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

Elabscience® FITC Labeling Kit

Catalog No: E-LK-F003B

Product size: 1 Reaction/3 Reactions/10 Reactions

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.

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Web: 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.

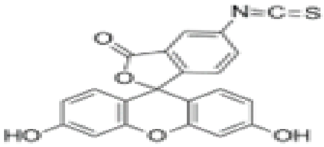
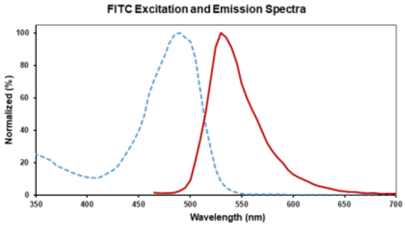
Introduction

Elabscience® FITC Labeling Kit provides all the reagents required for labeling, which can label proteins containing primary amino-group (-NH_2) molecules simply and effectively.

Characteristic

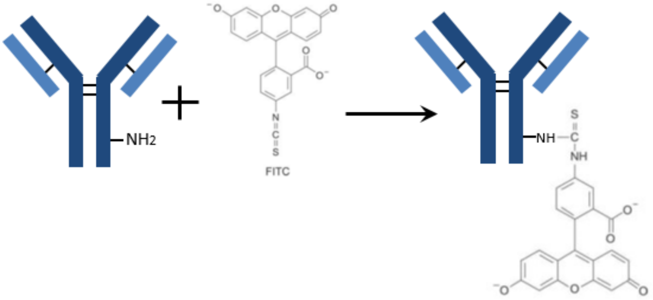
- ✓ **Fast**: The whole process takes only 90 min.
- ✓ **Convenient**: The dye has been activated and can be used directly. Filtration tube desalts without dialysis.
- ✓ **Flexible use**: It can be used for both micro-labeling and large-scale labeling, and can label 0.1-1 mg proteins each time.

Essential 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 coefficient of 280nm(CF280)	0.3
Dye spectrum	

Labeling Principle

Within a certain pH range, FITC specifically reacts with primary amino groups (N-terminal and lysine residue side chains) to form a stable thiourea bond, so as to realize the coupling of FITC with protein.

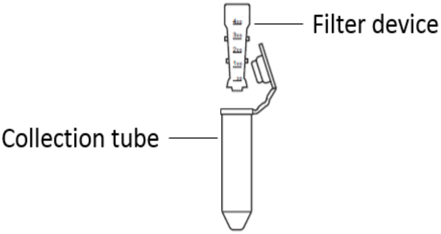


Components

Cat.	Products	1 Reaction	3 Reactions	10 Reactions	Storage
E-LK-F03L	FITC	0.3 mg ×1	0.3 mg ×3	0.3 mg ×10	-20°C, shading light
E-LK-012	Labeling Buffer III	10 mL	20 mL	20 mL ×2	2~8°C
E-LK-006	DMF	500 μL	500 μL	500 μL	2~8°C, shading light
E-LK-007	1 ×PBS(pH7.4)	10 mL	10 mL	10 mL ×2	2~8°C
E-LK-001B	10KD Filtration tube*	1 set**	3 set	10 set	RT

*The filtration tube is purchased from Millipore. Please refer to the appendix III for usage.

**1 set 10 KD Filtration tube (0.5 mL) consisted of one filter device and two collection tubes.



Storage

The unopened kit can be stored at 2~8°C for 1 year, and the dissolved FITC can be stored at -20°C or -80°C for 1 week.

Materials Not Supplied

1. Pipettor and tips (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL).
2. Ultraviolet spectrophotometer or nanodrop or multifunctional microplate reader.
3. 37°C incubator.
4. Centrifuge (centrifugal force up to 12,000 ×g).

Calculation of the usage amount of FITC:

The amount of FITC used in each reaction depends on the mass, concentration and molecular weight of the protein to be labeled. For the protein of 30 KD~100 KD, the recommended molecular ratio of FITC and protein using this kit is 10 : 1 ~ 35 : 1. The molecular ratio can be adjusted according to the molecular weight, or determined through experimental exploration.

