

APC Antibody Labeling Kit

【Catalog】 ALK-A002

【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.

US and Canada: **Tel:** +1 800-810-0816

Asia and Pacific: **Tel:** +86 400-682-2521

Web: <http://www.acrobiosystems.com>

E-mail: order@acrobiosystems.com

Catalog

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【Intended Use】

APC Antibody Labeling Kit uses a simple and quick process for APC conjugation of antibody. 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-A002-100 µg	100 µg antibody labeling
ALK-A002-500 µg	500 µg antibody labeling
ALK-A002-1 mg	1 mg antibody labeling

【Principle】

Allophycocyanin (APC) is a blue fluorescent protein which has an excitation wavelength of 640 nm and an emission wavelength of 661 nm. APC antibody Labeling kit provides a rapid and simple method to covalently link via disulfide bonds in the hinge region of antibody.



【Materials Provided】

Table 2. Materials provided

ID	Components	Size (1 kit)	Format	Storage
ALK02-C01	Modifier reagent	1 vial	powder	-20°C
ALK02-C02	Activated APC	1 vial	powder	-20°C
ALK02-C03	Quencher reagent	1 vial	powder	-20°C

【Storage】

The kit is stored at -20°C, 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;

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 Ig G subtype antibody molecules.

【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
<i>Note: ¹ These compositions may reduce labeling efficiency.</i>	

2. Components Preparation

2.1 Take out each component and equilibrate to room temperature.

Note: If the powder of the ALK02-C01 and ALK02-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 ALK02-C02 and ALK02-C03, store at 4 °C away from light.

Table 4. Dissolution volume of components

ID	Component	Dissolution volume		
		100 µg kit	500 µg kit	1 mg kit
ALK02-C02	Activated APC	35 µL	150 µL	300 µL
ALK02-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 ALK02-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 ALK02-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.

4. Antibody Conjugation

4.1 Add the dissolved ALK02-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
ALK02-C02	Activated APC	30 µL	150 µL	300 µL
ALK02-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 ALK02-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.

【Concentration Verification】

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

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

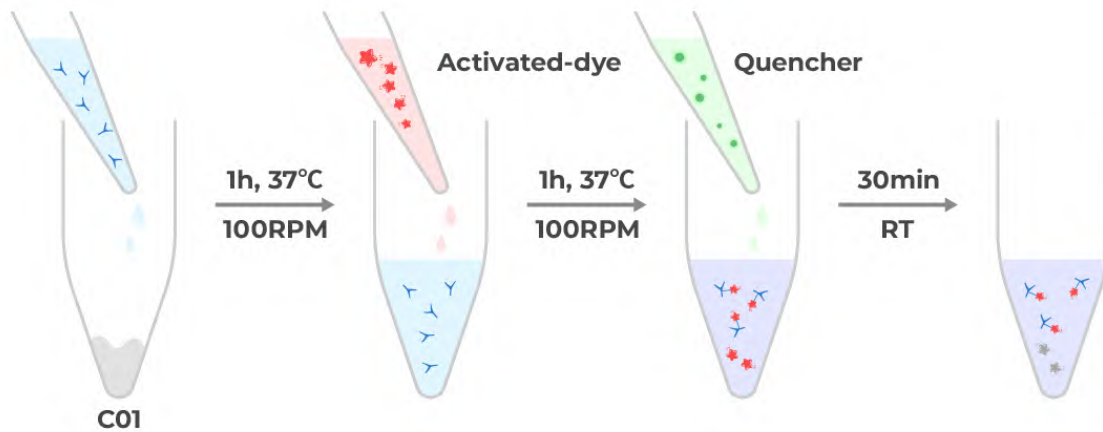
For example, if the antibody amount is 100 μg (2 mg/mL),

$$\text{Conjugate concentration} = \frac{100 \mu\text{g}}{50 \mu\text{L} + 30 \mu\text{L} + 4 \mu\text{L}} = 1190.48 (\mu\text{g/mL})$$

【Storage of Conjugate】

It is generally recommended to store APC-antibody conjugate at 4°C 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.

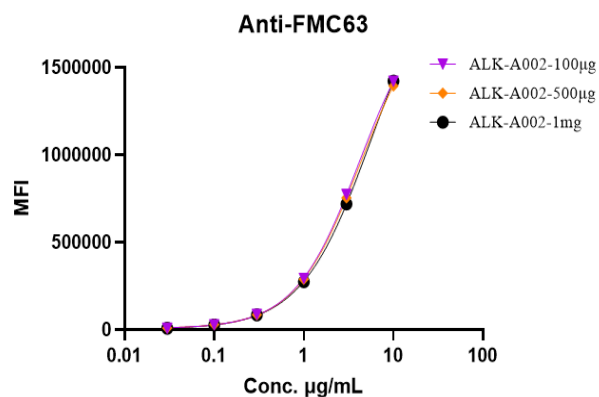
【Quick Guild】



【Typical Data】

The following figure shows the data of products using ALK-A002-100 µg / 500 µg / 1 mg.

Conc. (µg/mL)	ALK-A002-100µg	ALK-A002-500µg	ALK-A002-1mg
10	1420455	1394360	1422007
3	773367	754292	718764
1	293450	288295	274046
0.3	86673	85398	84897
0.1	28650	29231	26860
0.03	8628	8947	7996
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.