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

Catalog No: E-LK-F003

Product size: 3 Reactions

Elabscience® FITC Labeling Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Introduction

The FITC Labeling Kit of Elabscience offering a collection of reagents required for FITC labeling is designed to label antibody with amidogen (NH2-). The specially treated FITC in this kit is sufficient and has been activated for direct use. The reagents are enough for approximately 3 labeling reactions each containing 0.1-2 mg of antibody or other protein. Each kit includes 6 Filtration tubes for desaltination of antibody labeling without the need for dialysis. The whole procedure is simple and can be completed in 100 min with proficient operation.

Product Features

- ✓ **All-inclusive:** This kit provides all the reagents required for FITC labeling.
- ✓ **Quick:** The whole procedure takes only 100 min.
- ✓ **Convenient:** Desalination can be achieved with Filtration tube, dialysis or gel filtration is not necessary.
- ✓ **Flexible:** The procedure can be easily adapted to both micro and large scales, with 0.1-2mg of protein labeled each time.
- ✓ **Perfect results:** This kit has been optimized to determine the optimum labeling ratio of FITC to antibody, lowering the possibility of protein inactivation resulted from insufficient labeling or excess FITC labeling.

Product component and storage

The kit is stable for 1 year at 2-8 ℃ before opening.
The dissolved Reactive FITC can be stored at 2-8 ℃ for 1 week.

Component	3 Reactions
FITC	0.3 mg ×3 vials
Labeling Buffer	10 mL ×1 vial
DMF	500 μL ×1 vial
Filtration tube	0.5 mL ×6

Materials required but not included in this kit

1. Adjustable high-precision transferpettor (10μL, 50μL, 200μL, 1000μL)
2. Incubator(37 ℃)
3. Centrifuge(the centrifugal force can be up to 12,000×g)

Principle of the assay

The FITC reacts with the primary amine (N-terminal and the side chain of lysine residue) specifically, stable isothiourea bonds are formed.

Calculation on the amount of FITC

The volume of FITC used in every reaction depends on the amount and concentration of the protein to be labeled. With optimization, we determine that the optimum molar ratio of the FITC to protein is 20:1 when labeling 2 mg/mL of protein sample (IgG, 150KD).

1. Calculate the millimole of FITC to make the ratio of FITC to antibody is 20:1 when labeling 2 mg/mL antibody:

$$\text{mL protein} \times \frac{2\text{mg protein}}{\text{mL protein}} \times \frac{1\text{mmol IgG}}{150{,}000\text{ mg IgG}} \times \frac{20\text{ mmol FITC}}{\text{mmol protein}} = \text{ mmol FITC}$$

2. Calculate the microliters of 10mM FITC to add to the reaction:

$$\text{mmol FITC} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{10\text{ mmol}} = \ \mu\text{L FITC}$$

Example: About 13.3 μL of 10mM FITC solution is to be added for 0.5 mL of 2mg/mL IgG(150,000 MW) solution.

$$0.5\text{mL IgG} \times \frac{2\text{mg IgG}}{1\text{mL IgG}} \times \frac{1\text{mmol IgG}}{150{,}000\text{mg IgG}} \times \frac{20\text{mmol FITC}}{1\text{mmol IgG}} = 0.000133\text{mmol FITC}$$

$$0.000133\text{mmol FITC} \times \frac{1{,}000{,}000\mu\text{L}}{\text{L}} \times \frac{\text{L}}{10\text{mmol}} = 13.3\mu\text{L FITC Solution}$$

Preparation before experiment:

1. Read the manual carefully.
2. Calculate the volume of FITC to be added.
3. Bring the kit to room temperature 20min before experiment (**Note**: the unused FITC should be stored in the refrigerator).

Assay procedure (we label 1mg of protein in this assay)

1. Add 1mg of protein sample and corresponded volume of Labeling Buffer to a Filtration tube to make the volume is 0.5mL. Centrifuge at 12,000×g for 10min. **Note**: ①the maximum volume of Filtration is 0.5mL. ②the protein sample can be treated with centrifugal ultrafiltration first when at low concentration.
2. Dissolve FITC: add 30μL of DMF to the vial of FITC, let it stand for 10min until it dissolved fully. The concentration of FITC is 10mM.
3. Add 13.3μL of FITC and appropriate volume of Labeling Buffer to the Filtration tube, making the final concentration of the protein solution is 2mg/mL. Mix it thoroughly with a pipette and incubate the tube for 30min at 37℃.
4. Centrifuge at 12,000×g for 10min.
5. Add appropriate volume of Labeling Buffer to the Filtration tube to make the total volume is 0.5mL. Mix it thoroughly with a pipette and centrifuge at 12,000×g for 10min. Repeat this step once again.
6. Add 0.2mL of Labeling Buffer to the Filtration tube and mix it thoroughly with a pipette. Invert the filtration tube and put it into another centrifugal tube. Centrifuge at 6,000×g for 10min.
7. Collect the solution in the centrifugal tube, namely antibody labeled by FITC.

Precautions

1. This kit can be also used to label antigen, HRP and polypeptides with amidogen (NH_2-). The labeling ratio depends on the amount of amidogen.
2. DMF should be preserved airtight in a dry place. Seal it with the parafilm immediately after use.
3. The Filtration tube provided in the kit has a molecular weight cutoff(MWCO) of 10KD. So please be careful of the molecular weight of the antigen or polypeptide to be labeled.
4. In the Step 6 above, Labeling Buffer is used to collect the labeled protein. You can also use other buffer or protective agents as you like.
5. In the Step 3 above, for other quality antibodies, the final concentration of antibody should be controlled to 2 mg/ml strictly, then calculate the volume of FITC required according to the quantity of the antibodies.
6. This kit can be stored for 1 year before opening. Please use it within the expiration date.