Peroxidase Labeling Kit - NH_2 Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk11.pdf

General Information

Peroxidase Labeling Kit - NH_2 is for simple and rapid preparation of peroxidase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and peroxidase-labeled antigen for competitive EIA. NH_2-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 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.

Kit Contents

- NH_2-Reactive Peroxidase..... 3 tubes
- Reaction Buffer.....200 µl x 1
- Filtration Tube..... 3 tubes
- Washing Buffer ..... 4 ml x 1
- Storage Buffer..... 4 ml x 1

Capacity

Three samples labeling

- Sample requirement: Protein (Molecular weight > 50,000; amount: 50-200 µg)
  Small molecule (Molecular weight < 5,000)

Storage Condition

Store at 0-5°C. This kit is stable for 1 year at 0-5°C before opening.

Caution

After a NH_2-Reactive Peroxidase is taken out from the seal bag, keep the unused NH_2-Reactive Peroxidase(s) in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

Required Equipment

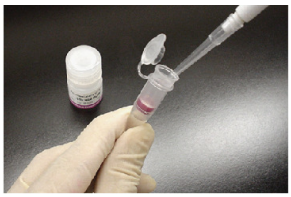
- 10 µl, 200 µl adjustable pipettes
- Microcentrifuge
- Incubator (37°C)
- Microtubes

Precaution

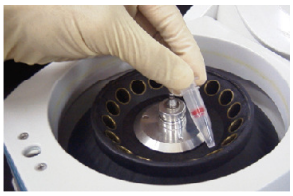
- If the target protein solution contains other proteins with molecular weight larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for peroxidase labeling, because it might interfere the labeling reaction.
- If the protein solution contains 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 0-5°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.

General Protocol -1

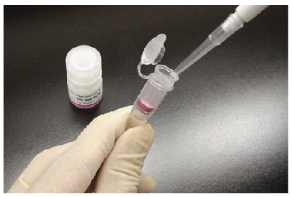
- Labeling for IgG -



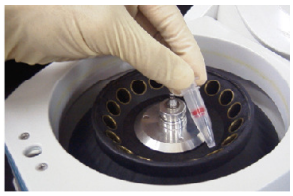
Step 1.
Add 100 µl Washing Buffer and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.



Step 2.
Pipette to mix and centrifuge at 8,000 x *g* for 10 min.[b]



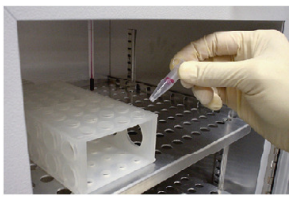
Step 3.
Add 100 µl Washing Buffer to the Filtration Tube again.



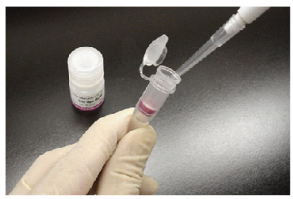
Step 4.
Centrifuge at 8,000 x *g* for 10 min again.[b]

Step 5.
Add 10 µl Reaction Buffer to NH_2-Reactive Peroxidase, and dissolve with pipetting.[c]

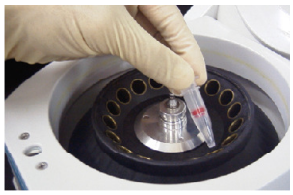
Step 6.
Add NH_2-Reactive Peroxidase solution to the Filtration Tube and pipette to mix.



Step 7.
Incubate the tube at 37°C for 2 h.



Step 8.
Add 100 µl Washing Buffer to the Filtration Tube.

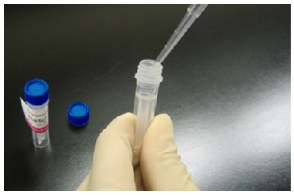


Step 9.
Centrifuge at 8,000 x *g* for 10 min.[b]

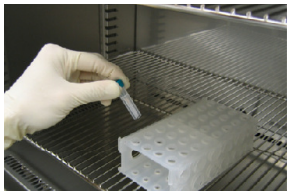
Step 10.
Add 200 µl Storage Buffer, pipette about 10 times to recover the conjugate.[d] Transfer the solution to a microtube (not included in this kit), and store the solution at 0 - 5°C[e].

a) 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.
b) If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
c) NH_2-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Peroxidase solution.
d) 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) We recommend using Storage Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.

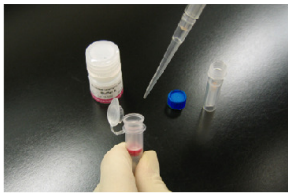
- Labeling for Small Molecule with Amino Group -



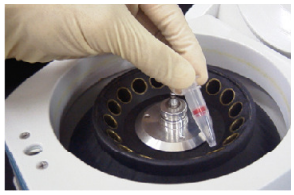
Step 1.
Prepare 50 µl of 1 mmol/l amine compound solution[a] with Reaction Buffer. Add this solution to a tube of NH_2-Reactive Peroxidase.



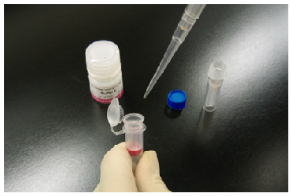
Step 2.
Pipette to dissolve NH_2-Reactive Peroxidase completely, and incubate the tube at 37°C for 1 h.



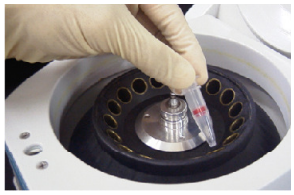
Step 3.
Add 100 µl Washing Buffer to the reaction solution, and transfer the solution to a Filtration Tube.



Step 4.
Centrifuge at 8,000 x *g* for 10 min.[b]



Step 5.
Discard the filtrate, add 200 µl Washing Buffer to the tube.



Step 6.
Centrifuge at 8,000 x *g* for 10 min.[b] Add 200 µl Washing Buffer and centrifuge again.

Step 7.
Add 200 µl Storage Buffer, and pipette about 10 times to dissolve the conjugate.[c] Transfer the solution to a microtube (not included in this kit), and store at 0 - 5°C.[d]

a) If the amine compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mmol/l solution, and mix 5 µl of this solution with 45 µl Reaction Buffer.
b) If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
c) One to two target molecules should be conjugated with one peroxidase molecule.
d) We recommend using Storage Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.

Q & A

◆ **Can I use this kit to label antibody which is commercially available?**
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 use this kit. Contact us for the purification procedure, if you need.

◆ **How long is the peroxidase labeled protein stable?**
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.

◆ **Can I use this kit for other proteins?**
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 µg 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.

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

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

◆ **Does NH_2-Reactive Peroxidase form an oligomer during the labeling reaction?**
No. Since all amino groups of NH_2-Reactive Peroxidase are blocked, no oligomerization is occured.

If you require assistance, please contact Dojindo customer service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun,Kumamoto 861-2202, Japan
Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies, Inc.
30 W Gude Dr, Suite 260, Rockville, MD 20850
Tel: +1-301-987-2667, Fax: +1-301-987-2687