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

FITC Labeling Kit

Catalog No.: RE80001

Size: 20mg/40mg/60mg

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email（Sale） order@enkilife.com

✉Email（Techsupport） techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Product Introduction

This FITC labeling kit provides all the reagents required for FITC labeling, which can simply and effectively label proteins containing primary amino (-NH2) molecules.

Product Features

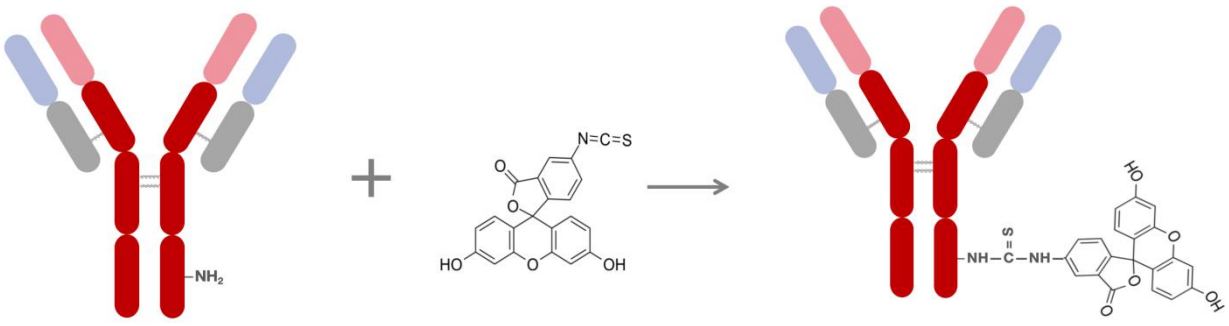
●Convenience: Desalting and removing free FITC by centrifugation, no dialysis or gel filtration required.
●Accuracy: This kit is equipped with an indicator to ensure complete removal of free FITC.

Basic information

Structural formula	
Molecular weight	389
Excitation/Absorption maximum (nm)	494
Emission maximum (nm)	518
Molar extinction coefficient ε (L· mol−1· cm−1)	68000
Correction factor at 280 nm(CF280)	0.3

Labeling Principle

Within a certain pH range, FITC reacts specifically with primary amino groups (N-terminus and lysine residue side chains) to form stable thiourea bonds, thereby achieving coupling with proteins,antibodies and peptides or other macromolecules.



Components

Components	Content in different sizes		
Active FITC	2 mg	4 mg	6 mg
Ultrafiltration Tube	1 vial		
DMSO	1.5 mL	3 mL	4.5 mL
Labeling Buffer	30 mL	60 mL	90 mL
Removal Reagent	1 mL	2 ml	3 ml
Storage Buffer	2 mL	4 mL	10 mL
Recommended Labeled Antibody Amount	0.2-20mg	1.0-40mg	5.0-60mg

Storage

The unopened kit can be stored at 2-8°C for one year, and the dissolved FITC can be stored at -20°C or -80°C for one week.

Calculation of FITC usage for antibody labeling

The amount of FITC reagent used in each reaction depends on the number and total amount of amino groups in the protein to be labeled. For example, our experimental data analysis shows that when labeling 2mg/ml antibody (IgG, 150kDa), adding 100μL of FITC (2mg/mL) per milliliter of antibody solution (2mg/mL) can achieve the best labeling effect; the labeling of other proteins can be based on actual conditions and refer to this ratio.

