

General Information

Phycobiliproteins are fluorescent proteins derived from cyanobacteria and eukaryotic algae. Their fluorescence is much higher than chemical fluorescent probes such as fluorescein and rhodamine. Because of this high fluorescence, phycobiliprotein labeled antibodies and other molecules can give greater sensitivity in flow cytometry and immunoblotting/immunostaining. R-Phycoerythrin (R-PE) is one of the phycobiliproteins, and it has an orange fluorescence at around 575 nm. R-Phycoerythrin Labeling Kit-NH₂ is for simple and rapid preparation of R-PE-labeled IgG. NH₂-reactive R-PE (a component of this kit) has activated ester groups, and can easily make a covalent bond with an amino group of the target molecule without any activation process. The filtration tube in this kit allows a quick buffer exchange and concentration of sample IgG solution. This kit contains all of the necessary reagents for R-PE labeling, including the storage buffer for conjugates.

Kit Contents

- NH₂-reactive R-PE 350 µg x 3 tubes
- WS buffer 4 ml x 1 bottle
- Reaction buffer 200 µl x 1 tube
- Filtration tube 3 tubes

Capacity

Three sample labeling

Sample requirements protein: molecular weight > 50,000; amount: 100 µg as IgG

Storage Condition

Store at 0-5 °C. This kit is stable for 6 months at 0-5 °C with protection from moisture.

Required Equipment and Materials

- 10 µl and 200 µl adjustable pipettes
- 0.5 ml microtubes
- Microcentrifuge
- Incubator (37 °C)

General Protocol for IgG Labeling



1 Add 100 µl WS buffer and the sample solution containing 100 µg IgG to a filtration tube.^{a)}



2 Centrifuge at 8,000-10,000 g for 10 min. Add 100 µl WS buffer and centrifuge once more.^{b)}



3 After finishing the centrifuge, add 10 µl Reaction buffer to NH₂-reactive R-PE and dissolve with pipetting.



4 Transfer the solution containing NH₂-reactive R-PE onto the membrane of the filtration tube where IgG is concentrated.



5 Rinse the entire surface of the membrane with the solution by pipetting and incubate the tube at 37 °C for 2 hr.



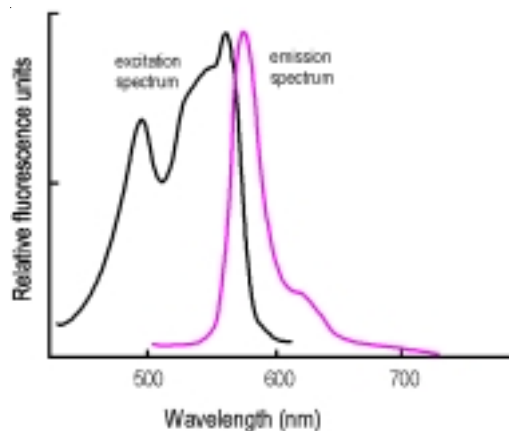
6 Add 190 µl WS buffer and pipette 10 to 15 times to recover the conjugate.^{c)} Transfer the solution to a 0.5 ml tube and store the solution at 0-5 °C.^{d)}

Precaution

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

- a) The volume of sample solution should be 100 µl or less. If the IgG concentration is lower than 0.5 mg/ml, repeat step 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate prior to go to the next centrifuge step.
- b) If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min. or increase the centrifuge speed.
- c) The concentration of the conjugate is 1.4-1.8 mg/ml. Dilute the R-PE-labeled IgG to prepare a solution with an appropriate concentration prior to using it for flow cytometry, immunoblotting, or immunostaining. One to two of R-PE molecules should be introduced onto one IgG molecule. Unconjugated R-PE should not interfere with a normal assay. If purification is necessary, use a gel permeation column or an affinity column for IgG.
- d) Generally, the R-PE-labeled IgG in WS buffer is stable for at least 2 months at 0-5 °C. For longer storage, add glycerol (final concentration: 50%), aliquot, and store at -20 °C. However, it is important to note that the stability will depend on the sample itself.

Spectrum of R-PE



Excitation wavelength : 566 nm
Emission wavelength : 578 nm

If you require assistance, please contact Dojindo technical service.

Dojindo Molecular Technologies, Inc.

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R-Phycoerythrin Labeling Kit - NH₂ Technical Manual
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Frequently Asked Questions

◆ Can I use this kit for other proteins?

