Peroxidase Labeling Kit - SH

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk09.pdf

General Information

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 an 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.

Kit Contents

- SH-Reactive Peroxidase ..... 3 tubes
- Solution A ..... 4 ml x 1
- Reaction Buffer ..... 200 µl x 1
- Filtration Tube ..... 3 tubes
- Reducing Agent ..... 3 tubes
- Solution B ..... 1 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 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 0-5°C.

Required Equipment

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

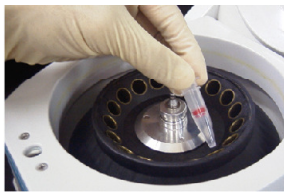
Precaution

- 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 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 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.

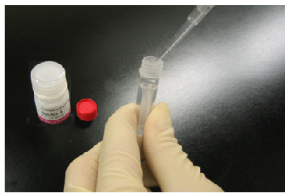
General Protocol -1

- Labeling for IgG[a] -

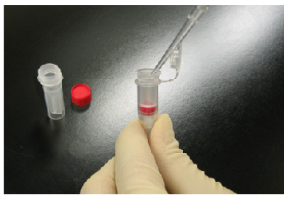
Step 1.
Add 100 µl Solution A and the sample solution containing 50-200 µg IgG[b] to a Filtration Tube.



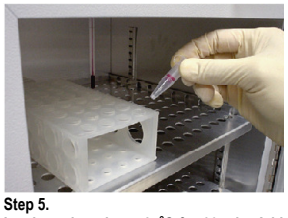
Step 2.
Pipette to mix and centrifuge at 8,000 x *g* for 10 min.[c]



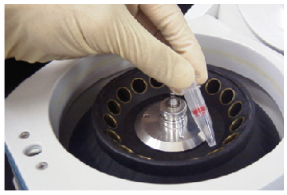
Step 3.
Add 150 µl Solution A to Reducing Agent,[d] and dissolve with pipetting.



Step 4.
Transfer 100 µl the Reducing Agent solution to the Filtration Tube, and pipette to dissolve the IgG.[e]



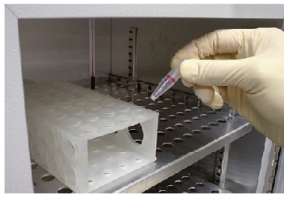
Step 5.
Incubate the tube at 37°C for 30 min. Add 100 µl Solution B and centrifuge at 8,000 x *g* for 10 min.[c]



Step 6.
Discard the filtrate, add 200 µl Solution B and centrifuge at 8,000 x *g* for 10 min again.[c]

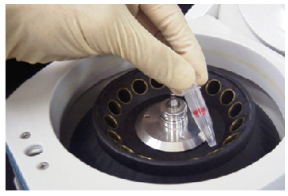
Step 7.
Add 50 µl Reaction Buffer to SH-Reactive Peroxidase, and dissolve it with pipetting.[f]

Step 8.
Add SH-Reactive Peroxidase solution to the Filtration Tube and pipette to mix.



Step 9.
Incubate the tube at 37°C for 1 h.

Step 10.
Add 100 µl Solution A to the tube.

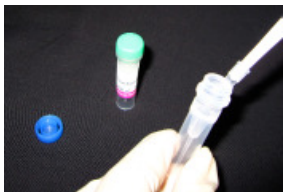


Step 11.
Centrifuge at 8,000 x *g* for 10 min.[c]

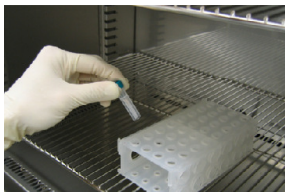
Step 12.
Add 200 µl Storage Buffer, pipette about 10 times to recover the conjugate.[g]
Transfer the solution to a microtube (not included in this kit), and store the solution at 0-5°C.[h]

a) If the target protein has free SH groups, skip the reducing procedure (Step 3-6).
b) 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.
c) If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
d) The reagent may be attached to the inner wall of the cap. Please open the cap carefully.
e) The reagent may cleave a disulfide bond of IgG except a hinge region.
f) SH-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 8 immediately after the preparation of the SH-Reactive Peroxidase solution.
g) 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.
h) We recommend using Storage Buffer to recover the conjugate. However, you can use an appropriate buffer for the downstream experiments.

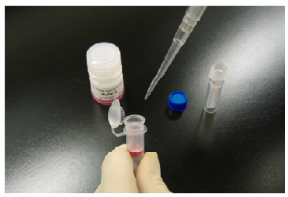
- Labeling for Small Molecule with Sulfhydryl Group -



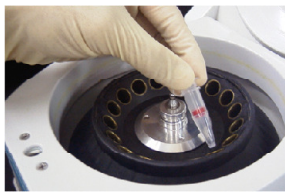
Step 1.
Prepare 50 µl of 1 mmol/l thiol compound solution[a] with Reaction Buffer. Add this solution to a tube of SH-Reactive Peroxidase.



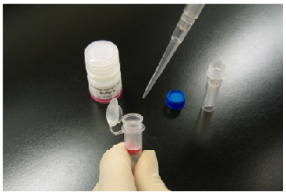
Step 2.
Pipette to dissolve SH-Reactive Peroxidase completely, and incubate the tube at 37 °C for 1 h.



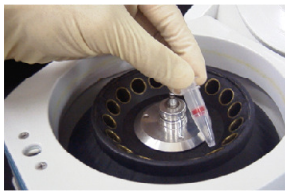
Step 3.
Add 100 µl Solution A to the reaction solution, and transfer the solution to a Filtration Tube.



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



Step 5.
Discard the filtrate, add 200 µl Solution A to the tube.



Step 6.
Centrifuge at 8,000 x *g* for 10 min.[b]
Add 200 µl Solution A 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 the solution at 0-5°C.[d]

a) If the thiol 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. However, you can use an appropriate buffer for the downstream experiments.

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 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.

◆ **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 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 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 at least one sulfhydryl 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.

If you require assistance, please contact Dojindo customer service.
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