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

# Fluor 770 Labeling Kit

Catalog No.: RE80016

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, including common fluorescent dyes from ultraviolet, visible spectrum to near-infrared spectrum, 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.

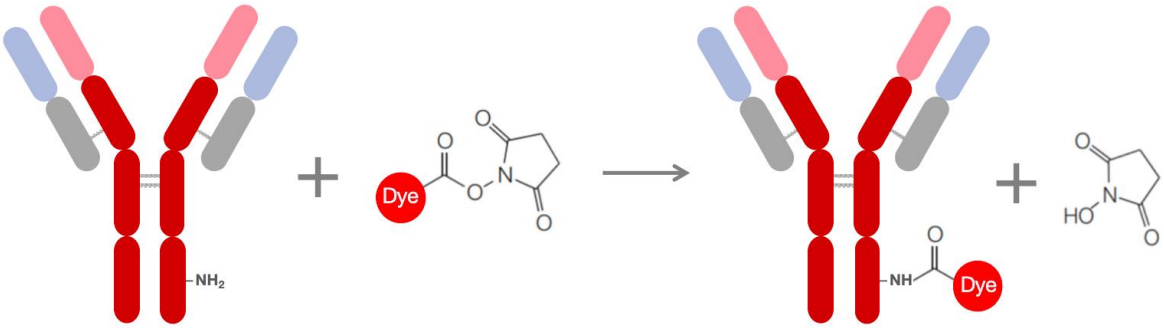
Fluor770 is a fluorescent dye with excitation and emission wavelengths of 783nm and 758nm, respectively. It forms more specific antibody-fluorophore conjugates with antibodies, with lower background.

## Product Features

- The kit is often used for direct labeling of primary antibodies, saving the use of secondary antibodies and their associated procedural 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 Fluor770 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 Fluor770 Labeled 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 Fluor770 to antibody is 23:1 (the range is 8:1 to 23:1).

Example: To label 0.1 mg of protein (concentration approximately 2 mg/mL), using a molar ratio of 23:1 for Fluor770 and protein (IgG, 150 KD), the molar concentration of Fluor770 is 7.7 mM. The calculation method for the amount of Fluor770 to be added is as follows:

1. Calculate the amount of substance n of Fluor770:

$$n_{\text{Fluor770}} = \text{n}_{\text{protein}} \times 23 = 0.1\ mg \div 150000\ mg/mmol \times 23 = 0.000015333\ mmol$$

2. Calculate the volume of Fluor770 needed (V):

$$V_{\text{Fluor770}} = n_{\text{Fluor770}} \div C_{\text{Fluor770}} = 0.000015333\ mmol \div 7.7\ mM = 2\ \mu\text{L}$$

