Alkaline Phosphatase Labeling Kit - NH_2 (for 1 mg) Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk59.pdf

General Information

Alkaline Phosphatase Labeling Kit-NH_2 is for simple and rapid preparation of alkaline phosphatase-labeled IgG for enzyme immunoassays (EIA), immunoblotting or immunostaining and alkaline phosphatase-labeled antigen for competitive EIA. NH_2-Reactive Alkaline phosphatase (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 alkaline phosphatase labeling, including the Storage Buffer for conjugates.

Kit Contents

- NH_2-Reactive Alkaline phosphatase ..... 1 tube
- Reaction Buffer .....1.2 ml x 1
- Filtration Tube ..... 1 tube
- Washing Buffer.....10 ml x 1
- Storage Buffer .....10 ml x 1
- 15 ml Tube (for counterbalance)..... 1 tube

Capacity

One sample labeling

- Sample requirement: Protein (Molecular weight > 50,000; amount: 1 mg)
  Small molecule (Molecular weight < 5,000)

Storage Condition

Store at 0-5°C. This kit is stable for 6 months at 0-5°C before opening.

Caution

Once a seal bag is opened, keep the unused NH_2-Reactive Alkaline Phosphatase in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

Required Equipment

- 200 µl and 1 ml adjustable pipettes
- Centrifuge and rotor for 15 ml tube
- Incubator (37°C)
- Microtube (> 2 ml)

Precaution

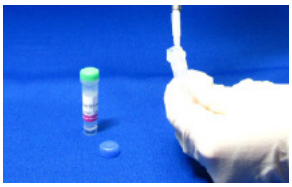
-If the target protein solution contains other proteins with molecular weight larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for alkaline phosphatase labeling, because it might interfere the labeling reaction.

-If the protein solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.

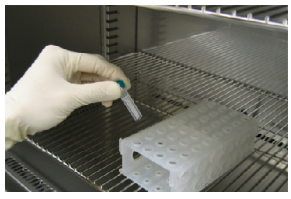
-The droplets which induced from the dry inhibitor of membrane, are occasionally found inside Filtration Tube while storing the kit at 0-5°C or after returning to room temperature. This phenomenon does not affect the performance.

General Protocol -1

- Labeling for Small Molecule with Amino Group -



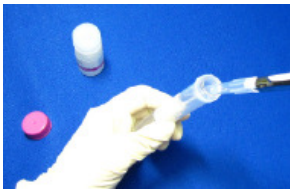
Step 1.
Prepare 0.5 ml of 1 mmol/l amine compound solution[a] with Reaction Buffer. Add this solution to NH_2-Reactive Alkaline Phosphatase.



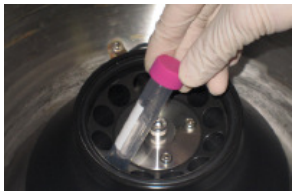
Step 2.
Pipette to dissolve NH_2-Reactive Alkaline Phosphatase completely, and incubate the tube at 37°C for 1 h.

Step 3.
Add the reaction solution prepared at Step 2 and 1 ml Washing Buffer to a Filtration Tube. Prepare a 15 ml Tube.[b]

Step 4.
Centrifuge at 6,000 x *g* for 20 min if using a fixed angle rotor.[c]



Step 5.
Discard the filtrate. Add 2 ml Washing Buffer to the Filtration Tube.



Step 6.
Centrifuge at 6,000 x *g* for 20 min if using a fixed angle rotor.[b, c] Add 2 ml Washing Buffer to the tube, and centrifuge again.

Step 7.
Add 2 ml Storage Buffer, and pipette 10-15 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.[e]

a) If the amine compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare 10 mmol/l 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 Tube with water. Use this 15 ml Tube for counter-balance.

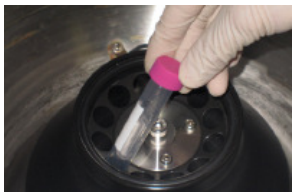
c) Centrifuge at 4,000 x *g* if using a swinging bucket rotor. If more than 100 µl of the solution still remains on the membrane after the centrifugation, spin for another 10 min. If a maximum centrifugal force is less than 6,000 x *g* , additional spin time should be requied (ex. 2,000 x *g* for 50 - 60 min).

d) One to two target molecules should be conjugated with one alkaline phosphatase molecule.

e) We recommend using Storage Buffer to recover the conjugate. However, you can use an appropriate buffer for the downstream experiments.

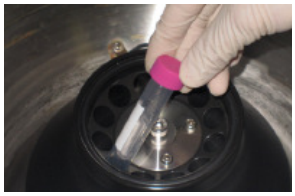
- Labeling for IgG -

Step 1.
Add 1 ml Washing Buffer and the sample solution containing 1 mg IgG[a] to a Filtration Tube. Prepare a 15 ml Tube.[b]

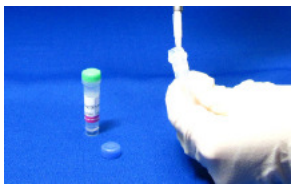


Step 2.
Pipette to mix and centrifuge at 6,000 x *g* for 30 min if using a fixed angle rotor.[b, c]

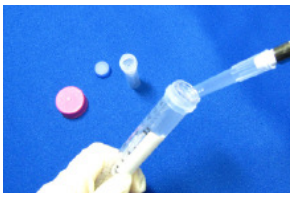
Step 3.
Add 1 ml Reaction Buffer to the Filtration Tube again.



Step 4.
Centrifuge at 6,000 x *g* for 30 min again.[c]



Step 5.
Add 50 µl Reaction Buffer to NH_2-Reactive Alkaline Phosphatase, and dissolve it with pipetting.[d]



Step 6.
Add NH_2-Reactive Alkaline Phosphatase solution to the IgG in the Filtration Tube.

Step 7.
Pipette several times and Incubate the tube at 37°C for 2 h.

Step 8.
Add 1.9 ml Storage Buffer and pipette 10-15 times to recover the conjugate.[e]
Transfer the solution to a microtube (not included in this kit), and store the solution at 0-5°C.[f]

a) 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 1 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 Tube with water. Use this 15 ml Tube for counter-balance.

c) Centrifuge at 4,000 x *g* if using a swinging bucket rotor. If more than 100 µl of the solution still remains on the membrane after the centrifugation, spin for another 10 min. If a maximum centrifugal force is less than 6,000 x *g*, additional spin time should be requied (ex. 2,000 x *g* for 50 - 60 min).

d) NH_2-Reactive Alkaline Phosphatase is unstable in Reaction Buffer. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Alkaline Phosphatase solution.

e) One to three molecules of alkaline phosphatase should be introduced onto one IgG molecule. Unconjugated alkaline phosphatase should not interfere with normal immunoassays. If purification is necessary, use a gel permeation column or an affinity column for IgG.

f) 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 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 alkaline phosphatase 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, please store at -20°C.

◆ **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 1 mg 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 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.

◆ **Does NH_2-Reactive Alkaline Phosphatase form an oligomer during the labeling reaction?**

No. Since all amino groups of NH_2-Reactive Alkaline Phosphatase are blocked, no oligomerization is possible.

If you require assistance, please contact Dojindo customer service.

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