KAMIYA BIOMEDICAL COMPANY

Peroxidase-SH Labeling Kit

For the rapid Peroxidase labeling of protein for EIA and Immunoblotting/Immunostaining

Cat. No. KT-332

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

Peroxidase-SH Labeling Kit is for the rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA) and immunoblotting/immunostaining. It can also be used for the preparation of peroxidase-labeled antigen for competitive EIA. SH-reactive peroxidase (a component of this kit) has maleimide groups and can easily make a covalent bond with a sulfhydryl group of the target molecule without any activation process. If the target is a small molecule, the conjugate can be purified with the Filtration Tube included in this kit. Peroxidase-SH Labeling Kit contains all of the necessary reagents for peroxidase labeling, including the reducing agent for preparation of reduced IgG and the storage buffer for the conjugates.

COMPONENTS

- Peroxidase, SH-reactive 3 X 100 µg
- Reducing Agent 3 tubes
- Solution A 4 mL
- Solution B 1 mL
- Reaction Buffer 200 µL
- Storage Buffer 4 mL
- Filtration Tubes 3 tubes.

Materials or equipment required but not provided

- 0.5 mL microtubes.
- 10 µL and 200 µL adjustable pipettes.
- Microcentrifuge
- 37˚C Incubator

SAMPLE REQUIREMENT

Proteins: Molecular weight >50, 000; amount: 50-200 µg (IgG)
Small Molecule: Molecular weight <5,000

PROCEDURE

Labeling of IgG (Protein)

1. Add 100 µL of Solution A and the sample solution containing 50-200 µg of IgG to the Filtration Tube.
2. Mix the solution with a pipette several times and then centrifuge at 8,000-10,000 g for 10 minutes.
3. Add 150 µL Solution A to Reducing Agent and dissolve it by vortexing.
4. Transfer 100 µL of the Reducing Agent solution from above to the membrane of the Filtration Tube where the IgG is concentrated.
5. Pipette the mixture up and down several times to mix and then incubate the tube at 37˚C for 30 minutes.
6. Add 100 µL of Solution B to the tube and centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate, add 200 µL of Solution B and centrifuge again.
7. Add 50 µL of Reaction Buffer to the Peroxidase and dissolve it by pipetting.
8. Transfer the Peroxidase solution onto the membrane of the Filtration Tube where the IgG is concentrated.
9. Pipette several times to mix and then incubate the tube at 37˚C for 1 hour.
10. Add 100 µL of Solution A to the tube and centrifuge at 8,000-10,000 g for 10 minutes
11. Add 200 µL of Storage Buffer to the membrane where the IgG is concentrate and pipette 10 to 15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube and store the solution at 4˚C.

Precautions

IgG or peroxidase–conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling with this kit. IgG solution can be purified by IgG purifications kits (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for labeling.

The recommended amount of IgG is 100 μg in a volume of 100 μL or less. If the antibody concentration is lower than 0.5 mg/mL, repeat step 1 and 2 until the total IgG accumulation becomes 50-200 μg. If the volume of the filtrate becomes 400 μL or more during the process, discard the filtrate prior to going on to the next centrifuge step.

If solution still remains on the membrane after centrifugation, spin another 5 minutes or increase the centrifuge speed.

The concentration of the conjugate is 0.5-1.3 mg/mL. Dilute the peroxidase-labeled reduced IgG to prepare a solution with an appropriate concentration prior to using it for enzyme immunoassay, immunoblotting or immunostaining. One to two molecules of peroxidase should be introduced onto one reduced IgG molecule. Unconjugated peroxidase should not interfere with normal immunoassays. If purification is necessary, use a permeation column or an affinity column for IgG.

Generally the peroxidase-labeled reduced IgG in Storage Buffer is stable for at least 2 months at 4˚C. For longer storage, add glycerol (final concentration 50%), aliquot and store at -20˚C. However, it is important to note that the stability will depend on the sample itself.

Labeling of Small Molecule

1. Prepare 50 μL of 1 mM thiol compound solution (sample) with Reaction Buffer and add the solution to a tube of SH-reactive Peroxidase. Pipette several times to mix and incubate at 37˚C for 1 hour.
2. Add 100 μL Solution A to the sample and transfer the entire solution to the Filtration Tube.
3. Centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate and add 200 μL of Solution A to the tube and centrifuge at 8,000-10,000 g for 10 minutes. Add 200 μL of Solution A and centrifuge again.
4. Add 200 μL of Storage Buffer and pipette 10-15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube and store the solution at 4˚C.

Precautions

If the thiol compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare a 10 mM solution. Mix 5 μL of the solution with 45μL Reaction Buffer.

If solution still remains on the membrane after the centrifugation, spin again for 5 minutes or increase the centrifuge speed.

The concentration of the conjugate is 400-500 μg/mL (10-12.5 μM). One to two target molecules should be conjugated with one peroxidase molecule.

The peroxidase-labeled small molecule is stable for at least 6 months at 4˚C.

STORAGE

Store all components at 4˚C. Stable for a year at 4˚C with protection from moisture.

FAQ

Q. Can I use this kit with $F(ab)_2$?
A. Yes, please follow the labeling protocol for IgG. The recovery should be over 80%.

Q. Can I use this kit for other proteins or peptides?
A. Yes, if the molecular weight of the reduced form is greater than 50,000 or less than 5,000 and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. If the molecular weight is greater than 50,000, follow the labeling protocol for IgG and use 0.5-1.0 nmol of sample protein. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules.

Q. Can I use this kit on oligonucleotides?
A. Yes, if the molecular weight is less than 5,000 and it has at least one SH group. Follow the label for small molecules.

Q. What is the minimum amount of IgG that can be labeled with this kit?
A. The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. However, even 10 µg of IgG can be labeled using 1/5 volume of SH-reactive Peroxidase solution in step 8.

Q. How many peoxidase molecules per reduced IgG are introduced?
A. Average number of peroxidase molecules per reduced IgG is 1 to 2.

Q. Do I have to use a filtration tube prior to labeling the protein?
A. If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg/mL, or about 70 µM, there is no need to use the filtration tube. Just mix 10 µL of the sample solution with Solution B and add the mixture to a vial of the SH-reactive Peroxidase.

Q. Do I have to use Storage buffer included in the kit?
A. No, you do not have to use storage buffer from the kit. You can choose a buffer that is appropriate for your experiment.

FOR RESEARCH USE ONLY

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