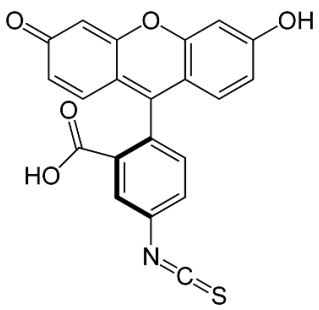
LinKine™ FITC Labeling Kit

Cat #: KTL0210 Size: 3×100 μg/1 mg

	FITC Labeling Kit		
REF	**Cat #:** KTL0210	LOT	**Lot #:** Refer to product label
	Applicable samples: labeling biomolecules, especially proteins and other ligands that contain a free amino group		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle



Fluorescein isothiocyanate (FITC) is a derivative of fluorescein used in wide-ranging applications including flow cytometry. FITC is the original fluorescein molecule functionalized with an isothiocyanate reactive group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive towards nucleophiles including amine and sulfhydryl groups on proteins. FITC is the most commonly used fluorescent dye for the preparation of green fluorescent conjugates with the λEx/λEm of 492 /520 nm.

LinKine™ FITC Labeling Kit is designed for preparing FITC conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The FITC provided in our kit has can be directly used for conjugation. LinKine™ FITC Labeling Kit contains ready-to-use components to prepare FITC labeled molecules.

Application example: The coupled direct-labeled primary antibody does not require a secondary antibody in the immunoassay, thus eliminating tedious incubation and washing steps, saving time.

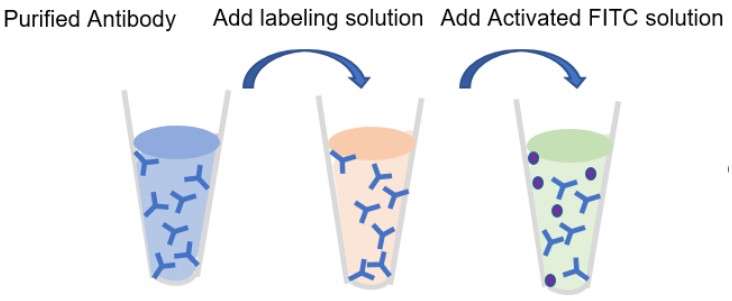


Figure 1: Schematic diagram of LinKine™ FITC Labeling Kit

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	3×100 μg	1 mg	
Activated FITC Solution	7.5 μL	25 μL	-20℃, protected from light

FITC Labeling Solution	15 μL	50 μL	-20℃
Purification Column (0.5 mL, 50 KD)	3	2	RT
Booklet	1	1	RT

Note: The purified column is suitable for samples with molecular weights ranging from 100 KD to 200 KD; If there are unused reagents left, please seal them and store them at -20℃ in time.

Materials Required but Not Supplied

- Prepared samples to be labelled
- Precision pipettes, disposable pipette tips
- Deionized water, PBS (pH 7.4)

Sample Preparation

The initial concentration of the sample to be labeled should be higher than 2 mg/mL. It is suggested that the protein concentration is above 2.5 mg/mL. Otherwise, increasing protein concentration is required before the experiment. The components (or storage buffer) of the sample to be labeled should meet the following requirements:

(1) Do not contain amino components, otherwise it will affect the coupling effect.

(2) A small amount of BSA will not affect the coupling effect. it is suggested to use PBS as storage buffer.

(3) If the sample contains substances that may interfere with the labeling, it is suggested to replace the buffer with PBS. The specific method is as below.

Add the sample to the ultrafiltration tube and add 200-150 μL PBS. Centrifuge at 12,000 g, 4℃, 10 min, and discard the filtrate. Then PBS was added again, and centrifuge at 12,000 g, 4℃, 10 min. After centrifugation, the inner core of the ultrafiltration tube is taken out, placed it in a clean outer tube, and centrifuge at 4,000 g, 4℃, 2 min to collect the sample.

List of components (or storage buffer) requirements of the sample to be labeled:

pH	6.5-8.0
Amine Free Buffer	MES, PBS, HEPES
Chelating Agents (e. g. EDTA)	✓
Glycerol	< 50%
BSA	< 0.1%
Glycine	✗
Components Containing Amino	✗

Assay Procedure

Note: Please enlarge or reduce the volume of each component of the kit in the reaction system according to the actual sample volume.

1. Setting up your conjugation reactions

The recommended optimal labeling sample size and labeling system for different specifications are as follows:

Size	Amount of Sample	Optimal Labeling System
3×100 μg	3×100 μg	3×50-3×75 μL
1 mg	1 mg	500-750 μL

The following operation steps are based on the 3×100 μg specification. For other specifications, please adjust the dosage

accordingly. If you need to adjust the volume, please keep the sample size unchanged and change the volume of FITC labeling solution, Activated FITC solution and deionized water in the labeling system.

