ALK04-EN.01

pH-Sensitive Fluorescent Dye Antibody Labeling Kit (Em660, 100μg/Reaction)

【Catalog】ALK-A004

【Size】3 Reactions

Please read this manual carefully before performing the experiment.

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

ALK04-EN.01

Catalog

【Intended Use】

pH-sensitive Dye Antibody Labeling Kit (Em660) provides a rapid and simple method for achieving covalent coupling between pH-sensitive fluorescent dye and antibody molecules. The conjugation reaction requires simple purification via spin column chromatography with straightforward operation, and the prepared dye-labeled antibody can be used for the detection of antibody internalization efficiency in cells.

【Principle】

The pH-sensitive dye (Em660) cannot only significantly enhance the fluorescence signal in an acidic environment but also minimize background noise effectively, which has an excitation wavelength of 643 nm and an emission wavelength of 660 nm.

pH-Sensitive Fluorescent Dye Antibody Labeling Kit provides a rapid and simple method to covalently label antibodies with pH-sensitive fluorescent dye by targeting primary amine groups.

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【Materials Provided】

Table 1

Catalog Number	ID	Components	Size (1 kit)	Storage
Box A ALK-A004	C01	Activated pH-Sensitive Fluorescent Dye (Em660)	1 vial x 3	-20℃
	C02	1M NaHCO3	1 vial	-20℃
	C03	Quencher Reagent	1 vial	-20℃

Table 2

Catalog Number	ID	Components	Size (1 kit)	Storage
Box B ALK-A004-1	C01	1xPBS (pH7.2-7.4), 11% Trehalose	5 mL	2-8℃
	C02	Desalting Spin column, 0.5 mL	3 per	2-8℃

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【Storage】

The transport temperature for the kit is 2-8°C.

- Box A: Unopened kit should be stored at -20℃ upon receiving.
- Box B: Unopened kit should be stored at 2-8℃ upon receiving.

Find the expiration date on the outside packaging and do not use reagents past their expiration date.

【Unsupplied Reagents or Equipment】

Thermostatic shaking incubator

Vortex mixer

Tubes: 1.5-2mL, 15mL

Centrifuge

Deionized or distilled water

1xPBS buffer (pH7.2-7.4)

UV/VIS Spectrophotometer.

【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;

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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.

【Conjugation Protocol】

1. Preparation of antibody solution

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

Note: *Keep the antibody solution away from ammonium ions, Tris, glycine, histidine, glutathione, ethanolamine, triethylamine, imidazole or other primary amino groups substances, as well as BSA, gelatin or other protective proteins.*

2. Components Preparation

2.1 Take out each component in Box A and equilibrate to room temperature (20-25°C).

2.2 Add 200 μL of deionized water to the vials of **C02-1M NaHCO3** to dissolve the powder, and then mix thoroughly.

2.3 Remove the cap from the vial of **C01-Activated pH-Sensitive Fluorescent Dye (Em660)**.

Note: *The C02 and C03 reagents should be stored at -20°C after use. Crystals precipitated from the 1M NaHCO3 solution after freezing can be completely dissolved by vortexing upon returning to room temperature.*

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3. Antibody Conjugation

3.1 Add 50 μL of prepared antibody solution into the **C01** lyophilized dye powder. Resuspend gently by repeatedly pipetting for a few seconds to ensure the powder is fully dissolved.

3.2 Add 5 μL **1M NaHCO3 solution** into 50 μL antibody reaction mixture and mix them well by repeatedly pipetting. (At this point, the solution will turn reddish-brown.)

3.3 Shake the reaction mixture at 100-300 rpm at room temperature (20-25°C) for 1 hour, avoid from light.

Note: *Optionally, you may transfer the antibody reaction mixture to a clean microcentrifuge tube to proceed with the reaction.*

4. Reaction Quenching

4.1 After conjugation reaction, add 2 μL **C03- Quencher Reagent** into the reaction mixture and mix them well.

4.2 Keep the reaction mixture at room temperature (20-25°C) for 30 minutes, avoid from light.

4.3 After the quenching reaction is complete, add 50 μL of 1xPBS buffer (pH7.2-7.4) to the reaction mixture, and the mixture is then ready for purification and buffer exchange.

5. Purification and Buffer exchange

5.1 Take out the Box B and prepare the C01-buffer (1xPBS (pH7.2-7.4), 11%Trehalose) and the Desalting Spin column (0.5 mL).

5.2 Remove the column's bottom closure and loosen the cap (do not remove the cap).

5.3 Place the column in a 2.0mL collection tube. Centrifuge in a centrifuge with a fixed angle rotor at 1000 × g for 1 minute to remove storage solution.

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5.4 Place a mark on the side of the column where the compacted resin is slanted upward. Place the column in the microcentrifuge with the mark facing outward in all subsequent centrifugation steps.

5.5 Carefully add 300µL of C01-buffer dropwise to the center of the resin bed surface without disturbing the settled resin. Centrifuge at 1000 × g for 1 minute to remove buffer.

5.6 Repeat Step 5.5 twice, discarding buffer from the collection tube.

5.7 Place the column in a new collection tube, remove cap and gently apply the antibody reaction mixture (from step 4.3) to the center of the resin bed surface.

