KAMIYA BIOMEDICAL COMPANY

Peroxidase-NH_2 Labeling Kit

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

Cat. No. KT-222

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
Peroxidase-NH_2 Labeling Kit is a simple and rapid method for preparing peroxidase-labeled IgG for enzyme immunoassays (EIA) and immunoblotting/immunostaining. It can also be used for preparing peroxidase-labeled antigen for competitive EIA. NH_2-reactive peroxidase (a component of this kit) has activated ester groups and can easily make a covalent bond with an amino 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. The Filtration Tube is also used to remove small molecules such as sodium azide, Tris buffer and amine compounds from the sample that may interfere with the assay or labeling reaction. This kit contains all of the necessary reagents for peroxidase labeling and storage buffer for the conjugates.

COMPONENTS
- Peroxidase, NH_2-reactive 3 X 100 µg
- Reaction Buffer 200 µL
- Wash Buffer 4 mL
- 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 Sample
1. Add 100 µL of Wash Buffer and the sample containing 50-200 µg of IgG into the Filtration Tube.
2. Centrifuge at 8,000-10,000 g for 10 minutes. Add 100 µL Wash Buffer and centrifuge again.
3. Add 10 µL Reaction Buffer to the Peroxidase and dissolve it by pipetting.
4. Transfer the solution containing the Peroxidase onto the membrane where the IgG is concentrated.
5. Rinse the entire surface of the membrane with the solution by pipetting and incubate the tube at 37˚C for 2 hours.
6. Add 100 µL of Washing Buffer to the tube. If the volume of the filtrate is 300 µL or more, discard the filtrate prior to centrifugation.
7. Centrifuge at 8,000-10,000 g for 10 minutes
8. Add 200 µL of Storage Buffer to the membrane where the IgG is concentrated 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 peroxide 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 IgG to prepare a solution with an appropriate concentration prior to using it for enzyme immunoassay, immunoblotting or immunostaining. One to three molecules of peroxidase should be introduced onto one 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 amine compound solution with Reaction Buffer and add the solution to a tube of Peroxidase, NH2-reactive. Pipette several times to mix and incubate at 37˚C for 1 hour.
2. Add 100 µL Wash Buffer to the reaction sample and transfer the entire solution to a Filtration Tube.
3. Centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate and add 200 µL of Wash Buffer to the tube and centrifuge again. Add 200 µL Washing buffer 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 amine 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 6 months 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 primary or secondary amino group. If the molecular weight is greater than 50,000, follow the labeling protocol for IgG and use 0.5-1 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 a reactive or secondary amino 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 still be labeled using this kit, the background will be higher.

Q. How many peoxidase molecules per IgG are introduced?
A. Average number of peroxidase molecules per reduced IgG is 1 to 3.

Q. Does unconjugated NH-reactive peroxidase still have an activated ester after the labeling reaction to IgG?
A. No, it is completely hydrolyzed during the reaction.

Q. Does NH reactive peroxidase form an oligomer during the labeling reaction?
A; No, since all the amino groups of the peroxidase, NH_2-reactive are blocked, no oligomerization is possible.

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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