Cat. No. 311

FluProLab®

Protein-NH2 Labeling Kit-Fluorescein
Product Information

Quick Facts

1. Storage conditions before opening： **4 ℃, protected from light.**
2. Keep the unused NHS-Fluorescein in the bag, seal tightly and store at **-20 ℃**.
3. The reaction temperature and time：**37 ℃/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 potentially interfering small molecules, such as Tris buffer and other amine compounds, which can interfere with the detection or labeling reaction. The labeling process is very simple: 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 preservation solution for storing the labeled protein.

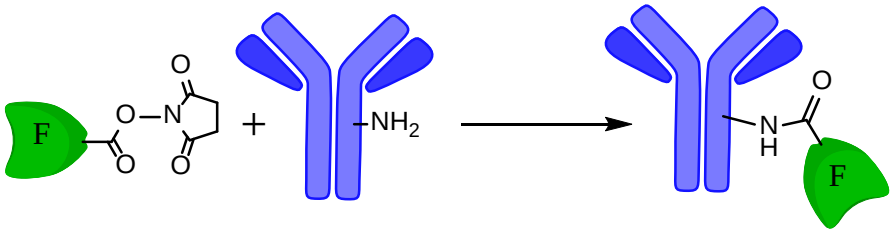

Fig. 1. The labeling reaction of NHS ester-activated fluoresceins and antibodies .

Kit Contents, Shipping, and Storage Information

Reagents	Description	3 reactions	Shipping & Storage
A	NHS-Fluorescein	150 μL **GREEN** labeled brown plastic vial X3	4 °C with protection from light.
B	Washing buffer	4 mL **RED** labeled plastic bottle X1	
C	Reaction buffer	1 mL **Yellow** labeled plastic bottle X1	
D	Dried DMSO	150 μL **BLUE** labeled plastic bottle X3	
E	Storage buffer	1 mL **PURPLE** 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 μg**.

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

※ 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 μL adjustable pipettes.
1.2 Incubators (37 ℃).
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 μL of wash buffer (**Reagent B**) to the dialysis tube, and add 25–100 μL of the target protein solution (the protein concentration should < 2000 μg/mL, so that the total protein is controlled at the range of 50–200 μg.) Pipette several times to mix. Centrifuge at 8000 x *g* for 10 minutes. Add another 100 μL of washing buffer (**Reagent B**), pipette several times to resuspend the protein. Centrifuge at 8000 x *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 μg).

<Note>The volumn below the dialysis membran of tube is approximately 300 μL. Please remove the liquid just before the liquid level touches the dialysis membran.

2.2.2 Preparation of NHS-Fluorescein working solution:

Allow this kit to stand at room temperature for at least 30 minutes. Pipette 100 μL 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 100 μL of reaction buffer (**Reagent C**) to the dialysis tube (step **2.2.1)**, pipette several times, and resuspend the protein. Add **5-20** μL of the NHS-fluorescein/DMSO working solution (step **2.2.2**), pipette several times to mix. Incubate at 37°C for 10–30 minutes.

<Note> If the total amount of protein is ~ 200 μg, added 20 μL 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 µL of washing buffer (**Reagent B**) to the dialysis tube, pipette several times to mix, and centrifuge at 8000 x *g* for 10 minutes. Add another 200 µL 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 µL 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:

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

A_{500}：absorbance at 500 nm.

A_{280}：absorbance at 500 nm.

ε ：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 x *g* for 10 minutes), above the dialysis membrane, there is no liquid remain, add 200 µL of storage buffer (**Reagent E**), and pipette more than ten times to ensure that the labeled protein is completely dissolved. Transfer this solution to a microtube (not provided with the product).

<Note> The storage buffer (**Reagent E**) provided in this kit contains a high concentration of glycerol (NaN_3-free) and can preserve proteins for 1 year at -20°C. It can be stored for even longer at -80°C.

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

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

聯絡人 Contact: Peter Hsu