R-Phycoerythrin Labeling Kit-NH2

Catalog Number KA0012

1 Kit

Version: 01

Intended for research use only

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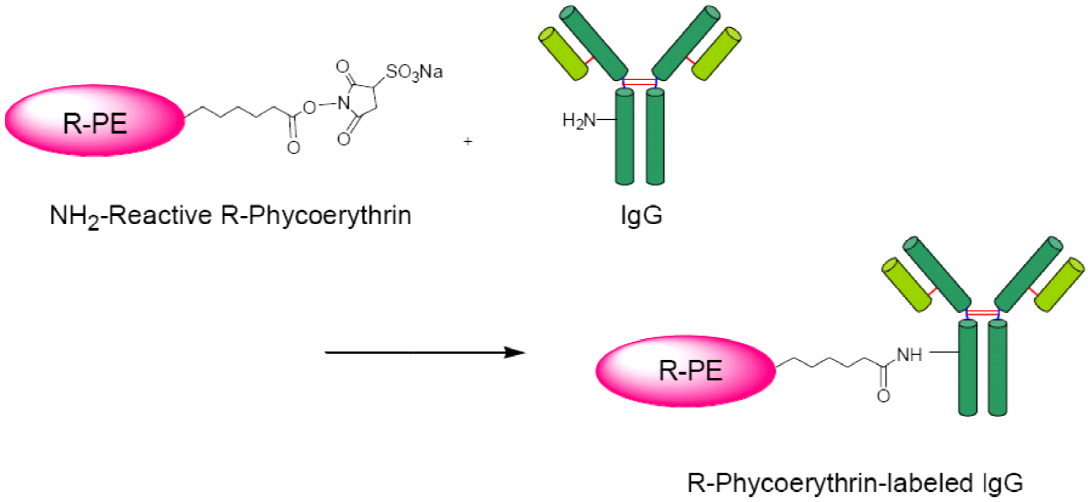
Introduction

Background

R-Phycoerythrin Labeling Kit-NH2 is primarily used for the preparation of R-Phycoerythrin-labeled antibody for immunostaining and cellular proteins for tracing. NH2-Reactive R- Phycoerythrin, a component of this kit, has succinimidyl group (NHS), and can easily make a covalent bond with amino groups of IgG or other proteins without any activation process. Filtration Tube included in this kit is used for removing small molecules in the sample protein such as Tris buffer and amine compounds that interfere with the assay or labeling reaction. The maximum excitation and emission wavelengths of the R-Phycoerythrin-labeled proteins are 564 nm and 575 nm, respectively. This kit contains all of the necessary reagents for labeling, including the storage buffer for conjugate.

Principle of the Assay



NH_2-Reactive R-Phycoerythrin

IgG

R-Phycoerythrin-labeled IgG

General Information

Materials Supplied

List of component

Component	Amount
NH2-Reactive R-Phycoerythrin	3 tubes
WS Buffer	4 ml x 1 bottle
Reaction buffer	200 µl x 1 tube
Filtration tube	3 tubes

Capacity

Sample requirements: molecular weight > 50,000; amount: 50-200 µg

Storage Instruction

- ✓ Store at 0-5 ℃. This kit is stable for six months at 0-5 ℃ with before opening.
- ✓ After a NH2-Reactive R- Phycoerythrin is taken out from the seal bag, keep the unused NH2-Reactive R-Phycoerythrin(s) in the bag, seal tight and store at -20℃.
- ✓ Store the other components at 0-5 ℃.

Materials Required but Not Supplied

- ✓ 10 µl and 200 µl adjustable pipettes
- ✓ Incubator (37 ℃)
- ✓ Microtubes
- ✓ Microcentrifuge

Precautions for Use

If the target protein solution contains other proteins with molecular weights larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for R-Phycoerythrin-labeled, because it might interfere the filtering or labeling reaction. If the protein solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.

Assay Protocol

Assay Procedure

Labeling for IgG

1. Add 100 µl WS Buffer and the sample solution containing 50- 200 µg IgG a) to a Filtration Tube.
2. Pipette to mix and centrifuge at 8,000 x g for 10 min. b)
3. Add 100 µl WS Buffer to a Filtration Tube again.
4. Centrifuge at 8,000 x g for 10 min again. b)
5. Add 10 µl Reaction Buffer to NH2-Reactive R-Phycoerythrin, and dissolve with pipetting. c)
6. Add NH2-Reactive R-Phycoerythrin solution to the IgG concentrated on the Filtration Tube.
7. Incubate the tube at 37℃ for 2 hours after pipetting to mix.
8. Add 190 µl WS Buffer, and pipette about 10 times to recover the conjugate. d) Transfer the solution to a microtube(not included in this kit), and store at 0-5℃.e)

Note:

a. *The volume of IgG 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 solution still remains on the membrane after the centrifugation, spin for another 5 min, or increase the centrifuge speed.*
c. *NH2-Reactive R-Phycoerythrin can be hydrolyzed by water. Proceed to Step 6 immediately after the preparation of the NH2-Reactive R-Phycoerythrin solution.*
d. *One to two R-Phycoerythrin should be introduced into one IgG molecule. Unconjugated R-Phycoerythrin remained in the solution might cause background increase with immunoassay. If purification is necessary, purify the conjugate using a gel permeation column or an affinity column for IgG.*
e. *For longer storage, add equal volume of glycerol to the sample solution and store it at -20℃.*

Resources

Troubleshooting

1. 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.
2. What is the minimum amount of protein that can be labeled using this kit?
   We recommend using 50 µg as a minimum amount. Though 10 µg protein can be labeled using this kit, the background might be increased.
3. Does NH2-Reactive R-Phycoerythrin form an oligomer during the labeling reaction?
   No. Since all amino groups of NH2-Reactive R-Phycoerythrin are blocked, no oligomerization is occurred.
4. Can I use this kit to lavel small molecule such as oligopeptide?
   Yes. The sample compound must have reactive amino group and its molecular weight should be lower than 5,000. You can skip the washing procedures (Step 1 - Step 4.) Simply add the sample solution to the Filtration Tube and proceed to Step 5. The sample solution should not include ather small amine compounds such as Tris. If you use the sample with the molecular weight higher than 5,000 up to 50,000, contact our technical service.
5. Can I use the R-Phycoerythrin conjugated protein that is precipitated in storage?
   Yes. The precipitated protein should be removed by centrifugation at 10,000 x g for 10 min, and use the supernatant.
6. Is there any notice for treatment of living cells with the R-Phycoerythrin conjugated protein?
   We recommend using PBS including 2-10% FBS for preparation of cell suspension to maintain the best cell condition.
7. Does recovery buffer (WS buffer) have harmful effect to living cells?
   No. WS buffer contains stabilizing agent (surfactant) that is controlled of its concentration without cytotoxicity. If you are concerned about the additive in WS buffer, you can use your own buffer currently used instead of WS buffer.