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

EnAb™ Horseradish Peroxidase(HRP) Antibody Labeling Kit

Catalog Number: RE80004p

Sizes: 80 μg /400 μg /4mg

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

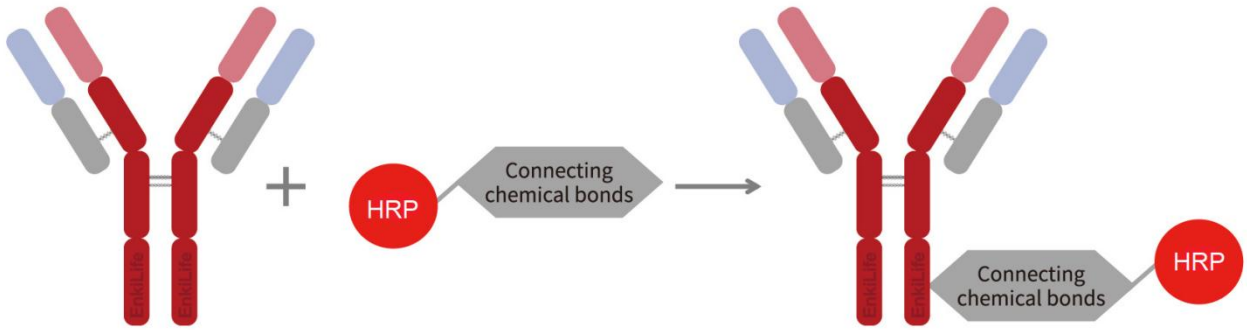
HRP is an oxidorereductase extracted from the roots of horseradish (Armoracia rusticana), belonging to the peroxidase superfamily. It can catalyze the oxidation reaction of hydrogen peroxide (H_2O_2) on the substrate to generate colored or fluorescent products. It is often used in signal amplification detection, such as ELISA,WB, in situ hybridization, and molecular biology. Applications of biosensors, etc. Different from the commonly used coupling methods, this EnkiLife HRP antibody labeling kit utilizes the cysteine characteristics on the antibody and the free amino groups on HRP, and adopts the directional docking coupling technology to covalently couple the antibody molecule with the HRP enzyme.

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 HRP configured in this kit is of high purity and high activity, and has a higher catalytic efficiency for substrates.
- This reagent uses directional coupling technology, and neither the HRP 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 HRP to covalently couple the antibody molecule with the HRP using a directed docking coupling technique.



Product composition

Product composition	Content of components in different specifications			
	80 μg antibody	400 μg antibody	4mg antibody	Storage temperature
Activated HRP	1 tube	5 tube	5 tubes	-20 °C, away from light
Labeling Buffer H	5 mL	5 mL	5 mL	4 °C, away from light
Antibody Modification Reagents	60 μL	60 μL	60 μL×2	-20 °C, away from light
Ultrafiltration Tube * ,50K MWCO	1 set **	1 set **	1 set **	RT
Blocking reagent	300 μg	300 μg	300 μg×4	-20 °C, away from light
DMSO (dissolve blocking reagent)	50 μL	50 μL	200 μL	-20 °C, away from light
Labeled protein storage solution	1mL	1mL	5mL	2-8 °C
Recommended amount of labeled antibody	Each tube is labeled with 20-80μg, and the best is 20μg	Each tube is labeled with 20-80μg, and the best is 20μg	Each tube is labeled with 200-800μg, and the best is 200μg	

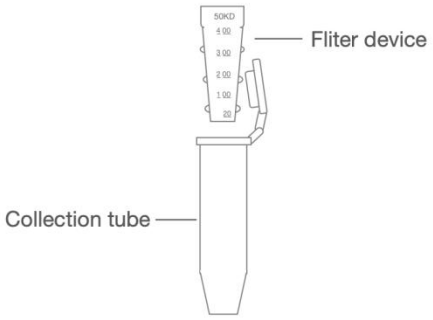
*Ultrafiltration tube instructions:

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 H**, 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 H.

Labeling steps (applicable to labeling 1mg-4mg antibody)

1. **Ultrafiltration concentration of antibody samples** : Take 1mg 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 60-100µL 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 20 µL of antibody modification reagent to 1mg of antibody , mix gently and react at 37°C for 60 minutes.
3. **HRP and antibody coupling:** Add 50 µL of labeling buffer to each of 5 tubes of activated HRP (each tube labeled with 200 µg-800 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 & Purify:** Add 30μL DMSO to a 300μg blocking reagent tube and mix to dissolve, then add 30μL blocking reagent according to 1mg antibody, mix and react at 37°C in the dark for 30 minutes to block the unreacted active groups . Transfer the labeled mixture back into a 50K MWCO ultrafiltration tube and recover the HRP-labeled mixture after ultrafiltration 3 to 5 times with the labeling buffer.
5. **Storage:**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 100μg-400μg 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 30-50μL 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. **HRP and antibody coupling:** Add 5 tube of activated HRP (each tube is labeled with 20μg-80μg antibody) to 10μL 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 & Purify:** Add 30μL DMSO to a 300μg 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.Transfer the labeled mixture back into a 50K MWCO ultrafiltration tube and recover the HRP-labeled mixture after ultrafiltration 3 to 5 times with the labeling buffer.
5. **Storage:** 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 20μg-80μg 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. **HRP and antibody coupling:** Add 1 tube of activated HRP (each tube is labeled with 20μg-80μg antibody) to 10μL 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 1.5h or at 4°C in the dark overnight .

4. **Block & Purify:** Add 30μL DMSO to a 300μg 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.Transfer the labeled mixture back into a 50K MWCO ultrafiltration tube and recover the HRP-labeled mixture after ultrafiltration 3 to 5 times with the labeling buffer.

5. **Storage:** 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) .

Precautions

1. This kit is most suitable for labeling conventional IgG antibodies (150kDa). 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 HRP 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 Ultrafiltration tube instructions

1. The 50K MWCO ultrafiltration tube equipped with this kit has a maximum filter capacity of 500μL 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 20ug of antibody to 20-30μL, 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.