(This kit is for research use only, not for clinical diagnosis!)

EnAb™ R-Phycoerythrin(R-PE) Antibody Quick Labeling Kit

Catalog Number: RE80005p

Sizes: 40 μg /200 μg /1 mg

Please read the instructions carefully before use. If you have any questions, please contact us via the following methods:

Web: https://www.enkilife.com/

E- mail: order@enkilife.com techsupport@enkilife.com

Tel: 0086-27-87002838

Product Introduction

EnkiLife protein fluorescent dye labeling kits provide a variety of commonly used fluorescent dye types on the market, including common phycoerythrin (R-PE), allophycocyanin (APC), peridinin-chlorophyll-protein complex (Percp) fluorescent dyes and a variety of tandem dye types of the three fluorescent dyes, which can meet a variety of application requirements, such as flow cytometry, microarray analysis, immunofluorescence, ELISA, etc. All protein fluorescent dye labeling kits contain all the reagents required for labeling, and are mainly used for antibody labeling.

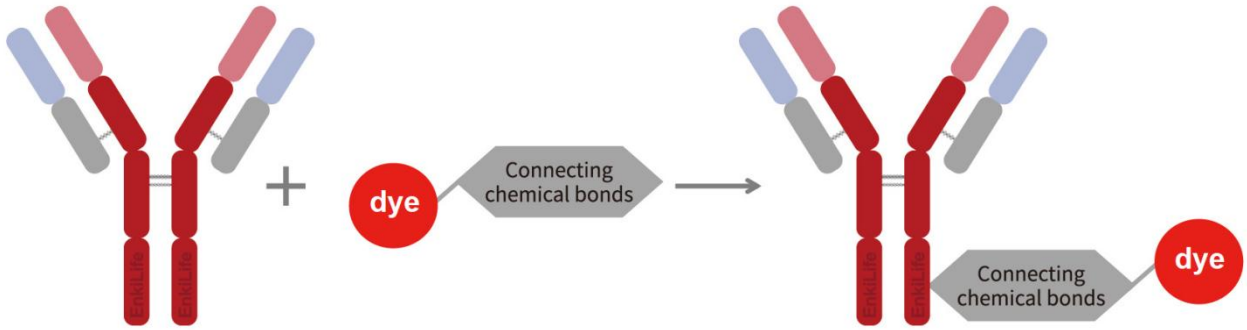
Dye characteristics: R-Phycoerythrin (R-PE) is a phycobiliprotein isolated from red algae. As a fluorescent dye for biological detection, it is nearly a hundred times more sensitive than traditional organic fluorophores. With a molecular weight of 240 kDa, PE has several subtypes. The R-PE used in this kit has a primary absorption peak at 565 nm, secondary absorption peaks at 496 and 545 nm, and a maximum emission wavelength of 575 nm. Like other phycobiliproteins, R-PE has a high extinction coefficient and high quantum efficiency, is stable in nature, and has low background interference. As a protein, R-PE can be conjugated to target antibodies or proteins using conventional protein cross-linking techniques, with its spectral characteristics remaining unchanged.

Features

- The kit has complete components and is easy to operate. You can obtain high-quality conjugated antibodies by simply following the operating steps.
- The dye configured in this kit is a high-purity, high-activity fluorescent protein with higher fluorescence efficiency.
- This reagent uses directional coupling technology, and neither the fluorescent protein nor the antibody will self-couple, ensuring the specificity and uniformity of the conjugate.
- The cross-linker used in the kit has an extended arm to ensure that the application effect is not affected by protein steric hindrance.

Labeling principle

This kit utilizes the cysteine characteristics of the antibody and the free amino groups on the dye to covalently couple the antibody molecule with the dye using a directed docking coupling technique.