5.8 Centrifuge at 1000 × g for 1 minutes to collect the sample. Discard the desalting column after use.

Note1: *The purification of labeled antibody is based on the principle of size exclusion chromatography. Therefore, after applying your sample to the desalting column, it should be centrifuged as soon as possible to ensure thorough purification.*

Note2: *This Kit provides a* 1x*PBS (pH* 7.2-7.4*) buffer with 11% Trehalose for the preservation of labeled antibody. The buffer is suitable for sample preservation of freezing or lyophilization, and does not affect the cell viability test of labeled antibodies. Other buffers compatible with your specific antibody may also be considered.*

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【Determination of the Concentration and Degree of Labeling (DOL)】

Measure the UV absorbance of the conjugate at 280 nm (A280) and 643nm (A643) using a UV spectrometer to determine the concentration of the labeled antibody, and calculate the concentration using the following formula：

$$\text{Labeled antibody concentration } (M) = \frac{A280 - (A643 * CF)}{210000}$$

$$\text{Labeled antibody concentration } (\frac{\text{mg}}{\text{mL}}) = \frac{A280 - (A643 * CF)}{1.4}$$

- CF = correction factor = $A280\ (dye)/A643\ (dye)$=0.178
- For most mammalian antibodies (such as immunoglobulin), the molar extinction coefficient is ~210,000 M^{-1} cm^{-1}, and the absorption value of 1 mg/mL IgG at 280 nm is 1.4. **You can also use your own antibody extinction coefficient instead of the default parameters.**

Calculate the DOL using the following formula：

$$DOL = \frac{A643}{54352 * \text{Labeled antibody concentration } (M)}$$

- The molar extinction coefficient of dye is 54352 M^{-1} cm^{-1}.

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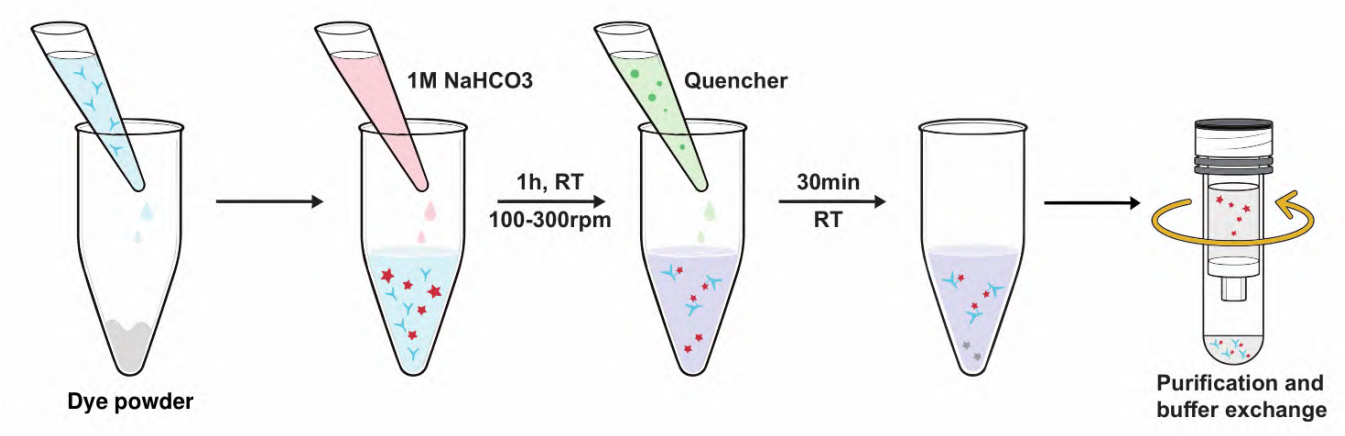
【Storage of Conjugate】

After conjugation, dilute the labeled antibody to a concentration below 0.5 mg/mL to prevent precipitation that may occur due to the inherent instability of some antibodies.

Prior to cell-based experiments, the labeled antibody should be filter-sterilized using a 0.2 μm filter.

It is generally recommended to store the labeled antibody at or below -20°C in small aliquots, protected from light, to prevent repeated freeze-thaw cycles.

【Quick Guide】



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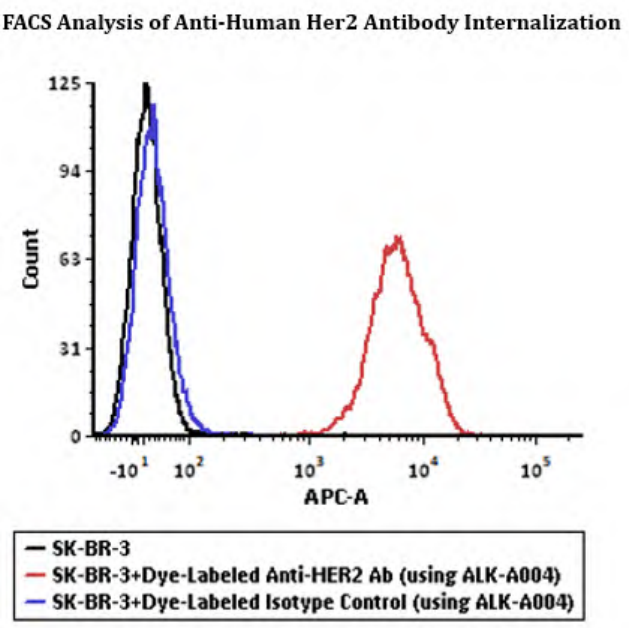
【Typical Data】

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

Figture 1: Anti-Her2 Ab and Human IgG1 isotype control were labeled using pH-Sensitive Fluorescent Dye Antibody Labeling Kit (Cat. No. ALK-A004). SK-BR-3 cells were treated with Dye Labeled Anti-Her2 Ab and Dye Labeled Isotype Control separately at 37℃ for 4 hours, then analysis by Flow cytometric. APC signal was used to evaluate the internalization signal.



Figture 1

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