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

Fluor 350 Labeling Kit

Catalog No.: RE80009

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. This series includes common fluorescent dyes from ultraviolet, visible spectrum to 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.

Fluor 350 is a fluorescent dye with excitation and emission wavelengths of 344nm and 448nm respectively. It forms a more specific antibody-fluorescein conjugate with antibodies and has a lower background.

Product Features

●This kit is often used for direct labeling of antibodies, eliminating the use of secondary antibodies and the related operating steps.

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

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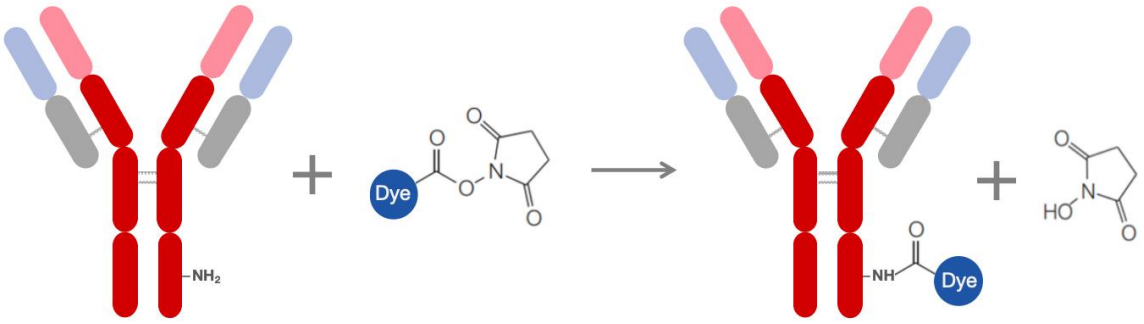
Labeling Principle

This kit mainly covalently binds the active bond of the fluorescein group 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 Fluor 350 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 Fluor 350 usage for antibody labeling

The amount of dye used in each reaction depends on the mass, concentration and molecular weight of the protein to be labeled. This kit is for antibody labeling, and the optimal molecular ratio of Fluor 350 to antibody is 23:1. (The molecular ratio of Fluor 350 to antibody ranges from 8:1 to 25:1)

Example: To label 0.1 mg of protein (concentration: about 2 mg/mL), when the molecular ratio of Fluor 350 to protein (IgG, 150 kDa) is 23:1, the molar concentration of Fluor 350 is 7.7 mM, and the calculation method for the amount of Fluor350 to be added is:

1.Calculate the required amount of substance n of Fluor 350:

$$n_{\text{Fluor350}} = n_{\text{protein}} \times 23 = 0.1\ mg \div 150000\ mg/mmol \times 23$$

$$= 0.000015333\ mmol$$

2.Calculate the required volume V of Fluor 350:

$$V_{\text{Fluor350}} = n_{\text{Fluor350}} \div C_{\text{Fluor350}} = 0.000015333\ mmol \div 7.7\ mM = 2\ \mu\text{L}$$

Operation process

Experimental preparation

1. Read the instruction manual carefully.

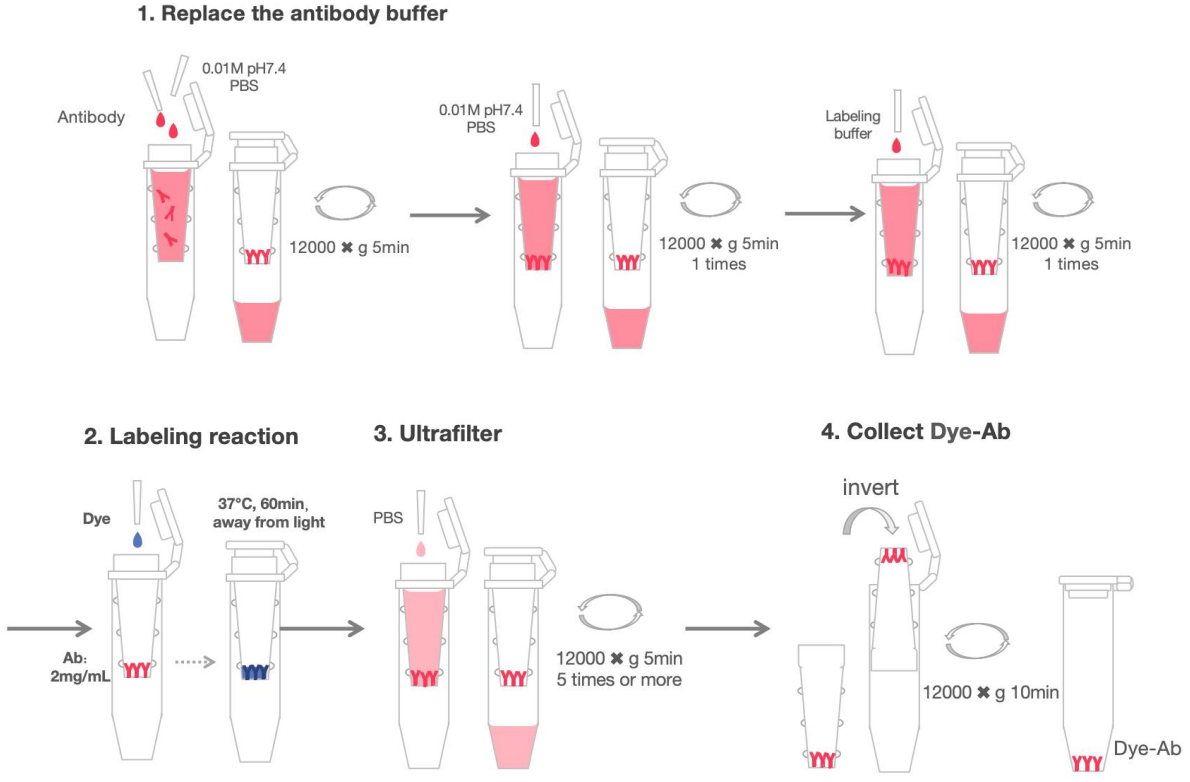
2. Prepare reagents and consumables: Take out the kit from the refrigerator 20 min in advance and equilibrate to room temperature (Note: Unnecessary reagent components should continue to be placed in the refrigerator).

