R-Phycoerythrin Labeling Kit - NH_2 Technical Manual

General Information

R-Phycoerythrin Labeling Kit - NH_2 is primarily used for the preparation of R-Phycoerythrin-labeled antibody for immunostaining and cellular proteins for tracing. NH_2-Reactive R-Phycoerythrin, a component of this kit, has succinimidyl ester groups, 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.

Kit Contents

- NH_2-Reactive R-Phycoerythrin ..... 3 tubes
- Reaction Buffer ..... 200 µl x 1
- WS Buffer ..... 4 ml x 1
- Filtration Tube ..... 3 tubes

Capacity

Three samples labeling
- sample requirement : molecular weight > 50,000; amount: 50-200 µg

Storage Condition

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

Caution

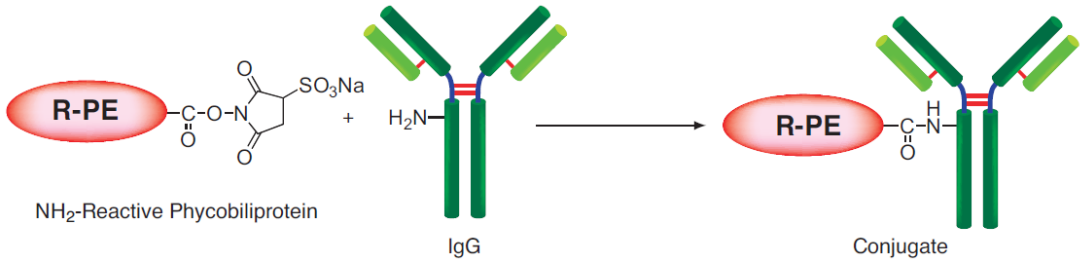
After a NH_2-Reactive R-Phycoerythrin is taken out from the seal bag, keep the unused NH_2-Reactive R-Phycoerythrin(s) in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

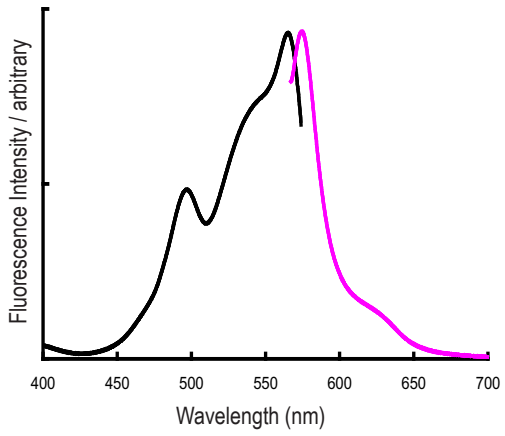
Required Equipment

- 10 µl and 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 R-Phycoerythrin labeling, 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.
- 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.

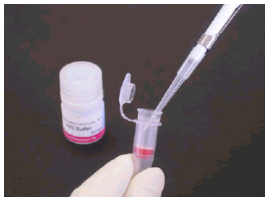




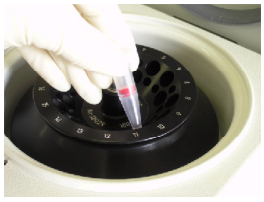
Maximum excitation wavelength : 564 nm
Maximum emission wavelength : 575 nm

Excitation and emission spectra of R-phycoerythrin-labeled protein

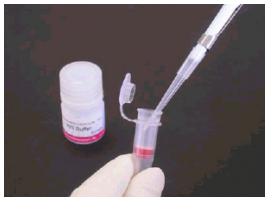
General Protocol - Labeling for IgG -



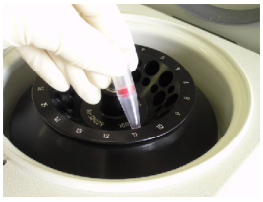
Step 1.
Add 100 µl WS Buffer and the sample solution containing 50 - 200 µg IgG[a] to a Filtration Tube.



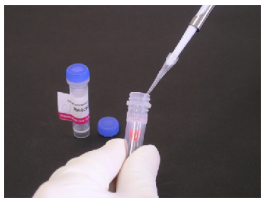
Step 2.
Pipette to mix and centrifuge at 8,000 x *g* for 10 min.[b]



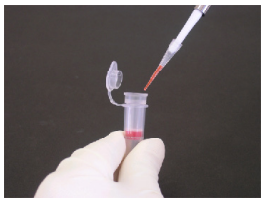
Step 3.
Add 100 µl WS Buffer to a Filtration Tube again.



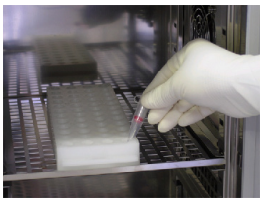
Step 4.
Centrifuge at 8,000 x *g* for 10 min again.[b]



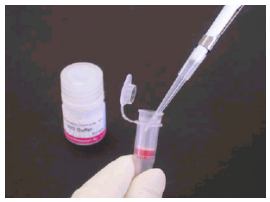
Step 5.
Add 10 µl Reaction Buffer to NH_2-Reactive R-Phycoerythrin, and dissolve with pipetting.[c]



Step 6.
Add NH_2-Reactive R-Phycoerythrin solution to the IgG concentrated on the Filtration Tube.



Step 7.
Incubate the tube at 37°C for 2 hours after pipetting to mix.



Step 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°C.[e]

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.
c) NH_2-Reactive R-Phycoerythrin can be hydrolyzed by water. Proceed to Step 6 immediately after the preparation of the NH_2-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) We recommend using WS 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 R-Phycoerythrin labeled protein stable?
The stability depends on the protein itself. For longer storage, add equal volume of glycerol to the sample solution and store at -20°C.

◆ 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.

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

◆ Can I use this kit to label 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 other 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.

◆ 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.

◆ 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.

◆ 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.

If you require an assistance, please contact Dojindo customer service.

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