# Peroxidase Labeling Kit-$NH_2$

## Introduction

Peroxidase Labeling Kit-NH2 is for simple and rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA. NH2-reactive peroxidase (a component of this kit) has an activated ester group, 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 that interfere with the assay or labeling reaction. This kit contains all of the necessary reagents for peroxidase labeling, including the storage buffer for conjugates.

**Description**

**HRP Labeling kit-NH2 # BT3772 1 rxn / 1 mg**

Kit contains:

| | | |
|---|---|---|
| – | NH2-reactive peroxidase | 1 mg x 1 |
| – | Washing buffer | 10 ml x 1 |
| – | Reaction buffer | 1,2 ml x 1 |
| – | Storage buffer | 10 ml x 1 |
| – | Filtration tube | 1 tube |
| – | 15 ml tube | 1 tube |

**Capacity:**

Protein (Molecular weight > 50000, IgG: 0,5 – 2 mg)
Small molecule (Molecular weight < 5000)

**Storage condition**

Store at 0-5 °C. This kit is stable for 6 months at 0-5 °C with protection from moisture.

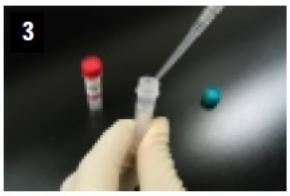
**Equipment and material non provided**

- 200 µl and 1 ml pipettes
- Incubator (37 °C)
- Centrifuge and rotor for 15 ml tube
- Microtube

## Labeling Procedure for IgG

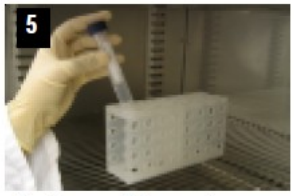

Add 1 ml Washing buffer and the sample solution containing 0.5-2 mg IgG to Filtration tube.[a] Prepare a 15 ml centrifuge tube.[b]


Centrifuge at 7,000 g for 30 min if using a fixed angle rotor. Add 1 ml Reaction buffer and centrifuge once more.[c]




Add 50 µl Reaction buffer to $NH_2$-reactive peroxidase and dissolve with pipetting.


Add the $NH_2$-reactive peroxidase solution to the IgG in the Filtration tube, which should be concentrated to about 50 µl.




Pipette several times and incubate the tube at 37 °C for 2 hrs.


Add 1 ml Washing buffer to the tube. If the volume of the filtrate is 4 ml or more, discard the filtrate prior to go to Step 7.


Centrifuge at 7,000 g for 30 min if using a fixed angle rotor.[b, c]


Add 2 ml Storage buffer and pipette 10 to 15 times to recover the conjugate.[d] Transfer the solution to a microtube, and store the solution at 0-5 °C.[e]

*Precaution*

IgG or peroxidase-conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to label peroxidase with this kit. IgG solution can be purified by IgG Purification Kits or a protein A/G column (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

*a) The recommended amount of IgG is 1 mg. The volume of sample solution should be 3 ml or less. If the volume of sample solution is larger than 3 ml, repeat step 1 and 2 until the total IgG accumulation becomes 0.5-2 mg. If the volume of the filtrate becomes 4 ml or more during the accumulation process, discard the filtrate prior to going to the next centrifuge step.*

*b) Measure the weight of the filtration tube. Prepare a same weight of 15 ml centrifuge tube with water. Use this 15 ml centrifuge tube to balance rotor.*

*c) If more than 50 µl of the solution still remains on the membrane after the centrifugation, spin for another 10 min. If using a swinging bucket rotor, centrifuge at 5,000 g.*

*d) 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 gel permeation column or an affinity column for IgG.*

*e) Generally, the peroxidase-labeled IgG in Storage buffer is stable for at least 2 months at 0-5°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 Procedure for Small Molecule with Amino Group


Prepare 0.5 ml of 1 mM amine compound solution with Reaction buffer,[a] and add the solution to $NH_2$-reactive peroxidase. Pipette several times to mix and incubate at 37 °C for 1 hr.


Add the reaction solution prepared at Step 1 and 1 ml Washing buffer to a Filtration tube. Prepare a 15 ml centrifuge tube.[b]


Centrifuge at 7,000 g for 20 min if using a fixed angle rotor.[c] Discard the filtrate. Add 2 ml Washing buffer to the tube, and centrifuge at 7,000 g for 20 min. Add 2 ml Washing buffer and centrifuge again.[b, c]


Add 2 ml Storage buffer and pipette 10 to 15 times to recover the conjugate.[d] Transfer the solution to a microtube, and store the solution at 0-5 °C.[e]

*a) If the amine compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mM solution, and mix 50 µl of this solution with 450 µl Reaction buffer.*

*b) Measure the weight of the filtration tube. Prepare a same weight of 15 ml centrifuge tube with water. Use this 15 ml centrifuge tube to balance rotor.*

*c) If more than 50 µl of the solution still remains on the membrane after the centrifugation, spin for another 10 min. If using a swinging bucket rotor, centrifuge at 5,000 g.*

*d) The concentration of the conjugate is about 400-500 µg per ml. One to two target molecules should be conjugated with one peroxidase molecule.*

*e) The peroxidase-labeled small molecule should be stable for at least 6 months at 0-5°C.*

## Frequently asked questions

- **Can I use this kit for other proteins or peptides?**
  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 0.5-1 nmol 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, contact our customer service at Interbiotech@interchim.com for more information.
- **Can I use this kit to label an oligonucleotide?**
  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.
- **Can I use this kit for Fab or Fab' labeling?**
  Yes, you can label Fab or Fab' using this kit. The recovery of the conjugate should be over 80%.
- **How many peroxidase molecules per IgG are introduced?**
  Average number of peroxidase molecule per IgG is 1 to 3.

- **Does unconjugated NH2-reactive peroxidase still have an activated ester after the labeling reaction to IgG?**
  No. It is completely hydrolyzed during the reaction. That is one of the reasons why blocking or purification steps are not necessary.
- **Does NH2-reactive peroxidase form an oligomer during the labeling reaction?**
  No. Since all amino groups of NH2-reactive peroxidase are blocked, no oligomerization is possible.
- **Do I have to use Storage buffer included with the kit?**

No, you do not have to use Storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment.

## Other information

For R&D use in vitro only.
Also available in kit format of 1mg labeling (#BT3772) and for 5/10mg labeling (#BT3773) .

**Related product:**
HRP labeling kit-SH (#BT7691)

For more information, please ask interbiotech@interchim.com