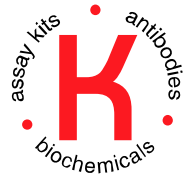

KAMIYA BIOMEDICAL COMPANY

Alkaline Phosphatase-SH Labeling Kit

For the rapid Alkaline Phosphatase labeling of proteins for EIA and Immunoblotting/Immunostaining

Cat. No. KT-330

For Research Use Only.

PRODUCT INFORMATION

Alkaline Phosphatase-SH Labeling Kit
Cat. No. KT-330

PRODUCT

Alkaline Phosphatase-SH Labeling Kit is for the rapid preparation of alkaline phosphatase-labeled IgG for enzyme immunoassays and immunoblotting / immunostaining. It can also be used for the preparation of alkaline phosphatase -labeled antigen for competitive EIA. SH-reactive Alkaline Phosphatase (AP) (a component of this kit) has maleimide groups 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 the Filtration Tube included in this kit. Alkaline Phosphatase-SH Labeling Kit contains all of the necessary reagents for alkaline phosphatase labeling, including the reducing agent for preparation of reduced IgG and the storage buffer for the conjugates.

COMPONENTS

- SH-reactive AP 3 X 100 µg
- Reducing Agent 3 tubes
- Solution A 4 mL
- Solution B 1 mL
- Reaction Buffer 200 µL
- Storage Buffer 4 mL
- Filtration Tubes 3 tubes.

Materials or equipment required but not provided

- 0.5 mL microtubes.
- 10 µL and 200 µL adjustable pipettes.
- Microcentrifuge
- 37˚C Incubator

SAMPLE REQUIREMENT

Proteins: Molecular weight >50,000; amount: 50-200 µg (IgG)
Small Molecule: Molecular weight <5,000

PROCEDURE

Labeling of Sample

1. Add 100 µL of Solution A and the sample solution containing 50-200 µg of IgG to the Filtration Tube.
2. Mix the solution with a pipette several times and then centrifuge at 8,000-10,000 g for 10 minutes.
3. Add 150 µL Solution A to Reducing Agent and dissolve it by pipetting.
4. Transfer 100 µL of the solution from step 3 to the membrane of the Filtration Tube where the IgG is concentrated.
5. Pipette the mixture up and down several times to mix and then incubate the tube at 37˚C for 30 minutes.
6. Add 100 µL of Solution B to the tube and centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate, add 200 µL of Solution B and centrifuge again.
7. Add 50 µL of Reaction Buffer to the SH-reactive AP tube and dissolve the contents by pipetting.
8. Transfer the SH-reactive AP solution onto the membrane of the Filtration Tube where reduced IgG is concentrated.
9. Pipette several times to mix and then incubate the tube at 37˚C for 1 hour.
10. Add 150 µL of Storage Buffer and pipette 10 to 15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube and store the solution at 4˚C.

Precautions

IgG or alkaline phosphatase–conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling with this kit. IgG solution can be purified by IgG purifications kits (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for labeling.

The recommended amount of IgG is 100 µg in a volume of 100 µL or less. If the antibody concentration is lower than 0.5 mg/mL, repeat step 1 and 2 until the total IgG accumulation becomes 50-200 µg. If the volume of the filtrate becomes 400 µL or more during the process, discard the filtrate prior to going on to the next centrifuge step.

If solution still remains on the membrane after centrifugation, spin another 5 minutes or increase the centrifuge speed.

The concentration of the conjugate is 0.5-1.3 mg/mL. Dilute the alkaline phosphatase-labeled reduced IgG to prepare a solution with an appropriate concentration prior to using it for enzyme immunoassay, immunoblotting or immunostaining. One to two molecules of alkaline phosphatase should be introduced onto one reduced 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.

Generally the alkaline phosphatase-labeled reduced IgG in Storage Buffer is stable for at least 2 months at 4˚C. For longer storage, store at -20˚C. However, it is important to note that the stability will depend on the sample itself.

Labeling of Small Molecule

1. Prepare 50 µL of 1 mM thiol compound solution with Reaction Buffer and add the solution to a tube of SH-reactive AP. Pipette several times to mix and incubate at 37˚C for 1 hour.
2. Add 100 µL Solution A to the reaction sample and transfer the entire solution to a Filtration Tube.
3. Centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate and add 200 µL of Solution A to the tube and centrifuge at 8,000-10,000 g for 10 minutes. Add 200 µL of Solution A and centrifuge again.
4. Add 200 µL of Storage Buffer and pipette 10-15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube and store the solution at 4˚C.

Precautions

If the thiol compound does not dissolve in aqueous solution, dissolve it with DMSO to prepare a 10 mM solution. Mix 5 µL of the solution with 45 µL Reaction Buffer.

If solution still remains on the membrane after the centrifugation, spin again for 5 minutes or increase the centrifuge speed.

The concentration of the conjugate is 400-500 µg/mL. One to two target molecules should be conjugated with one alkaline phosphatase molecule.

The alkaline phosphatase-labeled small molecule is stable for at least 6 months at 4˚C.

STORAGE

Store all components at 4˚C. Stable for a year at 4˚C with protection from moisture.

FAQ

Q. Can I use this kit with $F(ab')_2$?
A. Yes, please follow the labeling protocol for IgG. The recovery should be over 80%.

Q. Can I use this kit for other proteins or peptides?
A. Yes, if the molecular weight of the reduced form is greater than 50,000 or less than 5,000 and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. If the molecular weight is greater than 50,000, follow the labeling protocol for IgG and use 0.5-1 nmol of sample protein. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules.

Q. Can I use this kit on oligonucleotides?
A. Yes, if the molecular weight is less than 5,000 and it has at least one SH group. Follow the label for small molecules.

Q. What is the minimum amount of IgG that can be labeled with this kit?
A. The minimum amount is 50 μg. There is no significant difference in sensitivity and background between 50 μg and 200 μg of IgG. However, even 10 μg of IgG can be labeled using 1/5 volume of SH-reactive AP solution in step 8.

Q. How many alkaline phosphatase molecules per reduced IgG are introduced?
A. Average number of alkaline phosphatase molecules per reduced IgG is 1 to 2.

Q. Do I have to use a filtration tube prior to labeling the protein?
A. If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg/mL, or about 70 μM, there is no need to use the filtration tube. Just mix 10 μL of the sample solution with Reaction Buffer and add the mixture to a vial of the SH-reactive AP.

Q. Do I have to use Storage Buffer included in the kit?
A. No, you do not have to use storage buffer from the kit. You can choose a buffer that is appropriate for your experiment.

FOR RESEARCH USE ONLY

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