PE-Cy7 Antibody Conjugation Kit

Cat No: BIOK1986

Product Introduction

The immunofluorescence technique is to label the known antibody or antigen molecule with fluorescein. When the corresponding antigen or antibody reacts, a certain amount of fluorescein will be carried on the formed complex. Under the fluorescence microscope, the binding site of antigen and antibody that emits fluorescence can be seen to detect the antigen or antibody. The main characteristics of this technique are: strong specificity, high sensitivity and fast speed.

Fluorescein labeled antibody (FA) is a specific, sensitive, qualitative and localization immunochemical reagent widely used in immunopathology, cytochemistry, flow cytometry, pathology and clinical immunodiagnosis of autoantibodies. The antibody used for labeling requires high specificity and high affinity. Generally, IgG and IgM should be purified and extracted before labeling.

The fluorescein in this kit is activated RPE-Cy7. R-PE is a commonly used fluorescent labeling reagent isolated and purified from red algae. The subunit group of R-PE protein becomes（α β）6 γ , the molecular weight of each α、β subunit was 20kDa, the molecular weight of each γ subunit was 30kda and the total molecular weight was 240kda. R-PE has high absorbance and fluorescence violet yield, strong and stable fluorescence and high sensitivity. The maximum excitation wavelength of RPE-Cy7 is 565nm and 750nm, and the maximum fluorescence emission peak is 575nm and 773nm.

Size: 100 ug

Storage: 2-8℃. Keep away from light

Shelf life: 6 month

Composition	Specifications	Quantity	Storage
ActivationRPE-CY7	/	1	4℃ (protect from light)
Antibody modifying agent 1	/	2	-20-4℃
Antibody modifying agent 2	/	2	4℃
Blocking agent	/	1	-20-4℃
DMSO	20ul	1	Room temperature
Labeling buffer	1500ul	1	Room temperature
Antibody pretreatment desalination column	/	1	Room temperature (standby)
Antibody desalination column 1	/	1	Room temperature
Antibody desalination column 2	/	1	Room temperature

Precautions

1. The antibody modifying reagent 1 and the blocking agent in the kit shall be stored at -20℃ for a long time, and the other components shall be stored at 2-8℃ without freezing.

2. The components of the kit may be reversed during transportation, which may cause the liquid or dry powder reagent to stick to the tube wall or bottle cap. Please centrifuge before use to deposit the liquid or dry powder reagent attached to the tube wall or bottle cap to the bottom of the tube.

3. DMSO contained in the blocking agent is a kind of micro poison, which is permeable to human skin and irritating to eyes. Avoid contact with skin, eyes and mucosa when using.

4. NaN3 and BSA need to be removed from the antibody before labeling. Dialysis, concentration and concentration determination of the antibody will cause the loss of antibody amount. Therefore, the most appropriate antibody amount should be considered according to the specific situation when preparing the antibody before labeling.

5. Since the modified group of the antibody is easy to be re-oxidized, the modified antibody should be coupled with the activated RPE-Cy7 as soon as possible.

Steps

1. Antibody Preparation

1.1 The recommended antibody concentration is 2-10 mg / ml.

1.2 The antibody shall not contain BSA, NaN3 or other protein components.

1.3 The antibody storage buffer is PBS, and pH 7.2-7.4 has the best labeling effect. The antibody can be dialyzed to 0.1M Pb (containing 0.15m NaCl) and labeled after pH 7.2-7.4.

2. Antibody Modification

2.1 Take out antibody modifying agent 1, centrifuge at high speed for 5 min after shaking, throw the dry powder to the bottom of the tube, and add 3ul DMSO into the tube to dissolve the modifier.

2.2 Add 100ug of antibody to the solution of antibody modifying agent 1 dissolved in DMSO, repeatedly pipette with a pipette or mix well with vortex, and react at room temperature for 3 hours.

2.3 Use the antibody desalination column 1 to replace the modified antibody into the labeling buffer, and remove the free antibody modifying agent at the same time.

Use of Antibody Desalination Columns

1) Take out the desalination column, swell with 800ul deionized water for 10 minutes, and then centrifuge at 1000×g for 2 min to remove the collected liquid.
2) Add 200ul labeling buffer, standing for 10-30 s, 1000×g for 2 min and repeat the operation twice.
3) The modified antibody solution was added to the desalination column, followed by adding 50ul of labeling buffer, centrifuged at 1000 × g for 2 min, and the modified antibody solution was collected and recovered.

2.4 Take out antibody modifying agent 2, centrifuge at high speed for several seconds, throw the dry powder to the bottom of the tube, transfer the modified antibody solution into the tube of

antibody modifying agent 2, mix and dissolve it, and then react at room temperature for 20-30 min, then replace the modified antibody into the labeling buffer with antibody desalination column 2, remove the free antibody modifying agent 2, and collect the recovered solution to be the modified antibody;

3. Coupling of Activated R-PE and Antibody

3.1 The modified antibody solution is added to the activated RPE-Cy7 solution drop by drop, and gently mixed while adding. The reaction is coupled for 1-2 hours at room temperature in the absence of light, or it is placed overnight at 4℃ after 0.5 hours at room temperature in the absence of light.

3.2 Take out the blocking agent, centrifuge for a few seconds, throw the dry powder to the bottom of the tube, add 6ul DMSO to the tube, and repeatedly blow with a pipette until the dry powder is completely dissolved.

3.3 Add the blocking agent to the conjugate, repeatedly pipetting with a pipette or mixing with a vortexer, and incubate at room temperature for 30 min in the dark.

3.4 Transfer the conjugate to an EP tube and store it at 4℃ in the dark until use, or add a preservative at 4℃ and store it in the dark for 6 months to 1 year.

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