



**KAMIYA BIOMEDICAL COMPANY**

# **Peroxidase Labeling Kit-NH<sub>2</sub>**

**For simple and rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA**

**Cat. No. KT-222**

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

## PRODUCT INFORMATION

### Peroxidase Labeling Kit-NH<sub>2</sub> Cat. No. KT-222

#### PRODUCT

Peroxidase Labeling Kit-NH<sub>2</sub> is for simple and rapid preparation peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA. NH<sub>2</sub>-reactive peroxidase (a component of this kit) has succinimidyl 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 Filtration Tube included in this kit. Filtration Tube is also used for sample IgG in removing small molecules such as sodium azide, Tris buffer and amine compounds interfere with the assay or labeling reaction. This kit contains all of the necessary reagents for peroxidase labeling, including the Storage Buffer for conjugates.

#### COMPONENTS

- |  |            |
|--|------------|
| • NH <sub>2</sub> -Reactive Peroxidase | 3 tubes    |
| • Reaction Buffer                      | 200 µL x 1 |
| • Washing Buffer                       | 4 mL x 1   |
| • Storage Buffer                       | 4 mL x 1   |
| • 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

1. Add 100 µL Washing Buffer 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 100 µL Washing Buffer to the Filtration Tube again.
4. 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.
5. Add 10 µL Reaction Buffer to NH<sub>2</sub>-Reactive Peroxidase, and dissolve with pipetting. NH<sub>2</sub>-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 6 immediately after the preparation of the NH<sub>2</sub>-Reactive Peroxidase solution.
6. Add NH<sub>2</sub>-Reactive Peroxidase solution to the Filtration Tube and pipette to mix.
7. Incubate the tube at 37°C for 2 h.
8. Add 100 µL Washing Buffer to the Filtration Tube.
9. 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.
10. Add 200 µL 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. 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 gel permeation column or an affinity column for IgG. We recommend using Storage Buffer to recover the conjugate. You can choose any kind of buffer appropriate for your experiment.

### Labeling for Small Molecule with Amino Group

1. Prepare 50  $\mu\text{L}$  of 1 mmol/L amine compound solution with Reaction Buffer. Add this solution to a tube of  $\text{NH}_2$ -Reactive Peroxidase. If the amine compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mmol/L solution, and mix 5  $\mu\text{L}$  of this solution with 45  $\mu\text{L}$  Reaction Buffer.
2. Pipette to dissolve  $\text{NH}_2$ -Reactive Peroxidase completely, and incubate the tube at 37°C for 1 h.
3. Add 100  $\mu\text{L}$  Washing Buffer 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  $\mu\text{L}$  Washing Buffer 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  $\mu\text{L}$  Washing Buffer and centrifuge again.
7. Add 200  $\mu\text{L}$  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. You can use any kind of buffer appropriate for your experiment.

### 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 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.

### STORAGE

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

**CAUTION: After a  $\text{NH}_2$ -Reactive Peroxidase is taken out from the sealed bag, keep the unused  $\text{NH}_2$ -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 protein. 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 primary or secondary amino group. If the molecular weight is higher than 50,000, follow the labeling protocol for IgG, and use 50-200  $\mu\text{g}$  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 a reactive primary or secondary amino 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  $\mu\text{g}$  as a minimum amount. Though 10  $\mu\text{g}$  IgG can still be labeled using this kit, the background will be increased.

Q. Does NH<sub>2</sub>-Reactive Peroxidase form an oligomer during the labeling reaction?

A. No. Since all amino groups of NH<sub>2</sub>-Reactive Peroxidase are blocked, no oligomerization is occurred.

**FOR RESEARCH USE ONLY**

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