat the ultrafiltration operation 4 times.

4. Collect the product: Gently mix the solution in the ultrafiltration tube core and blow against the inner wall of the tube core, and transfer to a clean, light-protected centrifuge tube.

Preservation of Labeled Antibodies

Volume adjustment: Adjust to the appropriate concentration according to the experimental needs, you can add an appropriate amount of BSA, glycerol, and preservatives, aliquot and store at -20˚C in the dark; you can also mix the labeling product with the preservation solution provided in the kit at a volume ratio of 1:1, aliquot and store.

Storage: Labeled products containing preservatives can be stored stably at 4˚C in the dark for 1 month; at -20˚C, they can be stored stably for 6 months.

Precautions

A. The dissolved Fluor750 is recommended to be used in one go and should not be saved for the next use.

B. Choice of ultrafiltration tube specifications: The ultrafiltration tube provided in this kit

has a default cutoff of 30k MWCO, suitable for labeling antibodies. If you need to label other molecular weight substances, it is recommended to choose the ultrafiltration tube specification according to the principle that the molecular weight of the substance to be labeled is more than twice the molecular weight cutoff of the ultrafiltration tube, and contact us before placing an order.

C. Selection of the molar ratio of Fluor750 to antibody: The recommended molar ratio of Fluor750 to antibody (23:1) in this kit is for reference only, and experimenters can explore according to actual needs, with the recommended range of the molar ratio of Fluor750 to antibody being 8:1~23:1.

D. Scope of application of the kit: This kit can also be used to label other proteins containing free amino groups, and the specific labeling ratio should be 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 the antibody to be labeled: The optimal reaction concentration for antibody labeling is 2mg/ml, if the concentration is too low, it needs to be concentrated to 2mg/ml before the experiment.

F. Requirements for the reaction buffer: The reaction environment for the substance to be labeled should meet the following requirements. If your antibody buffer meets the following requirements, you can proceed directly with the labeling; if not, please use the labeling buffer or 0.01M pH7.4 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.
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 (<1mg/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℃.