Product composition

Product composition	Content of components in different specifications			
	40 μg antibody	200 μg antibody	1 mg antibody	Storage temperature
Activated **R-PE**	1 tube	1 tube	5 tubes	-20 °C, away from light
Labeling Buffer L	2 mL	2 mL	2 mL	4 °C, away from light
Antibody Modification Reagents	60 μL	60 μL	60 μL	-20 °C, away from light
Ultrafiltration Tube * ,50K MWCO	1 set **	1 set **	1 set **	RT
Blocking reagent	300 μg	300 μg	300 μg	-20 °C, away from light
DMSO (dissolve blocking reagent)	50 μL	50 μL	50 μL	-20 °C, away from light
Labeled protein storage solution	1 mL	1 mL	5 mL	2-8 °C
Recommended amount of labeled antibody	Each tube is labeled with 20-40 μg, and the best is 20 μg	Each tube is labeled with 50-200 μg, and the best is 100 μg	Each tube is labeled with 50-200 μg, and the best is 100 μg	

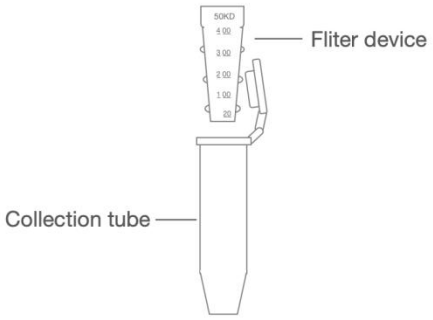
*Ultrafiltration tube instructions:

If the same biological molecule is labeled, the ultrafiltration tube membrane can be reused several times before it breaks.

If different biomolecules are labeled, different ultrafiltration tubes should be replaced to avoid cross contamination of biomolecules.

** If you need more of the 50K MWCO ultrafiltration tubes, please contact us for further supply.

** 1 set 50K MWCO ultrafiltration tube (0.5 mL) includes 1 filter device and 2 collection tubes .



Storage conditions

Unopened kits can be stored at -20˚C for 1 year. After opening, store at storage temperature. Dissolved dyes or reagents should be used as soon as possible. They can be stored at -20°C or -80°C for up to one week .

Operation process

Preparation before the experiment

1. Read the instruction manual carefully.
2. Take out the kit from the refrigerator 20 minutes in advance and allow the components of the kit to equilibrate to room temperature. (Note: Unnecessary reagent components should continue to be placed in the refrigerator).
3. DMSO may freeze at -20°C and needs to be left at room temperature for about 10 minutes to thaw.
4. Ultrafiltration tube infiltration (**for antibody ultrafiltration concentration and buffer replacement**): Add 450 µL **labeling buffer L**, leave at room temperature for 10 minutes for later use, and discard the labeling buffer before adding the labeled object (the ultrafiltration tube filter should be kept moist during the entire labeling process).
5. Antibody preparation: Purify and remove impurities from the labeled antibody, and determine the concentration and content. Please ensure that the antibody sample meets the standards in the following table

Components	Kit Labeling Compatibility
Sodium azide <0.1%, glycerol <50%, Tris <50mM, glycine <50mM, Proclin <0.5%, EDTA <10mM, trehalose <5%, sucrose <5%, or other small molecule additives	Yes, perform **ultrafiltration and concentration of antibody samples,** i.e. labeling step 1.
Antibodies in ascites, serum, and cell culture supernatant	No, perform the purification step before using this kit
Contains BSA>0.1%	No, use a BSA removal kit (such as RE80028: BSA Removal Kit) to purify the antibody before using this kit
Antibody concentration is less than 2 mg/mL	Yes, perform **ultrafiltration and concentration of antibody samples,** i.e. labeling step 1.
Antibody concentration ≥ 2 mg/mL, and does not contain BSA (< 0.1%), gelatin (< 0.1%), and small molecule additives.	Yes, no need for ultrafiltration concentration and buffer exchange. Directly dilute it to 1mg/mL using the labeling buffer L.