(1) Add 15 μL FITC labeling solution to the sample solution to be labeled and mix gently with a pipette.

(2) Pipette 7.5 μL of Activated FITC solution to the reaction solution in step (1), add deionized water to 150 μL (Note: this volume is the labeled system), mix gently, and let stand at 37℃ in the dark for 1 hr.

Note: Step (1)/(2) belong to the labeling steps.

(3) Add an appropriate amount of PBS (fixed volume to about 500 μL) to the reaction solution in step (2), mix gently, and move the solution to the purification column. Centrifuge at 12,000 g at 4℃ for 10 min.

(4) Discard the filtrate and add an appropriate amount of PBS (fixed volume to about 500 μL) to the purification column. Centrifuge at 12,000 g for 10 min at 4℃.

(5) Take out the purification column, put it upside down in a clean centrifuge tube, centrifuge at 4,000 g for 2 min at 4℃. The solution collected in the centrifuge tube is the coupling product.

Note: Step (3)/(4)/(5) are the purification steps.

2. Storage of conjugates

The conjugate can be stable at 4℃ in the dark for more than one month. For long-term preservation, the conjugate should be packed in small aliquots, placed at -20℃ in the dark and added the same volume of glycerol. Avoid repeated freezing and thawing.

Data Analysis

1. Calculation of Conjugates Concentration:

$C(mg/mL) = \{[A_{280}-(A_{max}\times Cf)]/1.4\}\times$dilution factor

Where: C: The concentration of conjugates collected in the experiment; Dilution factor: Dilution factor in photometric measurement (see “Note” as below for details); A_{280} and A_{max}: The absorbance at 280 nm and the absorbance at the maximum absorption wavelength (the maximum absorption wavelength of FITC is 492 nm); Cf: The correction factor. The Cf value of FITC is 0.254; 1.4: The extinction coefficient of IgG (mL/mg). The extinction coefficient of FITC is 7300.

2. Calculation of Degree of Labeling (DOL) of Conjugates:

$DOL=(A_{max}\times Mwt\times$dilution factor$)/(\varepsilon\times C)$

Where: A_{max}: The absorbance at the maximum absorption wavelength (the maximum absorption wavelength of FITC is 492 nm); Dilution factor: Dilution factor in photometric measurement; C: The concentration of conjugates collected in the experiment; Mwt: The molecular weight of the sample to be labeled; ε: The extinction coefficient of fluorescent dyes. The extinction coefficient of FITC is 7300.

Note: The conjugate eluted by the column may be too concentrated for absorbance detection directly, so it needs to be diluted to appropriate concentration (Within the concentration detected by protein analyzer). The dilution multiple (i.e., dilution factor) needs to be estimated from the initial sample mass and the total volume of eluted conjugate. If undiluted, the dilution factor is 1.

FAQ

Q1: After concentration, the sample concentration is still below 2 mg/mL?

A1: If the sample concentration is still less than 2 mg/mL after concentration, the volume of the labeling system can be adjusted appropriately, but the final concentration should be greater than 1 mg/mL. The volume of the FITC labeling solution in step (1) should be 10% of the volume of the labeling system. In step (2), deionized water may not be added and the volume of the Activated FITC solution remains unchanged. You can also make appropriate adjustments based on your own experiments.

Q2: How to select the appropriate purification column for different molecular weight of the sample to be labeled?

A2: The purified column is suitable for samples with molecular weights ranging from 100 KD to 200 KD. If the samples with molecular weights higher than 200 KD or less than 100 KD, it should better be equipped with more suitable size of the purified column.

Q3: Is the molecular weight of the sample to be labeled similar to FITC?

A3: If the molecular weight of the sample is similar to FITC, after completing the labeling step corresponding to this instruction, add 30 μL 0.5 μM NH_4Cl and incubate at 37℃ for 10 min to quench the free FITC without the need for purification steps.

Precautions

1. Different batch number, different manufacturers of components do not mix; Otherwise, the result may be abnormal.
2. When mixing or reconstitution, avoid bubbles. If there are unused reagents, please seal and store at -20℃ in time.
3. Frequently change the pipette tip to avoid cross-contamination between components.
4. Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

Recommended Products

Catalog No.	Product Name
KTL0100	LinKine™ HRP Labeling Kit
KTL0110	LinKine™ AP Labeling Kit
KTL0520	LinKine™ AbFluor™ 488 Labeling Kit
KTL0540	LinKine™ AbFluor™ 594 Labeling Kit
KTL0120	LinKine™ Biotin Labeling Kit
KTL0530	LinKine™ AbFluor™ 555 Labeling Kit
KTL0560	LinKine™ AbFluor™ 647 Labeling Kit
KTL0580	LinKine™ AbFluor™ 680 Labeling Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.