Yes, if the molecular weight is higher than 50,000 and it has a reactive primary or secondary amino group. Follow the protocol for IgG labeling with 0.5-1 nmol of sample protein.

◆ How many R-PE molecules per IgG are introduced?

The average number of R-PE molecule per IgG is 1 to 2.

◆ Does unconjugated NH₂-reactive R-PE still have an activated ester after the labeling reaction to IgG?

No. It is completely hydrolyzed during the reaction.

◆ Does NH₂-reactive R-PE form an oligomer during the labeling reaction?

No. Since all amino groups of NH₂-reactive R-PE are blocked, no oligomerization is possible.

◆ Do I have to use WS buffer included with the kit?

Yes. Use the WS buffer to prepare a stock solution of the conjugate. However, you can choose any kind of buffer appropriate to dilute the conjugate stock solution for your experiment.

◆ How long is the conjugate stable?

If you store at 4 °C, it is stable for over 2 months. For longer storage, add 100% volume of glycerol, aliquot, and store at -20 °C. However, please note that the stability depends on the protein itself.

Product Information

Product Name	Unit	Product Code
R-PE Labeling Kit-NH ₂	100 µg x 3 samples	LK23-10

Related Product Information

Product Name	Unit	Product Code	Product Name	Unit	Product Code
Peroxidase Labeling Kit-NH ₂	100 µg x 3 samples	LK11-10	SulfoRh101 Labeling Kit-NH ₂	100 µg x 3 samples	LK29-10
Peroxidase Labeling Kit-NH ₂	1 mg x 1 sample	LK11-12	Oyster-556 Labeling Kit-NH ₂	100 µg x 3 samples	LK04-10
Peroxidase Labeling Kit-NH ₂	10 mg x 1 sample*	LK11-20	Oyster-656 Labeling Kit-NH ₂	100 µg x 3 samples	LK07-10
Peroxidase Labeling Kit-SH	100 µg x 3 samples	LK09-10	HF-555 Labeling Kit-NH ₂	100 µg x 3 samples	LK14-10
Peroxidase Labeling Kit-SH	1 mg x 1 sample	LK09-12	HF-555 Labeling Kit-SH	100 µg x 3 samples	LK17-10
Peroxidase Labeling Kit-SH	10 mg x 1 sample*	LK09-20	HF-647 Labeling Kit-NH ₂	100 µg x 3 samples	LK15-10
ALP Labeling Kit-NH ₂	100 µg x 3 samples	LK12-10	HF-647 Labeling Kit-SH	100 µg x 3 samples	LK18-10
ALP Labeling Kit-NH ₂	1 mg x 1 sample	LK12-12	HF-750 Labeling Kit-NH ₂	100 µg x 3 samples	LK16-10
ALP Labeling Kit-SH	100 µg x 3 samples	LK13-10	HF-750 Labeling Kit-SH	100 µg x 3 samples	LK19-10
ALP Labeling Kit-SH	1 mg x 1 sample	LK13-12	B-PE Labeling Kit-NH ₂	100 µg x 3 samples	LK22-10
Biotin Labeling Kit-NH ₂	100 µg x 3 samples	LK03-10	B-PE Labeling Kit-SH	100 µg x 3 samples	LK25-10
Biotin Labeling Kit-NH ₂	1 mg x 1 sample	LK03-12	R-PE Labeling Kit-SH	100 µg x 3 samples	LK26-10
Biotin Labeling Kit-SH	100 µg x 3 samples	LK10-10	APC Labeling Kit-NH ₂	100 µg x 3 samples	LK21-10
Fluorescein Labeling Kit-NH ₂	100 µg x 3 samples	LK01-10	APC Labeling Kit-SH	100 µg x 3 samples	LK24-10
Rh110 Labeling Kit-NH ₂	100 µg x 3 samples	LK27-10	IgG Purification Kit-A	3 samples	AP01-10
Rh110 Labeling Kit-SH	100 µg x 3 samples	LK28-10	IgG Purification Kit-G	3 samples	AP02-10

* 5 mg x 2 samples can be labeled with this kit

ALP: alkaline phosphatase, Rh110: rhodamine 110, SulfoRh101: Sulfurhodamine 101, HF: HiLyte Fluor, APC: Allophycocyanin, B-PE: B-Phycoerythrin, R-PE: R-Phycoerythrin
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HiLyte Fluor is a trade mark of AnaSpec, Inc.