

PhoProLab®

Protein-NH₂ Labeling Kit-Orange Eu Product Information

Quick Facts

1. Storage conditions before opening : **4 °C, protected from light.**
2. Keep the unused activated Eu complex in the bag, seal tightly and store at **-20 °C.**
3. The reaction temperature and time : **37 °C/ 2 hours.**
4. Measure the phosphorescence intensity at **620 nm** ; Excitation at **335 nm.**

Introduction

This assay kit uses activated Europium (Eu) complexes to label antibodies or proteins for immunostaining or cellular protein tracing. The activated Eu complexes can directly form covalent bonds with target antibody proteins or other macromolecules containing primary amine groups. The kit includes dialysis tubes to remove unreacted complexes and other small amine compounds, such as tris and glycine, which can interfere with the detection or labeling reaction. The labeling process is very easy: add activated Eu complexes (Reagent A) to the protein solution which remain above the dialysis membrane and incubate at 37 °C for 2 hours. Excess unreacted Eu complexes can be removed through the centrifugation. The maximum excitation and emission wavelengths of the labeled protein are 335 nm and 620 nm, respectively. The large Stokes shift of 285 nm, with no overlap between the absorption and emission spectra, resulting in good phosphorescent stability. Under continuous excitation at 335 nm for one hour, the emission intensity remains almost constant. Furthermore, the Eu complexes lifetime of phosphorescent photon is 1.0 ms, allowing for effective differentiation of endogenous fluorescent signals from the sample by using time-resolution spectroscopic equipment. This kit contains all the necessary reagents for labeling, including a storage buffer for storing the labeled protein.

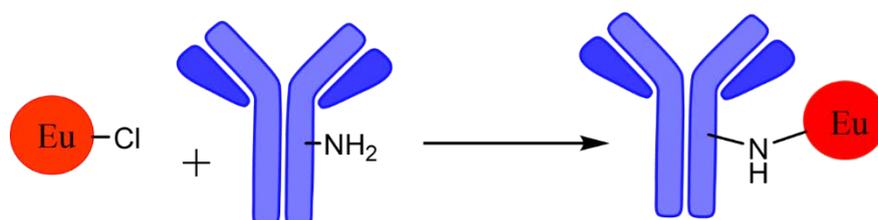


Fig. 1. The labeling reaction of activated Eu complexes and antibodies .

Kit Contents, Shipping, and Storage Information

Reagents	Description	3 reactions	Shipping & Storage
A	Eu complexes	1.5 mL GREEN labeled plastic vial X3	4 °C with protection from light.
B	Washing buffer	5 mL RED labeled plastic bottle X1	
C	Reaction buffer	5 mL YELLOW labeled plastic bottle X1	
D	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 °C. The other reagents can be stored at 4 °C.
- ※ Do not use reagent D to resuspend the protein and calculate the Eu complex/Protein ratio

(step 2.2.5). Please use reagent **B** to resuspend the protein, calculate the ratio, and then process step 2.2.6 to preserve the labeled protein with reagent **D**.

Protocol

1. Materials Required but not Provided :

- 1.1 200 μ L adjustable pipettes.
- 1.2 Incubators (37 °C).
- 1.3 Microtubes.
- 1.4 Microcentrifuge.(8000 x *g*)

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 solution used in the target protein may contain interfering substances such as tris; please remove it. Add 100 μ L of wash buffer (**Reagent B**) to the dialysis tube, and add 25–100 μ L of the target protein solution (the protein concentration should be < 2000 μ g/mL, so that the total protein is controlled at the range of 50–200 μ g.) Pipette several times to mix. Centrifuge at 8000 x *g* for 10 minutes. Add another 200 μ L 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>When using a centrifuge, please remember to keep the rotor balanced to ensure stable operation. If solution still remains above the dialysis membrane, the centrifugation can be extended for another 5 minutes.

<Note>If the concentration of target protein solution is low, the centrifugation step can be repeated several times to collect enough protein (50-200 μ g).

<Note>The volume below the dialysis membrane of tube is approximately 400 μ L. Please remove the liquid before the liquid level touches the dialysis membrane.

