



KAMIYA BIOMEDICAL COMPANY

Peroxidase Labeling Kit-SH

For rapid preparation of peroxidase-labeled IgG

Cat. No. KT-332

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT

Peroxidase Labeling Kit-SH is for rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA. SH-Reactive Peroxidase (a component of this kit) has a maleimide group, 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 Filtration Tube included in this kit. This kit contains all of the necessary reagents for peroxidase labeling, including the Storage Buffer for conjugates.

COMPONENTS

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|--------------------------|---------|
| • SH-Reactive Peroxidase | 3 tubes |
| • Reducing Agent | 3 tubes |
| • Solution A | 4 mL |
| • Solution B | 1 mL |
| • Reaction Buffer | 200 µL |
| • Storage Buffer | 4 mL |
| • Filtration Tube | 3 tubes |

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microtubes
- 10 µL, 200 µL adjustable pipettes
- Microcentrifuge
- Incubator (37°C)

CAPACITY

Three samples labeling

Sample requirement:

Protein (Molecular weight >50,000; amount: 50-200 µg)

Small Molecule (Molecular weight <5,000)

PROCEDURE

Labeling for IgG

If the target protein has free SH groups, skip the reducing procedure (Step 3-6).

1. Add 100 µL Solution A and the sample solution containing 50-200 µg IgG to a Filtration Tube. The volume of sample solution should be less than 100 µL. If the IgG concentration is lower than 0.5 mg/mL, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.
2. Pipette to mix and centrifuge at 8,000 x g for 10 min. If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
3. Add 150 µL Solution A to Reducing Agent, and dissolve with pipetting. The reagent may be attached to the inner wall of the cap. Please open the cap carefully.
4. Transfer 100 µL the Reducing Agent solution to the Filtration Tube, and pipette to dissolve the IgG. The reagent may cleave a disulfide bond of IgG except a hinge region.
5. Incubate the tube at 37°C for 30 min. Add 100 µL Solution B and centrifuge at 8,000 x g for 10 min. If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
6. Discard the filtrate, add 200 µL Solution B and centrifuge at 8,000 x g for 10 min again. If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
7. Add 50 µL Reaction Buffer to SH-Reactive Peroxidase, and dissolve it with pipetting. SH-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 8 immediately after the preparation of the SH-Reactive Peroxidase solution.
8. Add SH-Reactive Peroxidase solution to the Filtration Tube and pipette to mix.
9. Incubate the tube at 37°C for 1 h.

10. Add 100 μ L Solution A to the tube.
11. Centrifuge at 8,000 x g for 10 min. If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
12. Add 200 μ L Storage Buffer, pipette about 10 times to recover the conjugate. Transfer the solution to a microtube (not included in this kit), and store the solution at 4°C. Two to four molecules of peroxidase should be introduced to one IgG molecule. Unconjugated peroxidase should not interfere with normal immunoassays. If purification is necessary, use a gel permeation column or an affinity column for IgG. We recommend using Storage Buffer to recover the conjugate. However, you can use an appropriate buffer for the downstream experiments.

PRECAUTION

If the target protein solution contains other proteins with molecular weight larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for peroxidase labeling, because it might interfere the labeling reaction.

If the protein solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.

The amount of Reducing Agent is optimized for the preparation of the reduced IgG. Please examine the necessary amount of Reducing Agent for the reduction of other proteins.

The droplets which induced from the dry inhibitor of membrane, are occasionally found inside Filtration Tube while storing the kit at 4°C or after returning to room temperature. This phenomenon does not affect the performance.

This kit includes microtubes containing solutions. Since there is a possibility that the droplets might attach to the inside walls or caps, please shake them down prior to open.

Labeling for Small Molecule with Sulfhydryl Group

1. Prepare 50 μ L of 1 mmol/L thiol compound solution with Reaction Buffer. Add this solution to a tube of SH-Reactive Peroxidase. If the thiol compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mmol/L solution, and mix 5 μ L of this solution with 45 μ L Reaction Buffer.
2. Pipette to dissolve SH-Reactive Peroxidase completely, and incubate the tube at 37°C for 1 h.
3. Add 100 μ L Solution A to the reaction solution, and transfer the solution to a Filtration Tube.
4. Centrifuge at 8,000 x g for 10 min. If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
5. Discard the filtrate, add 200 μ L Solution A to the tube.
6. Centrifuge at 8,000 x g for 10 min. If the solution still remains on the membrane after the centrifugation, spin for another 5 min. Add 200 μ L Solution A and centrifuge again.
7. Add 200 μ L Storage Buffer, and pipette about 10 times to dissolve the conjugate. Transfer the solution to a microtube (not included in this kit), and store the solution at 4°C. One to two target molecules should be conjugated with one peroxidase molecule. We recommend using Storage Buffer to recover the conjugate. However, you can use an appropriate buffer for the downstream experiments.

STORAGE

Store at 4°C. This kit is stable until the expiration date at 4°C before opening.

Caution: After a SH-Reactive Peroxidase is taken out from the seal bag, keep the unused SH-Reactive Peroxidase(s) in the bag, seal tightly and store at -20°C. Store the other components at 4°C.

FAQ

Q. Can I use this kit to label antibody which is commercially available?

A. Yes. However, if antibody solution contains other proteins such as serum albumin or gelatin, labeling reaction might be interfered by that proteins. Purification of the antibody solution with affinity chromatography is necessary prior to using this kit. Contact us for the purification procedure, if you need.

Q. How long is the peroxidase labeled protein stable?

A. The stability depends on the protein itself. In the case of labeling for goat IgG, the labeled IgG is stable at 4°C for 2 months. However, for longer storage, add equal volume of glycerol to the sample solution and store at -20°C.

Q. Can I use this kit for other proteins?

A. Yes, if the molecular weight is higher than 50,000 or lower than 5,000, and it has a reactive sulfhydryl group, or a disulfide group that can be reduced without losing activity. If the molecular weight is higher than 50,000, follow the labeling protocol for IgG, and use 50-200 μ g of sample protein. If it is lower than 5,000, follow the labeling protocol for

small molecules. If the molecular weight is lower than 50,000 but higher than 5,000, please contact us.

Q. Can I use this kit to label oligonucleotides or peptides?

A. Yes, if the molecular weight is less than 5,000, and it has at least one sulfhydryl group. Follow the labeling protocol for small molecules.

Q. What is the minimum amount of IgG that can be labeled with this kit?

A. We recommend using 50 μg as a minimum amount. Though 10 μg IgG can still be labeled using this kit, the background will be increased.

FOR RESEARCH USE ONLY

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