

FluProLab®

## Protein-NH<sub>2</sub> Labeling Kit-Green Fluorescein Product Information

### Quick Facts

1. Storage conditions before opening : **4 °C, protected from light.**
2. Keep the unused NHS-Fluorescein in the bag, seal tightly and store at **-20 °C.**
3. The reaction temperature and time : **37 °C/10~30 mins.**
4. Measure the fluorescence intensity at **525 nm** ; Excitation at **500 nm.**

### Introduction

This assay kit uses NHS ester-activated fluorescein to label antibodies or proteins for immunostaining or cellular protein tracing. The activated fluorescein can directly form covalent bonds with target antibody proteins or other macromolecules containing primary amine groups. The kit includes dialysis tubes to remove unreacted fluorescein and other amine molecules, such as tris and glycine, which can interfere with the detection or labeling reaction. The labeling process is very easy: add NHS-Fluorescein (Reagent **A**) to the protein solution which remain above the dialysis membrane and incubate at 37°C for 10–30 minutes. Excess unreacted fluorescein molecules can be removed through the centrifugation. The excitation and emission wavelengths of the fluorescein-labeled protein are 500 nm and 525 nm, respectively. This kit contains all the necessary reagents for labeling, including a storage buffer n for storing the labeled protein.

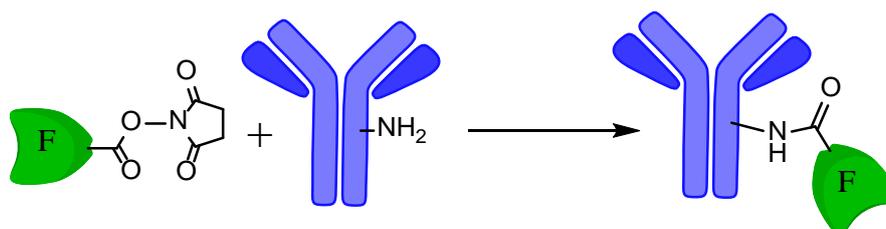


Fig. 1. The labeling reaction of NHS ester-activated fluoresceins and antibodies .

### Kit Contents, Shipping, and Storage Information

Reagents	Description	3 reactions	Shipping & Storage
<b>A</b>	NHS-Fluorescein	150 $\mu$ L <b>GREEN</b> labeled brown plastic vial X3	4 °C with protection from light.
<b>B</b>	Washing buffer	4 mL <b>RED</b> labeled plastic bottle X1	
<b>C</b>	Reaction buffer	1 mL <b>YELLOW</b> labeled plastic bottle X1	
<b>D</b>	Dried DMSO	150 $\mu$ L <b>BLUE</b> labeled plastic bottle X3	
<b>E</b>	Storage buffer	1 mL <b>PURPLE</b> labeled plastic bottle X1	
Dialysis tubes X3 pc			

※ Each reaction of this product can label proteins with a molecular weight > 50,000, with a weight of approximately 50-200  $\mu$ g.

※ Upon receiving this product, please take out the reagent **A** and store it at sealed bad and protected from light at -20 °C. The other reagents can be stored at 4 °C.

※ **Do not** use reagent **E** to dissolve the protein and calculate the Fluorescein/Protein ratio (step 2.2.5), because reagent **E** will affect the results. Please use reagent **B** to dissolve the protein, calculate the ratio, and then proceed to step 2.2.6 to preserve the protein.

## Protocol

### 1. Materials Required but not Provided :

- 1.1 10 and 200  $\mu\text{L}$  adjustable pipettes.
- 1.2 Incubators (37  $^{\circ}\text{C}$ ).
- 1.3 Microtubes.
- 1.4 Microcentrifuge.

### 2. Experimental Protocols :

#### 2.1 Precaution :

- 2.1.1 If the target protein solution contains other proteins with a molecular weight > 10,000, such as serum albumin, collagen, etc., please be sure to purify the protein solution, otherwise the interfering protein will affect the labeling reaction.
- 2.1.2 If the target protein solution contains insoluble substances, centrifuge and take the supernatant for labeling.
- 2.1.3 When this product is taken out of the refrigerator, water droplets may occasionally condense on the wall of the dialysis tube. This is a normal phenomenon and will not affect the labeling performance.

