ALK01-EN.02

R-PE Antibody Labeling Kit

【Catalog】ALK-A001

【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.

ALK01-EN.02

Catalog

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【Intended Use】

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

【Catalog Number and Specification】

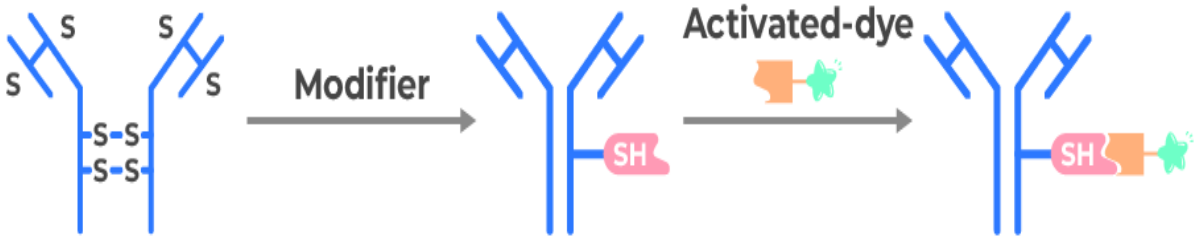
Table 1. Catalog number and Specification

Catalog Number	Specification
ALK-A001-100 μg	100 μg antibody labeling
ALK-A001-500 μg	500 μg antibody labeling
ALK-A001-1 mg	1 mg antibody labeling

【Principle】

R-Phycoerythrin (PE) is an orange fluorescent protein which has an excitation wavelength of 488 nm/561 nm and an emission wavelength of 575 nm.

The interchain disulfide bonds of the antibody can be modified to generate active cysteine residues, which are then conjugated with the preactivated PE to obtain the PE-antibody conjugate.



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【Materials Provided】

Table 2. Materials provided

Catalog Number	ID	Components	Size (1 kit)	Format	Storage
ALK-A001-100 μg	ALK01-C01A	Modifier reagent	1 vial	powder	-20℃
	ALK01-C02A	Activated PE			
	ALK01-C03A	Quencher reagent			
ALK-A001-500 μg	ALK01-C01B	Modifier reagent	1 vial	powder	-20℃
	ALK01-C02B	Activated PE			
	ALK01-C03B	Quencher reagent			
ALK-A001-1 mg	ALK01-C01C	Modifier reagent	1 vial	powder	-20℃
	ALK01-C02C	Activated PE			
	ALK01-C03C	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;

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Vortex mixer;

Tubes: 1.5mL, 10mL;

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 is used for fluorescence labeling of lg G subtype antibody molecules.

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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 pH 7.2-7.4 1×PBS buffer.

Keep the antibody solution away from cysteine, glutathione or other free sulfhydryl substances, as well as BSA, gelatin or other protective proteins.

Table 3. Buffer composition requirements

Buffer composition	Yes or No
buffer without sulfhydryl groups	Yes
pH 6.5-7.5	Yes
EDTA	<5 mM
trehalose	≤10%
cysteine	No
glutathione	No
mercaptoethanol or DTT	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.

Note: *If the powder of the ALK01-C01 and ALK01-C03 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 According to Table 4, add deionized water to dissolve component ALK01-C02 and ALK01-C03, store at 4 ℃ away from light.

Table 4. Dissolution volume of components

ID	Component	Dissolution volume		
		100 μg kit	500 μg kit	1 mg kit
ALK01-C02	Activated PE	70 μL	350 μL	700 μL
ALK01-C03	Quencher reagent	50 μL	50 μL	50 μL

Note 1: *The dissolved volume is sufficient to meet the subsequent need.*

Note 2: *Please pipette up and down the ALK01-C02 solution a few times to mix well.*

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

3. Antibody Modification

3.1 Add the prepared antibody solution into the ALK01-C01 tube, and mix them well by repeatedly pipetting up and down or vortexing the vial for a few seconds.