Example: Label 1 mg protein (concentration about 2 mg/mL), when the molecular ratio of FITC and protein (50KD) is 17.1 : 1, the molar concentration of FITC is 25.7 mM (refer to the preparation of FITC), the calculation of the amount of FITC to be added is below:

1. Calculate the amount of substance required of FITC:

$$n_{\mathrm{FITC}} = \mathrm{n}_{\mathrm{protein}} \times 17.1 = \frac{1\,mg}{50000 mg/mmol} \times 17.1 = 0.000342 mmol$$

2. Calculate the required volume of FITC:

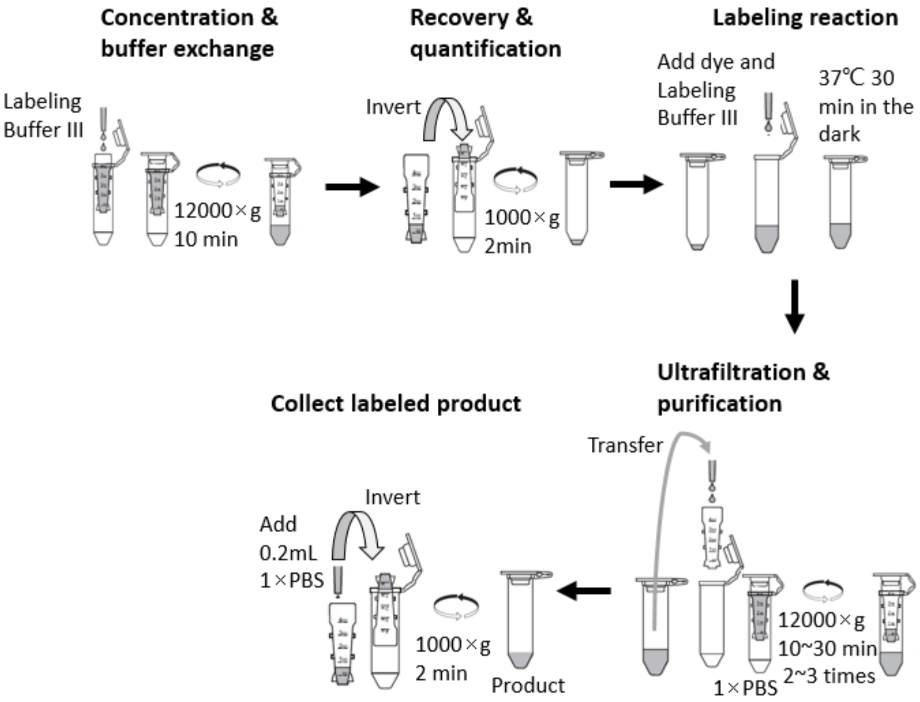
$$V_{\mathrm{FITC}} = \frac{n_{\mathrm{FITC}}}{c_{\mathrm{FITC}}} = \frac{0.000342\mathrm{mmol}}{25.7\,mM} = 13.3\ \mu L$$

Experimental Operation

■ Experiment preparation

1. Read the instructions carefully.
2. Bring all reagents to room temperature for 20 min before use (note: the reagent components are temporarily unused are still in the refrigerator).
3. Infiltration of ultrafiltration tube: Add 500 μL of Labeling Buffer III into the dry filter device, stand at room temperature for 10 min, and discard Labeling Buffer III before adding the reagent to be labeled (the filter device should remain moist throughout the labeling process).
4. Preparation of FITC: Dissolve 0.3 mg of FITC with 30 μL of DMF, and stand for 10 min until it is fully dissolved. At this time, the concentration of FITC is 25.7 mM, and cover the tube for later use.

■ Labeling process



■ Labeling procedure (This procedure is used to label 1 mg protein)

1. **Concentration & buffer exchange:** Put the filter device in the collection tube, add 1 mg of protein to be labeled into the filter device, add Labeling Buffer III to the final volume of 0.5 mL, cover the filter tube, centrifuge at 12,000 ×g for 10 min, and discard the liquid in the collection tube.