#### 2.2 General Protocol :

##### 2.2.1 Replace the buffer system of protein solution:

The buffer system used in the target protein solution may contain interfering substances such as Tris, please remove it. Add 100  $\mu\text{L}$  of wash buffer (**Reagent B**) to the dialysis tube, and add 25–100  $\mu\text{L}$  of the target protein solution (the protein concentration should < 2000  $\mu\text{g}/\text{mL}$ , so that the total protein is controlled at the range of 50–200  $\mu\text{g}$ .) Pipette several times to mix. Centrifuge at 8000  $\times g$  for 10 minutes. Add another 200  $\mu\text{L}$  of washing buffer (**Reagent B**), pipette several times to resuspend the protein. Centrifuge at 8000  $\times g$  for 10 minutes to completely remove interfering substances.

<Note> If solution remains above the dialysis membrane after centrifugation, centrifugation can be extended for another 5 minutes.

<Note> If the concentration of target protein solution is low, this step can be repeated several times to collect enough protein (50–200  $\mu\text{g}$ ).

<Note> The volume below the dialysis membrane of tube is approximately 400  $\mu\text{L}$ . Please remove the liquid just before the liquid level touches the dialysis membrane.

##### 2.2.2 Preparation of NHS-Fluorescein working solution:

Allow this kit to stand at room temperature for at least 30 minutes. Pipette 100  $\mu\text{L}$  of dried DMSO (**Reagent D**) into the NHS-Fluorescein (**Reagent A**) tube. Pipette and vortex several times until the NHS-Fluorescein is completely dissolved. NHS-Fluorescein is highly sensitive to water; please proceed with the following labeling reaction immediately. Use the overnight NHS-fluorescein/DMSO working solution may result in failure of labeling.

##### 2.2.3 Process the labeling reaction :

Add 200  $\mu\text{L}$  of reaction buffer (**Reagent C**) to the dialysis tube (step 2.2.1), pipette several times, and resuspend the protein. Add 5–20  $\mu\text{L}$  of the NHS-fluorescein/DMSO working solution (step 2.2.2), pipette several times to mix. Incubate at 37 $^{\circ}\text{C}$  for 10–30 minutes.

<Note> If the total amount of protein is  $\sim$  200  $\mu\text{g}$ , added 20  $\mu\text{L}$  of working solution. Adjust the volume of addition working solution based on the total amount of protein needed to be labeled.

##### 2.2.4 Remove unreacted fluorescein:

Add 100  $\mu\text{L}$  of washing buffer (**Reagent B**) to the dialysis tube, pipette several times to mix, and centrifuge at  $8000 \times g$  for 10 minutes. Add another 200  $\mu\text{L}$  of washing buffer (**Reagent B**), pipette several times, and resuspend the protein. This washing step can be repeated 3-4 times to ensure the removal of unreacted fluorescein.

### 2.2.5 Calculate the Fluorescein/Protein ratio :

Add 200  $\mu\text{L}$  of washing buffer (**Reagent B**), pipette more than ten times to ensure complete dissolution of the labeled protein, and transfer this solution to a microtube (not provided with the product). Measure the absorbance of this solution at 280 nm and 500 nm. When the target protein is IgG, the molar absorption coefficient is 216,000. Meanwhile, the molar absorption coefficient of fluorescein is 60,000. Calculate the Fluorescein/Protein ratio with the following equation:

$$\text{Ratio(Fluorescein molecules per protein molecule)} = \frac{A_{500}/60,000}{(A_{280} - A_{500} \times 0.22)/\epsilon \text{ of protein}}$$

$A_{500}$  : absorbance at 500 nm.

$A_{280}$  : absorbance at 280 nm.

$\epsilon$  : molar absorption coefficient of protein at 280 nm.

<Note> Because the storage buffer (**Reagent E**) contains antibacterial agent, it can affect absorbance measurements and lead to incorrect calculation. **DO NOT** use the storage buffer (**Reagent E**) to recover the labeled protein in this step.

### 2.2.6 Recovery of labeled proteins:

After centrifugation( $8000 \times g$  for 10 minutes), above the dialysis membrane, there is no liquid remain, add 200  $\mu\text{L}$  of storage buffer (**Reagent E**), and pipette more than ten times to ensure that the labeled protein is completely resuspension. Transfer this solution to a microtube (not provided).

<Note> The storage buffer (**Reagent E**) provided in this kit contains a high concentration of glycerol (without  $\text{NaN}_3$ ) and can preserve proteins for 1 year at  $-20^\circ\text{C}$ . It can be stored for even longer at  $-80^\circ\text{C}$ .

<Note> You can use the protein preservative methods used in your laboratory, or aliquot the labeled protein to avoid repeated freeze-thaw cycles.

---

聚創新材料股份有限公司

IMT FORMOSA New Materials Co., Ltd.

電話 Phone: 0926-159317

電郵 Email: [polycreatives@outlook.com](mailto:polycreatives@outlook.com)

聯絡人 Contact: Peter Hsu

