

## Cy3 Conjugation Kit

### Order details

**CatNo:** ABCK103

**Size:** 1mg

### Product description

Fluorescein-labeled antibody is abbreviated as fluorescent antibody (FA), which is currently widely used in immunopathology, cytochemistry, flow cytometry, virology and autoantibody clinical immunodiagnosis of specific, sensitive, qualitative and localized immune Chemical reagents. Antibodies used for labeling require high specificity and high affinity. The antiserum used should not contain antibodies against normal tissues in the specimen. Generally, IgG and IgM need to be purified and extracted before labeling. The fluorescein in this kit uses high-quality imported Cy3. The cyanine dye Cy3 is one of the few near-infrared fluorescent labeling dyes. It produces a low red fluorescent background, high fluorescence intensity, and good fluorescence stability. Cy3 are usually used in biomolecule labeling, fluorescence imaging and other fluorescent biological analysis, such as labeling peptides, proteins and nucleic acids. Cy3 fluorescent labeling dye can significantly enhance the fluorescence intensity after binding to protein.

## Product description

Cy3	1 vial (Protect from light)
Buffer	50 ul
DMSO	150 ul
Purification column	2 vial
Collection tube	2 vial

## Steps

### 1. Antibody preparation

1.1. The recommended antibody concentration is between 1-2mg/ml.

1.2. Do not contain BSA or other protein components in the antibody.

1.3. The antibody buffer should not contain amino salts (such as Tris, NaN<sub>3</sub>), and the pH should be 6.5-8.5.

### 2. Antibody labeling

2.1. Take out the Cy3 and centrifuge for a few seconds, and shake the Cy3 powder in the tube to the bottom of the tube;

2.2. Add 50ul DMSO to the tube and pipette repeatedly or vortex until the Cy3 is completely dissolved;

2.3. Add an appropriate amount of adjustment buffer to the antibody (add 10ul adjustment buffer to per 100ul antibody);

2.4. Add the dissolved Cy3 to the antibody (add 4.0ul Cy3 to per 100ug of antibody), pipette repeatedly or vortex to mix.

2.5. Place the antibody-Cy3 mixture on a horizontal shaker or a rotating mixer, and react for 1 hour at room temperature while shaking (the reaction tube can be wrapped in tin foil).

Note: If a higher Cy3 coupling ratio is required, the coupling time of antibody and Cy3 can be extended appropriately.

### 3. Free Cy3 removal

3.1. Take out the purification column, load the molecular sieve packing, and centrifuge the purification column at 3000 rpm for 2 minutes.

3.2. Temporarily keep the buffer in the collection tube.

3.3. Move the purification column to a new collection tube, suck the antibody-Cy3 conjugate and place it on the surface of the packing in the purification column. If the sample volume is less than 50ul, you should first make up the liquid volume to 50ul with the buffer reserved in 3.2. The maximum sample volume should not exceed 100ul.

3.4. After the sample infiltrates the packing, centrifuge at 3000rpm for 2min. To ensure the removal effect, this operation can be repeated 1-2 times.

3.5. Store the antibody-Cy3 marker at 4°C until use. The final product can be stored at 4°C for 1 year.

### Note

1. Store the kit at 2-8°C, do not freeze.

2. The components of the kit may be upside down during transportation, causing liquid or dry powder reagents to stick to the tube wall or bottle cap. Please centrifuge before use to make the liquid or dry powder reagent attached to the tube wall or bottle cap settle to the bottom of the tube.

3. Cy3 needs to be prepared for immediate use, and the dissolved Cy3 cannot be stored for a long time.

4. The buffer in the purification column contains the toxic component sodium azide (NaN<sub>3</sub>). Avoid contact with skin, eyes and mucous membranes when using it.



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5. DMSO is slightly toxic, permeable to human skin and irritating to the eyes. Avoid contact with skin, eyes and mucous membranes when using it.
6. The dialysis, concentration and concentration determination of the antibody before labeling will all cause the loss of the amount of antibody. Therefore, when preparing the antibody before labeling, consider the most appropriate amount of antibody according to the specific situation.