PE-Cy5 Antibody Conjugation Kit

Cat No: BIOK1987

Product Introduction

Immunofluorescence technique is to label the known antibody or antigen molecule with fluorescein. When the corresponding antigen or antibody reacts with it, 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 technology 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, virology and clinical immunodiagnosis of autoantibodies.

The antibody used for labeling requires high specificity and high affinity. The antiserum used should not contain antibodies against normal tissues in the specimen. Generally, IgG and IgM should be purified and extracted before labeling.

Fluorescein PE-Cy5 in this kit is a tandem fluorescent dye obtained by coupling our high-quality R-phycoerythrin (R-PE) and small molecule fluorescent dye Cy5. PE-Cy5 is a commonly used fluorescent dye for flow cytometry. Its maximum excitation wavelength is 488nm and its maximum emission wavelength is 670nm.

Size: 500 ug*2

Storage: 2-8℃. Keep away from light

Shelf life: 6 month

Composition	Specifications	Quantity
PE-Cy5	125ul	2 (protect from light)
Fluorescent activator	/	2
Antibody modifying agent	/	2
Blocking agent	/	2
DMSO	400ul	1
Neutralization buffer	50ul	1
Fluorescent desalination column	0.5ml	4
Antibody desalination column	0.5ml	4
Desalination column collection tube	/	8

Precautions

1. The kit shall be stored at 2-8℃, and shall not be frozen.

2. The components of the kit may be reversed during transportation, which may cause 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. Fluorescent activator, antibody modifying agent and blocking agent need to be used and prepared now, and cannot be stored for a long time after the dry powder is dissolved.

4. 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.

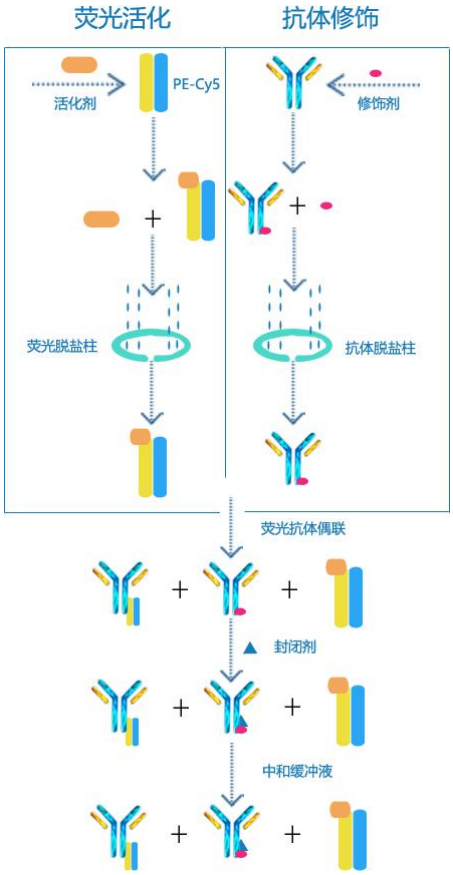
5. Dialysis, concentration and concentration determination of antibody before labeling will cause loss of antibody amount. Therefore, the most appropriate antibody amount shall be considered according to specific conditions when preparing antibody before labeling.

6. Fluorescent activation and antibody modification can be performed simultaneously.

7. This kit can be labeled twice (two identical or different antibodies can be labeled).

8. Since the groups carried by the modified antibody are easy to be re oxidized, the modified antibody needs to be coupled with the activated PE-Cy5 as soon as possible.

Marking Process Diagram



Steps

1. Antibody Preparation

1.1 The recommended antibody concentration is 2.5-4.0mg/ml.

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

1.3 The antibody storage buffer is PBS, and the labeling effect is best when the pH is 7.2-7.4. The antibody can be dialyzed to PBS, and the labeling can be performed after the pH is 7.2-7.4.

1.4 If the antibody storage buffer contains 0.02-0.1% sodium azide, it does not need to be removed and has no effect on labeling.

2. Antibody Modification

2.1 Take out the antibody modifying agent, centrifuge for several seconds, throw the dry powder to the bottom of the tube, add 100ul of deionized water or PBS, pH7.4 into the tube, blow repeatedly with a pipette gun or mix with vortex until completely dissolved.

2.2 Add 9.2ul of antibody modifying agent to 500ug of antibody (add 1.84ul of antibody modifying agent to every 100ug of antibody), mix well and stand at room temperature for 1.5h (PE-Cy5 can be activated at the same time).

2.3 Use antibody desalination column to replace the modified antibody into the labeling buffer and remove the free antibody modifier.

Use of Antibody Desalination Columns

a. Two antibody desalination columns were taken out and centrifuged at 4000rpm for 2min.

b. Temporarily retain the liquid in the collection tube.

c. Put the antibody desalination column into a new collection tube, divide the modified antibody into two equal parts and add them to two antibody desalination columns respectively. The antibody is added to the surface of the packing in the column. The loading volume is between 50-110ul.

d. After the sample infiltrates into the filler, centrifuge at 4000rpm for 2min.

NOTE: The recovery volume of antibody is 90-100% of the loading volume.

3. PE-Cy5 Activation

3.1 Take out the fluorescent activator, centrifuge for a few seconds, shake the dry powder to the bottom of the tube, add 35ul DMSO to the tube, and repeatedly pipet with a pipette or mix with vortex until the dry powder is completely dissolved.

3.2 Take out the PE-Cy5, centrifuge for a few seconds, throw the PE-Cy5 to the bottom of the tube, add 7.5ul of the dissolved fluorescent activator to the tube, and mix with a pipette or vortex repeatedly.

3.3 Centrifuge for a few seconds after mixing, throw the PE-Cy5 to the bottom of the tube, and let it stand at room temperature for 1 h.

3.4 Use a fluorescent desalination column to replace the activated PE-Cy5 into the labeling buffer, while removing the free fluorescent activator.

Use of Fluorescent Desalination Columns

a. Two fluorescent desalination columns were taken out and centrifuged at 4000rpm for 2min.

b. Temporarily retain the liquid in the collection tube.

c. Put the fluorescent desalination column into a new collection pipe, divide the activated pe-cy5 into two parts and add them to two fluorescent desalination columns respectively. Pe-cy5 is added to the surface of the packing in the column. The loading volume is between 50-110ul.

d. After the sample infiltrates into the filler, centrifuge at 4000rpm for 2min.

NOTE: The recovery volume of PE-Cy5 is 90-100% of the loading volume.

4. PECy5-Antibody Conjugation

4.1 Add the modified antibody in the collection tube to the activated PE-Cy5 dropwise, mix gently with a mixer while adding, and incubate for 2 h at room temperature in the dark.

4.2 Take out the blocking agent, centrifuge for several seconds, throw the dry powder to the bottom of the tube, add 100ul DMSO into the tube, blow repeatedly with a pipette gun or mix with vortex until the dry powder is completely dissolved.

4.3 Add 2ul of blocking agent to the conjugate, blow repeatedly with a pipette gun or mix with vortex, and then incubate for 30 min at room temperature in the dark.

4.4 Add an appropriate amount of neutralization buffer to the conjugate (add 2.5ul of neutralization buffer per 100ul of conjugate).

4.5 The conjugate can be transferred to a brown EP tube, stored in the dark at 4℃ for use, and stored in the dark at 4℃ for 1 year.

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