Labeling steps (applicable to labeling 250 µg-1 mg antibody)

1. **Ultrafiltration concentration of antibody samples** : Take 500 µg of the antibody to be labeled (purity> 90%), put it into the ultrafiltration tube filter , and add the labeling buffer that does not exceed the maximum volume of the ultrafiltration tube filter , 14000 × g centrifuge for 5 minutes and discard the filtrate ; repeat this step once depending on the additives in the antibody , and obtain 50-100µL after the last ultrafiltration . Invert the ultrafiltration tube filter into the collection tube and centrifuge at 14000 × g. Centrifuge at 14000 × g for 1 minute, collect the obtained antibody solution, and add the labeling buffer to the antibody concentration of about 4 mg/mL according to the total amount of antibody. At the same time, add an appropriate amount of 1× PBS to the filter element to keep it moist.

Note: If the concentration of the antibody to be labeled is greater than 6 mg/mL and does not contain impurities (such as the antibody buffer is PBS), directly use the labeling buffer to dilute the antibody to 4 mg/mL .

2. **Antibody modification** : Modify the antibody by adding 10 µL of antibody modification reagent to 500 µg of antibody , mix gently and react at 37°C for 60 minutes.
3. **Dye and antibody coupling:** Add 50 µL of labeling buffer to each of 5 tubes of activated dye (each tube labeled with 50 µg-200 µg of antibody), vortex to mix and dissolve, then add a total of 250 µL directly to the modified antibody solution, and react at 37°C in the dark for 1.5 hours or at 4°C in the dark overnight .

4. **Block & storage:** Add 30μL DMSO to a 300 μg blocking reagent tube and mix to dissolve, then add 15μL blocking reagent according to 500 μg antibody, mix and react at 37°C in the dark for 30 minutes to block the unreacted active groups . At this time, the reaction is complete , and the antibody preservation solution is selectively added to the appropriate concentration according to the subsequent application requirements (generally, the antibody concentration is required to be not less than 0.2 mg/mL , stored at 4°C in the dark, do not freeze, and can generally be stored stably for more than half a year) .

Labeling step (applicable to labeling 50 μg-200 μg antibody)

1. **Ultrafiltration concentration of antibody samples** : Take 100 μg of the antibody to be labeled (purity> 90%), put it into the ultrafiltration tube filter , and add the labeling buffer that does not exceed the maximum volume of the ultrafiltration tube filter , 14000 × g centrifuge for 5 minutes, discard the filtrate ; repeat this step once depending on the additives in the antibody , and obtain 30-50 μL after the last ultrafiltration . Invert the ultrafiltration tube filter into the collection tube and centrifuge at 14000 × g for 5 minutes. Centrifuge at 14000 × g for 1 minute, collect the antibody solution, and add the labeling buffer to the total amount of antibody to an antibody concentration of 1 mg/mL. At the same time, add an appropriate amount of 1× PBS to the filter element to keep it moist.

Note: If the concentration of the antibody to be labeled is greater than 2 mg/mL and does not contain impurities (such as the antibody buffer is PBS), directly use the labeling buffer to dilute the antibody to 1 mg/mL.

2. **Antibody modification** : Modify the antibody by adding 2 μL of antibody modification reagent to 100 μg of antibody , mix gently and react at 37°C for 60 minutes.
3. **Dye and antibody coupling:** Add 1 tube of activated dye (each tube is labeled with 50 μg-200 μg antibody) to 50μL labeling buffer, vortex to mix and dissolve, then add directly to the modified antibody solution, and react at 37°C in the dark for 1.5h or at 4°C in the dark overnight .
4. **Block & storage:** Add 30 μL DMSO to a 300 μg blocking reagent tube and mix to dissolve, then add 3μL blocking reagent according to 100μg antibody, mix and react at 37°C in the dark for 30 minutes to block the unreacted active groups . At this time, the reaction is complete. According to the subsequent application requirements, selectively add antibody preservation solution to the appropriate concentration (generally the antibody concentration is required to be not less than 0.2 mg/mL , store at 4°C in the dark, do not freeze, and it can generally be stored stably for more than half a year.