   Note:

   a) The maximum volume of the filter device is 0.5 mL.
   b) If the volume of 1 mg protein is greater than 0.5 mL, please add it in several times and concentrate it by centrifugation and ultrafiltration.
   c) If the protein to be labeled contains free amino groups (Tris, amino acids or other interferents, repeat ultrafiltration with Labeling Buffer III to ensure that it is removed fully).
2. **Recovery & quantification:** Invert the filter device into the collection tube, centrifuge at 1000 ×g for 2 min, collect the protein in the collection tube, take out the filter device, add an appropriate amount of Labeling Buffer III into the collection tube, make sure that the protein concentration is about 2 mg/mL. At the same time, add 0.5 mL Labeling Buffer III into the filter device and put it on a pipe rack for later use.
3. **Labeling reaction:** Immediately add 13.3 μL of 25.7 mM FITC to the protein solution, gently blow and mix fully, sealed with a lid, and incubate at 37 ℃ for 30 min in the dark.
4. **Ultrafiltration & purification:** Add an appropriate amount of 1 ×PBS into the above reaction solution to the final volume of 0.5 mL, gently mix and transfer the reaction solution to the filter device, make sure that the Labeling Buffer III in the filter device in step 2 should be discard (if the above reaction solution exceeds 0.5 mL, it can be transferred to the spin-dried filter device for several times after ultrafiltration), and cover the cap after matching with the collection tube, and centrifuge for 10~30 min at the speed of 12,000 ×g. Discard the liquid in the collection tube,

replenish 1×PBS to 500 μL in the filter device, and repeat the centrifugal ultrafiltration operation for 2~3 times until the color of the ultrafiltrate in the collection tube is almost colorless and transparent.

5. **Collect labeled product:** Add 0.2 mL 1×PBS into the filter device and pipet gently. Invert the filter device in another collection tube and centrifuge at 1000×g for 2 min. Collect the solution in the collection tube, which is the protein labeled by FITC.

■ (Optional) Determining the degree of labeling

1. Use an absorbing light scanning device to set the scanning range of 230 nm~800 nm.
2. Set 1×PBS to as blank control.
3. Take 2 μL FITC labeled sample, scan the absorption spectrum (230 nm to 800 nm) and record A280 and A494 data (1 cm optical path).

   Note: In this case, it is necessary to scan the absorption value in the range of 230nm~800nm, rather than measuring the value at A280 and A494. By observing the absorption spectrum curve, some abnormal values can be eliminated, such as the measurement error caused by bubbles in the sample.
4. The DOS and protein concentration can be calculated based on the molar extinction coefficient of FITC dye, the A280 correction value, the molar extinction coefficient of the protein, the molecular weight of the protein, etc. The calculation formula is as follows:

   $\text{DOS} = (A_{494} \times \varepsilon_{IgG})/(\varepsilon_{FITC} \times (A_{280} - CF_{280} \times A_{494}))$

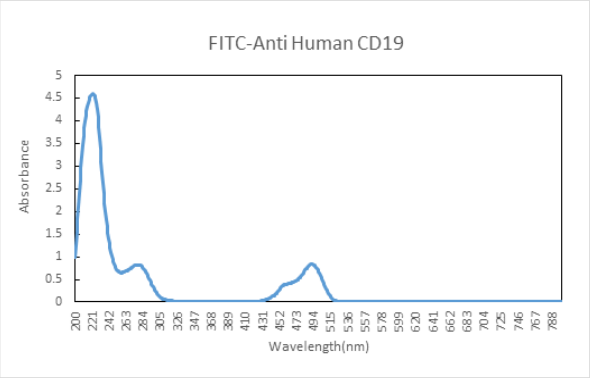
   $\text{Protein concentration (mg/mL)} = (A_{280} - CF_{280} \times A_{494}) \times 150000/\varepsilon_{IgG}$

Parameter	Implication	Value
DOS	The average number of fluorescein labeled per protein.	
A_{494}	The OD value of FITC dye at 494 nm wavelength with 1 cm optical path.	Refer to measurements
ε_{IgG}	The molar extinction coefficient of IgG ($L \cdot mol^{-1} \cdot cm^{-1}$).	210000
ε_{FITC}	The molar extinction coefficient of FITC ($L \cdot mol^{-1} \cdot cm^{-1}$).	68000
A_{280}	The OD value of FITC labeled protein at 280 nm wavelength with 1 cm optical path.	Refer to measurements
CF_{280}	The correction coefficient of OD value of FITC dye at 280nm wavelength	0.3

■ The storage and use of protein

Add 0.05~0.2% Proclin 300 or 0.05% sodium azide and stabilizer protein (such as 0.1% BSA) to the labeled protein, the protein can be stored at 2~8°C in the dark for 6 months. Or add the same volume of glycerol, the protein can be stored at -20°C for 6 months.

