KAMIYA BIOMEDICAL COMPANY

Fluorescein Labeling Kit - NH_2

For the preparation of fluorescein-labeled proteins for immunostaining and cellular proteins for tracing.

Cat. No. KT-223

For Research Use Only.

PRODUCT INFORMATION

Fluorescein Labeling Kit – NH_2
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PRODUCT

Fluorescein Labeling Kit-NH_2 is mainly used for the preparation of fluorescein-labeled proteins, such as IgG, for immunostaining and cellular proteins for tracing. NH_2-reactive fluorescein, a component of this kit, has a succinimidyl group (NHS) that reacts with an amino group of proteins or other molecules. This kit contains all of the necessary reagents for labeling. Each tube of NH_2-reactive fluorescein can label up to 200 µg of IgG, conjugating about 4 to 6 fluorescein molecules per IgG molecule. The labeling process is simple. Add the NH_2-reactive fluorescein to IgG solution on a filter membrane and incubate at 37°C for 10 minutes. The excess fluorescein molecules can be removed by a filtration tube. The excitation and emission wavelengths of the fluorescein-labeled IgG are 495 nm and 520 nm, respectively. This kit is for research use only.

COMPONENTS

- NH_2-reactive fluorescein 3 tubes
- Reaction Buffer 500 µL
- WS Buffer 4 mL
- Filtration Tubes 3 tubes

Materials or equipment required but not provided

- 0.5 mL microtubes
- 10 µL and 200 µL adjustable pipettes
- Microcentrifuge
- 37°C Incubator
- DMSO or ethanol

SAMPLE REQUIREMENT

For the labeling of 3 samples: Molecular weight >50,000, as IgG < 200 µg

PROCEDURE

Labeling of Sample

1. Add 100 µL of WS Buffer and the sample solution containing 100 µg of IgG to a Filtration Tube.
2. Centrifuge at 8,000-10,000 g for 10 minutes.
3. Add 10 µL DMSO to NH_2-reactive fluorescein and dissolve by pipetting.
4. Add 100 µL Reaction Buffer and 8 µL NH2-reactive fluorescein solution to the Filtration Tube and pipette to mix.
5. Incubate the tube at 37°C for 10 minutes.
6. Add 100 µL WS Buffer to the Filtration Tube and centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate.
7. Add 200 µL WS Buffer to the Filtration Tube and centrifuge at 8,000-10,000 g for 10 min. Repeat this step.
8. Add 200 µL WS Buffer and pipette 10 to 15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube, and store at 4°C.

Precautions

IgG or fluorescein-conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains other proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to fluorescein labeling with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

a) The volume of IgG solution should be less than 100 µL. If the antibody concentration is less than 1 mg/mL, repeat steps 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µL or more during the accumulation process, discard the filtrate prior to going to the next centrifuge step.

b) If solution still remains on the filter after the centrifugation, spin for another 5 minutes or increase the centrifuge speed.

c) NH_2-reactive fluorescein is on the bottom of the tube. Add 10 μL DMSO to the bottom of the tube, and pipette several times to dissolve. If DMSO is not available, you may use ethanol.
d) If the amount of IgG is 200 μg, add the entire NH_2-reactive fluorescein solution.
e) You do not have to use WS Buffer to recover fluorescein-conjugated IgG. You can choose any kind of buffers appropriate for your experiment.

STORAGE

Store all components at 4°C. Stable at 4°C with protection from moisture until kit expiration date.

Determination of Fluorescein / IgG Ratio

Measure the absorbance of the fluorescein-labeled IgG solution at 280 nm and 500 nm. Calculate the ratio using the following equation:

$$\text{Ratio (fluorescein molecules per IgG molecule)} = \frac{3 \times A_{500}}{A_{280} - 0.2 \times A_{500}}$$

A_{500}: absorbance at 500 nm
A_{280}: absorbance at 280 nm

FAQ

Q. Can I use this kit for other proteins?
A. Yes, if the molecular weight is greater than 50,000.

Q. Do I have to use the filtration tube prior to labeling the protein?
A. If the protein solution does not contain small molecules with an amino group and the concentration of the protein is 10 mg/mL, or about 70 μM, there is no need to use the Filtration tube. Mix 10 μL of the sample solution with 90 μL of Reaction buffer and add 8 μL NH2-reactive fluorescein (prepared at Step 3) to the mixture and follow the protocol starting at Step 4.

Q. How long is the fluorescein-labeled protein stable?
A. If you store at 4°C, it is stable for over 2 months. For longer storage, add 100% volume of glycerol, aliquot, and store at -20°C. However, please note that stability depends on the protein itself.

Q. What is the minimal amount of IgG that can be labeled with this kit?
A. The minimal amount is 10 μg; simply follow the protocol. The labeling ratio remains the same for 10 μg to 100 μg of IgG.

Q. Can I use this kit to label oligonucleotides or peptides?
A. No. Oligonucleotides and peptides may be too small to retain on the membrane filter of the filtration tube.

FOR RESEARCH USE ONLY

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