3.2 Shake the reaction mixture at 100 rpm at 37 °C for 1 hour, avoid from light.

Note: *If the shaking incubator was unavailable, please pipetting up and down the mixture every 0.5 hour.*

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4. Antibody Conjugation

4.1 Add the dissolved ALK01-C02 solution to the modified antibody solution according to the Table 5, and mix it well.

Note: *If 1mg of antibody was requested, please transfer the mixture to a 2-5 mL tube to ensure the adequate volume.*

4.2 Shake the reaction mixture at 100 rpm at 37 °C for 1 hour, avoid from light.

Table 5. Component adding volume

ID	Component	Adding volume		
		100 μg Kit	500 μg Kit	1 mg Kit
ALK01-C02	Activated PE	70 μL	350 μL	700 μL
ALK01-C03	Quencher reagent	4 μL	20 μL	40 μL
Note: *Please mix the solution well before pipetting.*				

5. Reaction Quenching

5.1 Mix well the dissolved ALK01-C03 component and add it to the reaction mixture according to the Table 5, and mix it well.

5.2 Keep the reaction mixture at room temperature for 0.5 hour, avoid from light.

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【Concentration Verification】

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

$$\text{Conjugate concentration}\left(\frac{\mu g}{uL}\right) = \frac{\text{antibody amount } (\mu g)}{\text{antibody solution volume } (\mu L) + C02 \text{ solution volume } (\mu L) + C03 \text{ solution volume } (\mu L)}$$

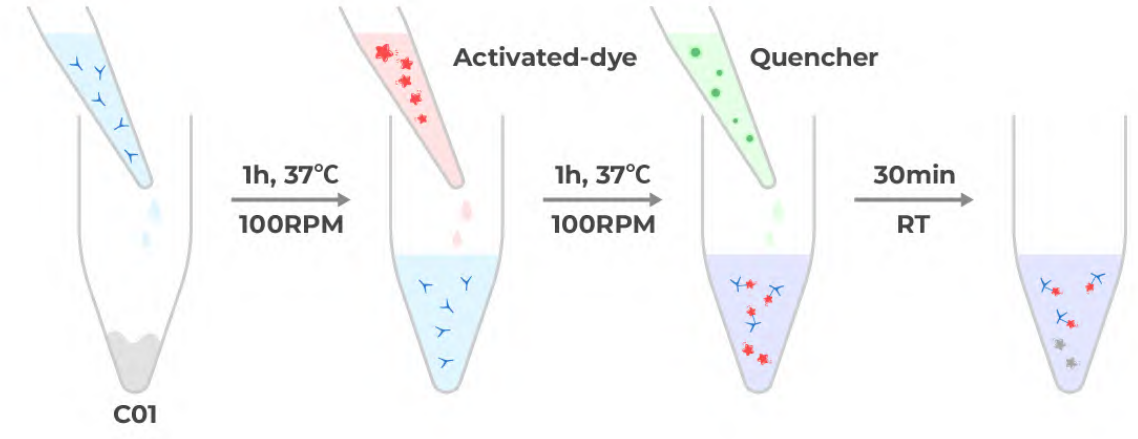
For example, if the antibody amount is 100 μg (2 mg/mL),

$$\text{Conjugate concentration} = \frac{100\ \mu g}{50\ \mu L + 70\ \mu L + 4\ \mu L} = 806.45\ (\mu g/mL)$$

【Storage of Conjugate】

It is generally recommended to store PE-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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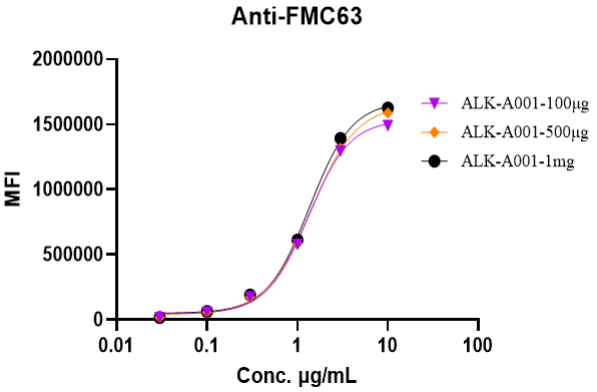
【Typical Data】

The following figure shows the data of products using ALK-A001-100 μg / 500 μg / 1 mg.

Conc. (μg/mL)	ALK-A001-100μg	ALK-A001-500μg	ALK-A001-1mg
10	1491329	1587439	1626276
3	1296473	1316610	1392038
1	579429	583929	613047
0.3	176515	174047	190314
0.1	58456	57977	64287
0.03	17347	17648	18230
blank	62		



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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