Operation process

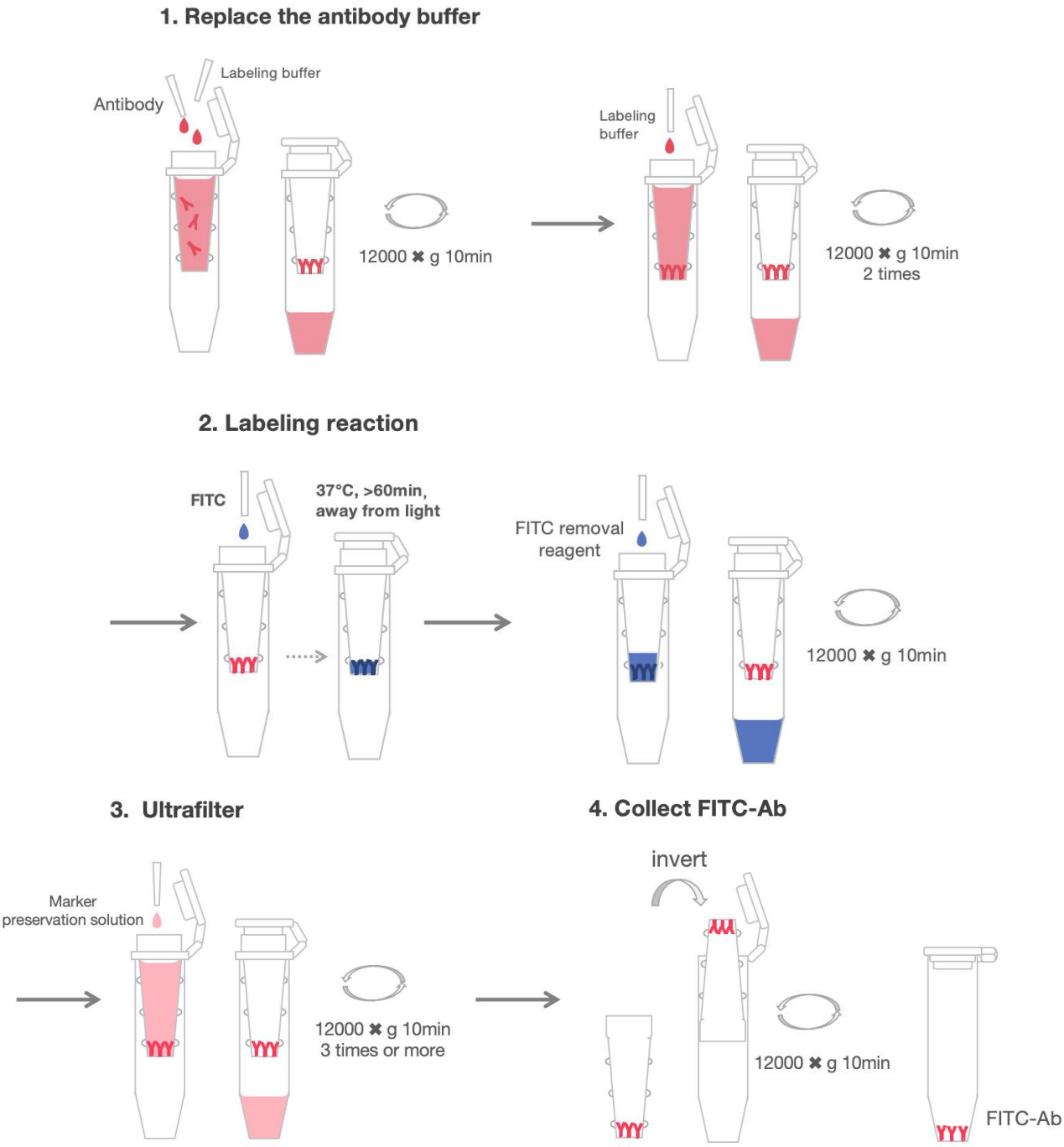
· Experimental preparation

1.Read the instruction manual carefully.
2.Take out the kit from the refrigerator 20 min in advance and equilibrate to room temperature.
3.Dissolve FITC with DMSO which needs to be melted at room temperature before use.

Tips:

A.It is best to use up the dissolved FITC at one time. If it is not used up, it can be sealed and placed in a -20℃ refrigerator. It can be used within a week, but the labeling efficiency will be reduced;
B.After using the FITC co-solvent, it needs to be sealed and stored immediately to prevent moisture absorption.

· Labeling procedures



· Labeling steps

(Taking the labeling of 1mg of antibody as an example)

1. Take 1 mg of the antibody to be labeled in an ultrafiltration tube, add a labeling buffer that does not exceed the maximum volume of the ultrafiltration tube, and centrifuge at 12,000 x g for 10 minutes; this step can be repeated multiple times; after the last

ultrafiltration is completed, an appropriate amount of labeling buffer can be added to adjust the antibody concentration to about 2 mg/mL.