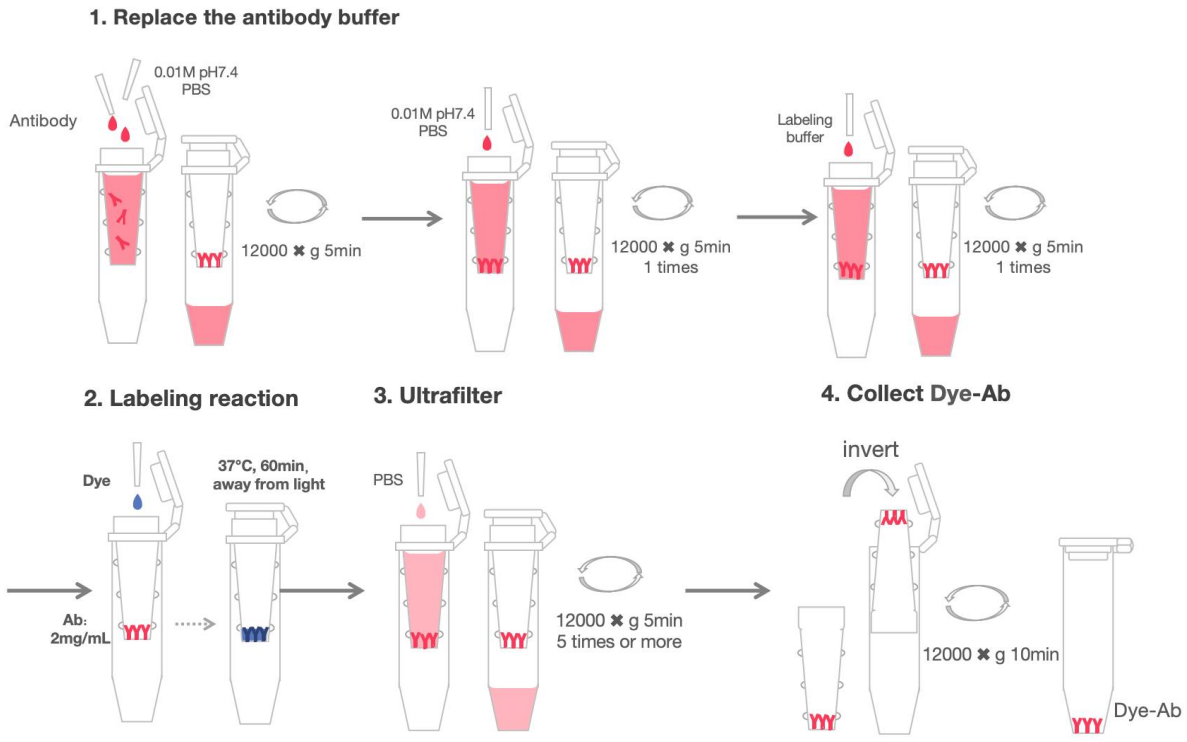
## Operation Process

### Preparation Before Experiment

1. Read the instruction manual 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 unused reagent components in the refrigerator).
3. Soak the ultrafiltration tube: Add labeling buffer to the dry ultrafiltration tube filter and let it stand at room temperature for 10 minutes, then discard the labeling buffer before adding the material to be labeled (the ultrafiltration tube filter should be kept moist throughout the labeling process).
4. Dissolve the activated Fluor770 powder (for example, with a product specification of 0.5 mg): Dissolve the Fluor770 powder with 4 μL DMSO, let it stand for 10 minutes until fully dissolved, at this time the concentration of Fluor770 is 7.7 mM, cap the tube and set aside for use.

### 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 the antibody buffer: Replace the buffer of the antibody to be labeled with the labeling buffer, then add labeling buffer to make the antibody concentration 2 mg/mL.
2. Labeling reaction: Take 2 μL of 7.7 mM Fluor770 solution and add it to the above antibody, gently mix, cover and seal, react at 37°C in the dark for 1 hour.
3. Ultrafiltration: Add an appropriate amount of PBS (about 450 μL) to the above reaction solution, gently mix, centrifuge at 4°C 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°C for 5 minutes at 12000 rpm. Repeat the ultrafiltration operation 4 times.
4. Collect Dy-Ab: Gently mix the solution inside the ultrafiltration tube and blow against the inner wall of the tube core, and transfer it to a clean, light-protected centrifuge tube.

## Storage of Labeled Antibody

- **Volume Adjustment**: Adjust to the appropriate concentration according to the experimental needs, and you can add an appropriate amount of BSA, glycerol, and preservatives, etc., to package and store at -20°C in the dark; you can also mix the labeling product with the preservation solution included in the kit at a volume ratio of 1:1, and then package 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.

## Notes

- Dissolved Fluor770 is recommended to be used in one go and should not be saved for the next use.
- Ultrafiltration tube specification selection: The ultrafiltration tube configured in this kit has a default cutoff of 30k MWCO, which is 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.

- Selection of Fluor770 to antibody molar ratio: The Fluor770 to antibody molar ratio (23:1) recommended in this kit is for reference only, and experimenters can explore according to actual needs, with the recommended range of Fluor770 to antibody molar ratio being 8:1 to 23:1.
- Scope 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.
- Requirements for the antibody to be labeled: The optimal reaction concentration for antibody labeling is 2 mg/ml. If the concentration is too low, it needs to be concentrated to 2 mg/ml before the experiment.
- 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

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|---|
| **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:**<br>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.<br>2. Low protein content (<1mg/ml) will affect labeling efficiency.<br>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.<br>4. Studies have shown that raising the pH to 9.0-9.4 significantly improves labeling efficiency and speed (only 10 minutes are needed).<br>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℃. |