Typical Results



Notes

1. Please select the appropriate kit according to the molecular weight of the protein to be labeled. The kit provides a 10 KD Filtration tube.
2. FITC is susceptible to moisture hydrolysis failure, and should be stored at -20 ℃ or -80 ℃ with the desiccant. In order to prevent water vapor from condensing into the dye, it is necessary to equilibrate the dye to room before the experiment.
3. The kit can also be used to label other proteins containing free amino groups. The specific labeling ratio is determined according to the number of available amino groups in the marker or set different molar ratios for labeling.

Related Products

Cat.No	Product
E-LK-R002	BSA Removal Kit

Troubleshooting

Symptoms	Causes	Comments
Low DOS value	The initial concentration of protein is inaccurate.	Quantify the protein according to the standard operating procedure, and refer to the instructions of the instrument manufacturer, or use other protein quantification methods such as BCA method.
	Initial protein contain a large number of interfering components for labeling, such as glycine, imidazole, Tris, sodium azide, thiomersal, proclin, etc.	Use dialysis, desalination or multiple ultrafiltration methods to fully remove the interfering components. In special cases, it may be necessary to quantify the interference components contained in the sample.
	The initial protein contains carrier proteins such as BSA, gelatin.	Use affinity purification or other chromatographic methods to remove the interfering components, or use the BSA removal kit, and then accurately quantify the protein concentration before labeling.
	The value measured is not accurate.	Quantify the measured values according to the standard operation methods.
	During labeling, DMF in the reaction system is excess, which interferes with labeling reaction.	Minimize the amount of DMF to dissolve dyes.
The proteins are not labeled with dye at all.	Improper operation, such as incomplete mixing of dye and protein, excessive ammonium ion or amino component in protein, or other improper operation.	Set a positive control.

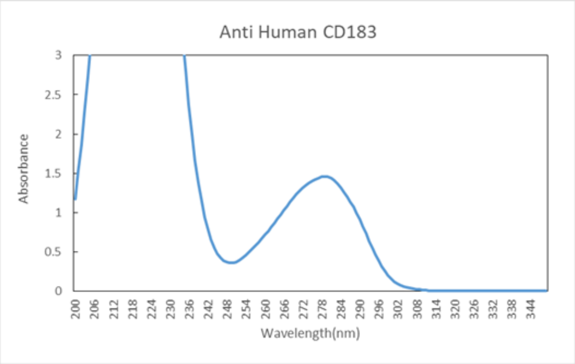
The proteins are not labeled with dye at all.	Improper preservation of dye.	Before labeling, the dye should not be mixed with water. When taking the dye, it should be kept at room temperature for about 5~10 min before unsealing.
	Improper use of ultrafiltration tube.	After ultrafiltration, there is less liquid in the filter device. Do not use the pipette to directly absorb the sample in the filter device. The sample should be centrifuged in an inverted state.
	Due to the difference of centrifuges, the rotation speed is too high during ultrafiltration.	Centrifuge speed is 12,000×g, not 12,000rpm
	Leakage of ultrafiltration tube.	Overload of filter to cause leakage.
Excessive DOS value (such as greater than 10)	The actual concentration of protein is low, such as protein precipitation, uneven concentration, resulting in excessive modification of dye.	Accurately measure the concentration according to the standard operation steps of the instrument and equipment after protein mixed fully.
	Excessive lysine residues of protein.	Reduce the amount of dye added or set different molar ratios for labeling.
	Inappropriate spectral measurements.	Refer to the instrument detection sensitivity, it is generally necessary to ensure that the theoretical concentration of protein is above 0.5 mg/mL after labeling. Measure the range of absorption spectrum, not the maximum absorption value, Determine the accuracy of the measured value based on the absorption spectrum curve, especially the baseline level of absorption spectrum curve.

