

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. Allophycocyanin (APC) is one of the phycobiliproteins, and it has a red fluorescence at around 660 nm. Allophycocyanin Labeling Kit-SH is for simple and rapid preparation of APC-labeled IgG. SH-reactive APC (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. 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 APC labeling, including a reducing agent for preparation of reduced IgG that has a SH group and a storage buffer for conjugates.

Kit Contents

- SH-reactive APC 200 µg x 3 tubes
- WS buffer 4 ml x 1 bottle
- Reaction buffer 200 µl x 1 tube
- Reducing agent 3 tubes
- RA solution 1 ml x 1 tube
- Filtration tube 3 tubes

Capacity

Three sample labeling

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

Store at 0-5 °C. This kit is stable for 1 year at 0-5 °C with protection from moisture.

Storage Condition

Required Equipment and Materials

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

General Protocol for IgG Labeling



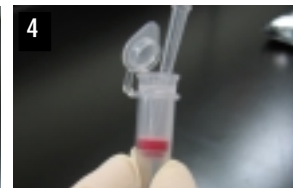
1 Add 100 µl WS buffer and the sample solution containing 100 µg IgG to the filtration tube. ^{a)}



2 Mix the solution with pipetting several times and centrifuge at 8000-10,000 g for 10 min. ^{b)}



3 Add 150 µl WS buffer to Reducing agent and dissolve it with pipetting several times.



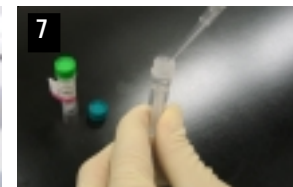
4 Transfer 100 µl of the solution from step 3 onto the membrane of the filtration tube where IgG is concentrated.



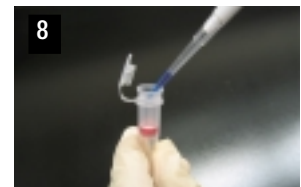
5 Pipette several times and incubate the tube at 37 °C for 30 min.



6 Add 100 µl RA solution to the tube and centrifuge at 8,000-10,000 g for 10 min. Discard the filtrate, add 200 µl RA solution, and centrifuge again. ^{b)}



7 Add 50 µl Reaction buffer to SH-reactive APC and dissolve with pipetting.



8 Transfer the SH-reactive APC solution onto the membrane of the filtration tube where reduced IgG is concentrated.



9 Pipette several times and incubate the tube at 37 °C for 1 hr.



10 Add 150 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

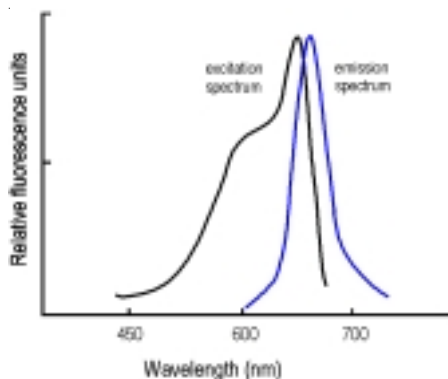
IgG or APC-conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to label B-PE with this kit. IgG can be purified by 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 antibody 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.

Spectrum of APC

- c) The concentration of the conjugate is 1.0-1.3 mg/ml. Dilute the APC-labeled reduced IgG to prepare a solution with an appropriate concentration prior to using it for flow cytometry, immunoblotting, or immunostaining. One to two APC molecules should be introduced onto one reduced IgG molecule. Unconjugated APC 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 APC-labeled reduced 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.



Excitation wavelength : 650 nm
Emission wavelength : 660 nm

If you require assistance, please contact Dojindo technical service.

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Allophycocyanin Labeling Kit - SH Technical Manual
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Frequently Asked Questions

◆ Can I use this kit for F(ab')₂?

Yes, follow the labeling protocol for IgG. The recovery of the conjugate should be over 80%.

◆ Can I use this kit for other proteins?

Yes, if the molecular weight of reduced form is greater than 50,000 and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. Follow the labeling protocol for IgG and use 0.5-1 nmol of sample protein.

◆ How many APC molecules per reduced IgG are introduced?

The average number of APC molecule per reduced IgG is 1 to 2.

◆ Do I have to use Filtration tube prior to labeling the protein?

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 Solution B and add the mixture to a vial of the SH-reactive APC.

◆ 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.

Product Information

Product Name	Unit	Product Code
APC Labeling Kit-SH	100 μg x 3 samples	LK24-10

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-NH ₂	100 μg x 3 samples	LK23-10
Biotin Labeling Kit-SH	100 μg x 3 samples	LK10-10	R-PE Labeling Kit-SH	100 μg x 3 samples	LK26-10
Fluorescein Labeling Kit-NH ₂	100 μg x 3 samples	LK01-10	APC Labeling Kit-NH ₂	100 μg x 3 samples	LK21-10
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: Sulforhodamine 101, HF: HiLyte Fluor, APC: Allophycocyanin, B-PE: B-Phycoerythrin, R-PE: R-Phycoerythrin
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HiLyte Fluor is a trademark of AnaSpec, Inc.