Tips:

A. The maximum volume and maximum molecular weight cutoff of the ultrafiltration tube. The maximum volume of the ultrafiltration tube in this example is 0.5 mL.

B. If the concentration of the antibody to be labeled is low, ultrafiltration centrifugation can be performed first.

C. If the labeled substance contains free amino groups (Tris, amino acids or other interfering substances, it is necessary to use labeling buffer to repeatedly ultrafilter to ensure that it is completely removed).

2. Add 50 μL of dissolved FITC to the above ultrafiltration tube and gently blow to mix. Place in a 37°C thermostat and incubate in the dark for 60 minutes.

3. After labeling, add 1/10 volume of FITC removal indicator to the above ultrafiltration tube.

4. Centrifuge at 12,000 x g for 10min.

5. Add an appropriate amount of marker preservation solution to the above ultrafiltration tube, gently blow and mix, and centrifuge at 12,000 x g for 10min. Repeat the operation several times until there is no blue in the ultrafiltration tube and the unlabeled FITC is completely removed; collect the solution in the ultrafiltration tube, which is the FITC-labeled antibody.

Calculation of label coupling ratio

$$F/P = \frac{MW}{389} \times \frac{A495/195}{[A280-(0.35\times A495)]/E0.1\%280} = \frac{A495\times C}{A280-(0.35\times A495)}$$

$$C = \frac{MW\times E0.1\%280}{389\times 195}$$

C is a constant for a certain protein.
MW is the molecular weight of the protein.
389 is the molecular weight of FITC.
195 is the absorbance of 1 mg/ml of coupled FITC at 495 nm (pH 13.0).
(0.35 x A495) is the correction factor for the absorbance of FITC at 280 nm.
E0.1% is the absorbance of 1.0 mg/ml of protein at 280 nm.

6. Add an appropriate amount of **Storage Buffer** to the FITC-labeled antibody , gently blow and mix,and stored at -20°C.

Tips:If the coupling ratio of the FITC-labeled antibody is to be calculated, it needs to be done before adding the Storage Buffer.

Notes

1. Before purchasing, customers need to select the most suitable model based on the molecular weight and labeling amount of the substance to be labeled.

Maximum labeling volume	Minimum molecular weight of labeling substance	The amount of antibody that can be labeled
0.5mL	3kDa	0.2 - 20 mg
4.0mL	3kDa	1.0 - 40 mg
15mL	3kDa	5.0 - 60 mg
0.5mL	10kDa	0.2 - 20 mg
4.0mL	10kDa	1.0 - 40 mg
15mL	10kDa	5.0 - 60 mg
0.5mL	30kDa	0.2 - 20 mg
4.0mL	30kDa	1.0 - 40 mg
15mL	30kDa	5.0 - 60 mg
0.5mL	50kDa	0.2 - 20 mg
4.0mL	50kDa	1.0 - 40 mg
15mL	50kDa	5.0 - 60 mg
0.5mL	100kDa	0.2 - 20 mg
4.0mL	100kDa	1.0 - 40 mg
15mL	100kDa	5.0 - 60 mg

1. This kit is suitable for labeling all macromolecular substances containing ε-amino groups (NH2-) (proteins, antibodies, and other compounds containing primary amino groups (NH2-)). The specific labeling ratio is determined according to the number of amino groups in the labeled substance.

2. The FITC cosolvent in this kit is DMSO. After use, it should be sealed and dried for storage.

3. This kit is equipped with a label preservation solution. The experimenter can also

choose a more suitable preservation solution according to the characteristics of the labeled substance.

4. The validity period of this kit before opening is 18 months. Please use it within the validity period.

5. The ultrafiltration tubes provided in this series of kits are divided into different molecular weight cutoffs and different maximum volumes. Customers need to choose the appropriate model according to the molecular weight and labeling amount of the substance they want to mark.