Low recovery of protein	Protein aggregated and precipitated during the labeling process.	Ammonium chloride (self-prepared) was added to a final concentration of 50 mM to terminate the reaction in time.
	Excessive protein concentration during ultrafiltration.	Do not load too much protein in filter device, such as more than 1 mg of protein.
	The protein cannot be completely dissolved in the labeling buffer.	Choose other labeling kits.

Declaration

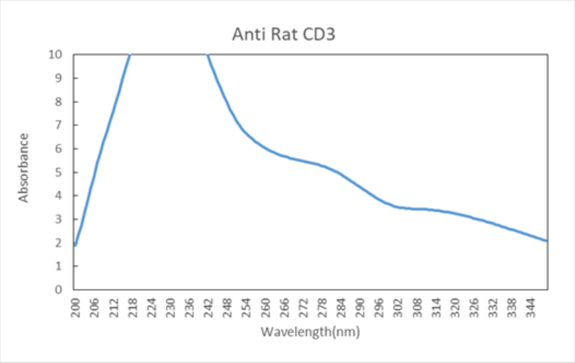
1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. The labeling kit can achieve good results for some common proteins such as IgG, Annexin V, protein A/G, 2019-nCov spike, NGAL, and some recombinant protein fragments, but there are many changes in different proteins, such as the solubility of the protein in different buffers, pH stability, temperature stability, protein purity, and the accessibility of the labeling site. Changes are more complex and there is a risk of labeling failure. Therefore, for the labeling of unknown proteins, it is recommended to use a small amount of labeling kit to test the feasibility of labeling.
4. Due to the failure of labeling, the protein is completely unavailable, this labeling kit does not carry full responsibility. We believe that our customers should be aware that the use of this labeling kit may impair the biological function of the protein in some cases.

Appendix Ⅰ: Normal absorbance curve of protein concentration (for reference only)



Description: 1 mg/mL Mouse anti human CD183, the protein type was Mouse IgG1, PBS (pH 7.2, no preservatives), measured by the Nano 100 spectrophotometer, the concentration curve was normal, and A280=1.454, in line with the labeled concentration.

Appendix Ⅱ: Abnormal absorbance curve of protein concentration (for reference only)



Description: 0.5 mg/mL Mouse anti rat CD3, protein type Mouse IgG3, containing protein stabilizer and sodium azide (≤ 0.09%), measured by Nano-100 spectrophotometer, the concentration curve was abnormal, A280=5.195, which does not meet the labeled concentration.

AppendixⅢ: Protein retention and concentrate recovery (from Millipore product manual)

(Cite from the User Guide of Millipore: https://www.emdmillipore.com/US/en/product/Amicon-Ultra-0.5-Centrifugal-Filter-Unit,MM_NF-UFC500324#documentation)

For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. Merck Millipore Ltd. (Millipore) recommends using a membrane with a NMWL at least two times smaller than the molecular weight of the protein solute that one intends to concentrate. Refer to the table below.

Marker/Concentration	Molecular Weight	Device NMWL	% Retention	Spin Time (min)
α-Chymotrypsinogen (1 mg/mL)	25,000	3K	＞95	30
Cytochrome C (0.25 mg/mL)	12,400		＞95	30
Vitamin B-12 (0.2 mg/mL)	1,350		＞42	30
α-Chymotrypsinogen (1 mg/mL)	25,000	10K	＞95	15
Cytochrome C (0.25 mg/mL)	12,400		＞95	15
Vitamin B-12 (0.2 mg/mL)	1,350		＞23	15
BSA (1 mg/mL)	67,000	30K	＞95	10
Ovalbumin (1 mg/mL)	45,000		＞95	10
Cytochrome C (0.25 mg/mL)	12,400		＜35	10
BSA (1 mg/mL)	67,000	50K	＞95	10
Ovalbumin (1 mg/mL)	45,000		~40	10
Cytochrome C (0.25 mg/mL)	12,400		＜20	10
Thyroglobulin (0.5 mg/mL)	677,000	100K	＞95	10
IgG (1 mg/mL)	156,000		＞95	10
Ovalbumin (1 mg/mL)	45,000		＜30	10

Spin Conditions: 40° fixed angle rotor, 14,000×g, room temperature, 500 µL starting volume, n=12.