2.2.2 Preparation of activated Eu complexes working solution:

Allow this kit to stand at room temperature for at least 30 minutes. Pipette 1 mL of Reaction buffer (**Reagent C**) into the Eu complexes (**Reagent A**) tube. Pipette and vortex several times until the Eu complexes is completely dissolved. Eu complexes is highly sensitive to water; please proceed with the following labeling reaction immediately. Use the overnight working solution may result in failure of labeling.

2.2.3 Process the labeling reaction :

Add 200 μ L working solution of step 2.2.2 to the dialysis tube (step 2.2.1), pipette several times to mix and resuspend the protein. Incubate at 37°C for 2 hours.

<Note> If the total amount of protein is ~ 200 μ g, added 200 μ L of working solution. Adjust the volume of addition working solution based on the total amount of protein, and maintain the total solution at 200 μ L by addition Reaction buffer (**Reagent C**). For

example, if you use 50 μL of working solution, you need to add 150 μL of reaction buffer (**Reagent C**).

2.2.4 Remove unreacted Eu complexes:

Add 100 μL 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 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 Eu complexes.

2.2.5 Calculate the Eu complexes/Protein ratio :

Add 200 μL of washing buffer (**Reagent B**), pipette more than ten times to ensure complete resuspension of the labeled protein, and transfer this solution to a microtube (not provided). Measure the absorbance of this solution at 280 nm and 335 nm. When the target protein is IgG, the molar absorption coefficient is 216,000. Meanwhile, the molar absorption coefficient of Eu complexes is 31,000. Calculate the Eu complexes /Protein ratio with the following equation:

$$\text{Ratio(Eu complex molecules per protein molecule)} = \frac{A_{335}/31,000}{(A_{280} - A_{335} \times 0.725)/\epsilon \text{ of protein}}$$

A_{335} : absorbance at 335 nm.

A_{280} : absorbance at 280 nm.

ϵ : molar absorption coefficient of protein at 280 nm.

<Note> **DO NOT** use the storage buffer (**Reagent D**) to resuspend the labeled protein in this step.

2.2.6 Recovery of labeled proteins:

After centrifugation ($8000 \times g$ for 10 minutes), there is no liquid remain above the dialysis membrane, add 200 μL of storage buffer (**Reagent D**), 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 D**) provided in this kit contains a high concentration of glycerol (without NaN_3) 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 used in your laboratory, or aliquot the labeled protein to avoid repeated freeze-thaw cycles.

Spectra :

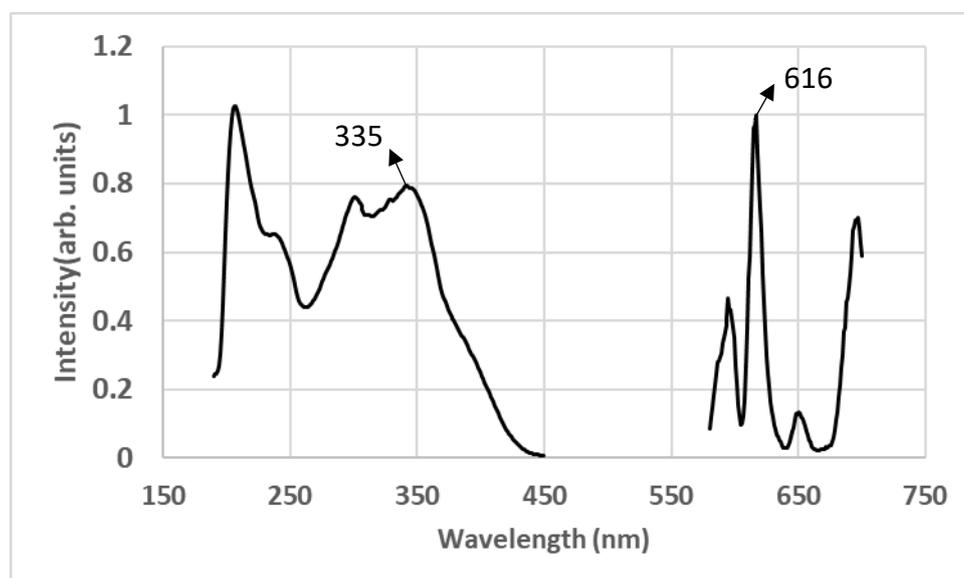


Fig. 2. UV-Vis and emission spectra of Eu complexes.

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