ALK03-EN.01

FITC Antibody Labeling Kit

【Catalog】ALK-A003

【Size】100 μg; 500 μg; 1 mg

Please read this manual carefully before performing the experiment.

For research use only, not for use in diagnostic or therapeutic procedures.

ALK03-EN.01

Catalog

【Intended Use】

FITC Antibody Labeling Kit provides a rapid and simple method for achieving covalently coupling of FITC and antibody molecules. The conjugated antibody can be used straight away without further purification step and antibody loss.

【Catalog Number and Specification】

Table 1. Catalog number and Specification

Catalog Number	Specification
ALK-A003-100 μg	100 μg antibody labeling
ALK-A003-500 μg	500 μg antibody labeling
ALK-A003-1 mg	1 mg antibody labeling

【Principle】

FITC is a green fluorescent labeling dye which has an excitation wavelength of 488 nm and an emission wavelength of 535 nm.

The pre-activated FITC dye can be covalently conjugated with the primary amino groups on the antibody surface to form a stable FITC-antibody conjugate.

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【Materials Provided】

Table 2. Materials provided

Catalog Number	ID	Components	Size (1 kit)	Format	Storage
ALK-A003-100 μg	ALK03-C01A	Activated FITC	1 vial	powder	-20℃
	ALK03-C02A	Quencher reagent			
ALK-A003-500 μg	ALK03-C01B	Activated FITC	1 vial	powder	-20℃
	ALK03-C02B	Quencher reagent			
ALK-A003-1 mg	ALK03-C01C	Activated FITC	1 vial	powder	-20℃
	ALK03-C02C	Quencher reagent			

【Storage】

The kit is stored at -20℃, the validity period is shown on the label of the outer box, please use within the validity period.

【Unsupplied Reagents or Equipment】

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

Thermostatic shaking incubator;

Vortex mixer;

Tubes: 1.5mL, 10mL;

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Timer;

Deionized or distilled water.

【Precautions】

1. For research use only, not for use in diagnostic procedures;
2. Please use the kit within the shelf life;
3. Components of different kits and different batches of kits should not be mixed;
4. This kit is used for the labeling of purified antibodies. Antibodies in ascites, serum, hybridoma or tissue culture may affect the labeling result;
5. This kit can be used to achieve fluorescence labeling for protein molecules which contain primary amino groups, but labeling conditions and efficiency depend on the characteristics of the protein.

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【Conjugation Protocol】

1. Preparation of antibody solution

Please select the appropriate specification of the kit based on the amount of the antibody to be labeled.

Prepare the antibody at 2 mg/mL by 1×PBS (pH 7.2-7.4) buffer.

Keep the antibody solution away from Tris, glycine, BSA, gelatin or other primary amino groups substances.

Table 3. Buffer composition requirements

Buffer composition	Yes or No
buffer without primary amino groups	Yes
pH 7.2-8.5	Yes
trehalose	⩽10%
ammonium salt ($(NH_4)_2SO_4$, CH_3COONH_4)	No
glutathione	No
Tris buffer	No
sodium azide	No [1]
glycerinum	No [1]
BSA or gelatin	No [1]
Note: [1] *These compositions may reduce labeling efficiency.*	

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2. Components Preparation

2.1 Take out each component and equilibrate to room temperature (23℃).

Note: *If the powder of the ALK03-C01 and ALK03-C02 tubes were attached to the side of the tube, please centrifuge them at 1000 g for 1 minute to collect the powder to the bottom.*

2.2 Add deionized water to dissolve component ALK03-C02, store at 4 ℃ away from light.

2.3 After the component is dissolved, it should be used within the same day.

3. Antibody Conjugation

3.1 Add the prepared antibody solution into the ALK03-C02 tube, and mix them well by repeatedly pipetting up and down or vortex the vial for a few seconds.

3.2 Shake the reaction mixture at 100 rpm at room temperature for 15-30 minutes, avoid from light.

4. Reaction Quenching

4.1 Add dissolved ALK03-C02 to the reaction mixture according to Table 4, and mix them well.

4.2 Keep the reaction mixture at room temperature for 30 minutes, avoid from light.

Table 4. Component adding volume

ID	Component	Adding volume		
		100 μg Kit	500 μg Kit	1 mg Kit
ALK03-C02	Quencher reagent	5 μL	25 μL	50 μL
Note: *Please mix the solution well before pipetting.*				

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【Concentration Verification】

The concentration of FITC-antibody conjugate is calculated according to the following formula:

$$\text{Conjugate concentration}\left(\frac{\mu g}{\mu L}\right) = \frac{\text{antibody amount } (\mu g)}{\text{antibody solution volume } (\mu L) + C02 \text{ solution volume } (\mu L)}$$

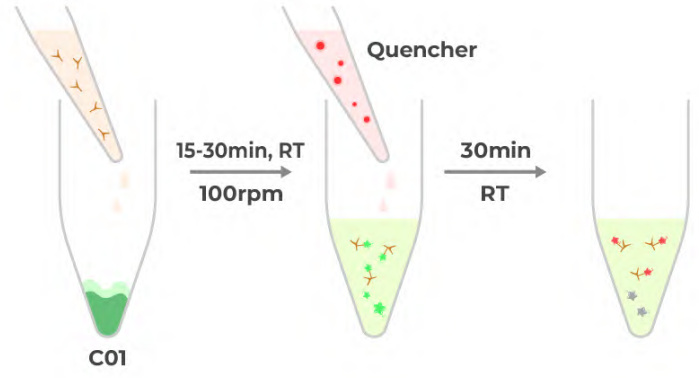
For example, if the antibody amount is 100 μg (2 mg/mL),

$$\text{Conjugate concentration} = \frac{100\ \mu g}{50\ \mu L + 5\ \mu L} = 1818.18\ (\mu g/mL)$$

【Storage of Conjugate】

It is generally recommended to store FITC-antibody conjugate at 4℃ and away from light for the presence of Proclin 300 in the reaction mixture. The optimal storage conditions should be determined based on the experiments, antibody stability, and other factors.

【Quick Guide】



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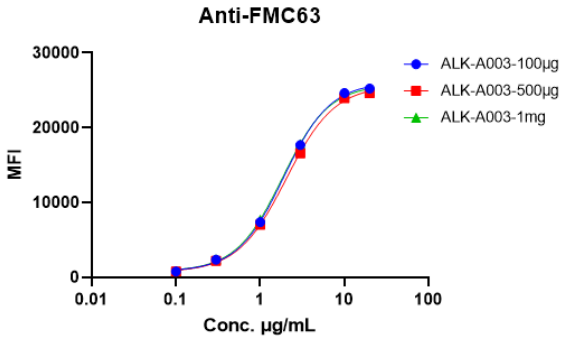
【Typical Data】

The following figure shows the data of products using ALK-A003-100 μg / 500 μg / 1 mg.

Conc. (μg/mL)	ALK-A003-100μg	ALK-A003-500μg	ALK-A003-1mg
20	25246	24616	24904
10	24635	23973	24537
3	17692	16578	17795
1	7392	7040	7680
0.3	2356	2211	2402
0.1	784	772	813
blank	40		



For each experiment, the specific MFI value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only.

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