Labeling step (applicable to labeling 20μg-40μg antibody)

1. **Ultrafiltration concentration of antibody samples** : Take 20 μg of the antibody to be labeled (purity> 90%), put it into the ultrafiltration tube filter , and add the labeling buffer that does not exceed the maximum volume of the ultrafiltration tube filter , 14000 × g centrifuge for 5 minutes and discard the filtrate ; repeat this step once depending on the additives in the antibody. After the last ultrafiltration , 20-30 μL is obtained . Invert the ultrafiltration tube filter into the collection tube and centrifuge at 14000 × g . Centrifuge at 14000 × g for 1 minute, collect the antibody solution, and add the labeling buffer to the total amount of antibody to an antibody concentration of 1 mg/ml. At the same time, add an appropriate amount of 1× PBS to the filter element to keep it moist.

Note: If the concentration of the antibody to be labeled is greater than 2 mg/mL and does not contain impurities (such as the antibody buffer is PBS), directly use the labeling buffer to dilute the antibody to 1 mg/mL.

2. **Antibody modification** : Modify the antibody by adding 0.5 μL antibody modification reagent to 20 μg of antibody , mix gently and react at 37°C for 60 minutes.

3. **Dye and antibody coupling:** Add 1 tube of activated dye (each tube is labeled with 20 μg-40 μg antibody) to 10 μL labeling buffer, pipette or vortex to mix and dissolve, then add directly to the modified antibody solution, react at 37°C in the dark for 1.5 h or at 4°C in the dark overnight .

4. **Block & storage:** Add 30μL DMSO to a 300μg blocking reagent tube and mix and dissolve, then add 0.6μL blocking reagent according to 20μg antibody, mix and react at 37°C in the dark for 30 minutes to block the unreacted active groups . At this time, the reaction is complete. According to the subsequent application requirements, selectively add antibody preservation solution to the appropriate concentration (generally the antibody concentration is required to be not less than 0.2 mg/mL , store at 4°C in the dark, do not freeze, and it can generally be stored stably for more than half a year.

Precautions

1. This kit is most suitable for labeling conventional IgG antibodies (150 kDa). IgM, IgA, IgE, IgY and other antibodies can also be labeled, but the conjugated antibodies obtained according to the steps of this kit may not have the best use effect, and the labeling efficiency is unknown. Various recombinant antibodies, antibody fragments or nanobodies have not been tested using this labeling kit.
2. The activated dye in this kit is vacuum freeze-dried. Once reconstituted, please use it all at once. If it is not used immediately after dissolution, its efficacy will decrease over time.
3. The sealant must be prepared before use, and the dry powder cannot be stored for a long time after it is dissolved.
4. The components of the test kit may be turned upside down during transportation, causing the liquid or dry powder reagents to stick to the tube wall or bottle cap. Please centrifuge before use to allow the liquid or dry powder reagents attached to the tube wall or bottle cap to settle to the bottom of the tube.
5. Do not mix components from different types of labeling kits.
6. Some chemical reagents are slightly toxic, please wear gloves when handling.

Appendix 1 Ultrafiltration tube instructions

1. The 50K MWCO ultrafiltration tube equipped with this kit has a maximum filter capacity of 500 μL and is marked with scales. If the user is using it for the first time, he can add a certain volume of marked buffer in advance, check the correspondence between the scale and volume, and increase or decrease the ultrafiltration centrifugation time.
2. Ultrafiltration centrifugation time is related to the characteristics of the protein sample or conjugate. The centrifugation concentration time of some antibodies needs to be adjusted according to actual conditions. For example, when ultrafiltration concentrates 20 ug of antibody to 20-30 μL, the centrifugation time can be appropriately extended to make the antibody concentration as close to 1mg/mL as possible.
3. The ultrafiltration membrane is the key component of the ultrafiltration tube. When blowing and mixing the protein solution, be careful not to touch the ultrafiltration membrane.

Appendix 2 Technical parameters of each protein fluorescent dye

Dye Name	Labeling Kit Catalog Number	Ex max/Em max
R-PE	RE80005p	496nm，565nm/575nm