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

4. Dissolve and activate Fluor 350 dry powder (taking the product specification of 0.5 mg as an example): Use 4 μL DMSO to dissolve Fluor 350 dry powder, let it stand for 10 min, and wait for it to be fully dissolved. At this time, the concentration of Fluor 350 is 7.7 mM. Cover the tube and set aside.

Labeling procedures

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

2. **Labeling reaction:** Add 2uL of 7.7 mM Fluor 350 solution 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 (about 450uL) to the above reaction solution, mix gently, and centrifuge at 4℃ 12000 x g for 5min. After centrifugation, remove the core and discard the solution in the outer tube, reinsert the core into the outer tube, add an appropriate amount of PBS (about 450uL) to the core, and centrifuge at 4℃ 12000 x g for 5min. Repeat the centrifugal ultrafiltration operation 4 times.

4. **Collect Dye-Ab:** Gently mix the solution in the ultrafiltration core and blow the inner wall of the core, and transfer it to a clean light-proof centrifuge tube.

Storage of labeled antibodies

Volume adjustment: Adjust to the appropriate concentration according to experimental needs, add appropriate amount of BSA, glycerol and preservatives, etc., and store in small portions at -20℃ away from light; you can also mix the labeling solution attached to the kit with the labeled product in a volume ratio of 1:1, and then store in small portions.

Storage: The labeled product containing preservatives can be stored stably at 4˚C away from light for 1 month; at -20℃, it can be stored stably for 6 months.

Notes

A. It is recommended to use the dissolved Fluor 350 once and not to save it for next use.

B. **Ultrafiltration tube specification selection:** The ultrafiltration tube configured in this kit has a default retention of 30k MWCO, which is suitable for labeling antibodies. If you need to label other molecular weight substances, it is recommended to select the

ultrafiltration tube specification according to the principle that the molecular weight of the substance to be labeled is more than 2 times the molecular weight of the ultrafiltration tube retention, and contact us before placing an order.

C. **Fluor 350 and antibody molecular ratio selection:** The Fluor 350 and antibody molecular ratio (23:1) recommended by this kit is for reference only. The experimenter can explore it according to actual needs. The recommended range of Fluor 350 and antibody molecular ratio is: 8:1~25:1.

D. **Scope of application of the kit:** 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.

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

F. **Reaction buffer requirements:** The reaction environment of the labeled substance should meet the following requirements. If your antibody buffer meets the following requirements, you can directly label it. If not, please use 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 components	×
Protective protein like BSA, etc	×

Frequently Asked Questions and Solutions

Q: What should I do if the concentration of the labeled molecule still does not reach 2 mg/ml after concentration and precipitation occurs after further concentration?
A: When labeling, try to reach this concentration as much as possible. If this concentration cannot be reached, appropriately increase the amount of activated fluorescent substance added. The best labeling effect can be determined by testing the gradual increase in the amount of fluorescent substance.
Q: Is the optimal molar ratio of the labeled molecule to the fluorescent dye only between 1:8 and 1:25?
A: This depends on the properties of different biological molecules, or more precisely, the number of amino groups on the surface of the biological molecules. The optimal labeling ratio can be determined based on gradient dosage tests.
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 to be labeled is more than 2 times the molecular weight cutoff of the ultrafiltration tube; for example, if the molecular weight of the labeled antibody is 150kDa, you can choose a molecular cutoff below 75kDa. The smaller the molecular weight cutoff, the slower the ultrafiltration. If the molecular weight is too small, it is recommended to use a higher precision purification method after labeling. For example, HPLC purification is recommended for a molecular weight of 10kDa.