Allophycocyanin Labeling Kit - SH

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk24.pdf

Generala Information

Allophycocyanin Labeling Kit - SH is primarily used for the preparation of allophycocyanin-labeled antibody for immunostaining and cellular proteins for tracing. SH-Reactive Allophycocyanin, a component of this kit, has maleimide group that reacts with sulfhydryl groups (SH) of reduced IgG or other proteins. This kit contains all of the necessary reagents for the labeling. Reducing Agent in this kit produces free sulfhydryl groups in the IgG molecule without loss of antibody affinity. The labeling process is simple. After the reducing reaction, add SH-Reactive Allophycocyanin to IgG solution on the membrane of Filtration Tube, and incubate at 37°C for 1 hour. The maximum excitation and emission wavelengths of the allophycocyanin-labeled proteins are 650 nm and 660 nm, respectively. This kit contains all of the necessary reagents for labeling, including the storage buffer for conjugates.

Kit Contents

- SH-Reactive Allophycocyanin .....3 tubes
- RA Solution .....1 ml x 1
- WS Buffer .....4 ml x 1
- Reducing Agent..... 3 tubes
- Reaction Buffer .....200 µl 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

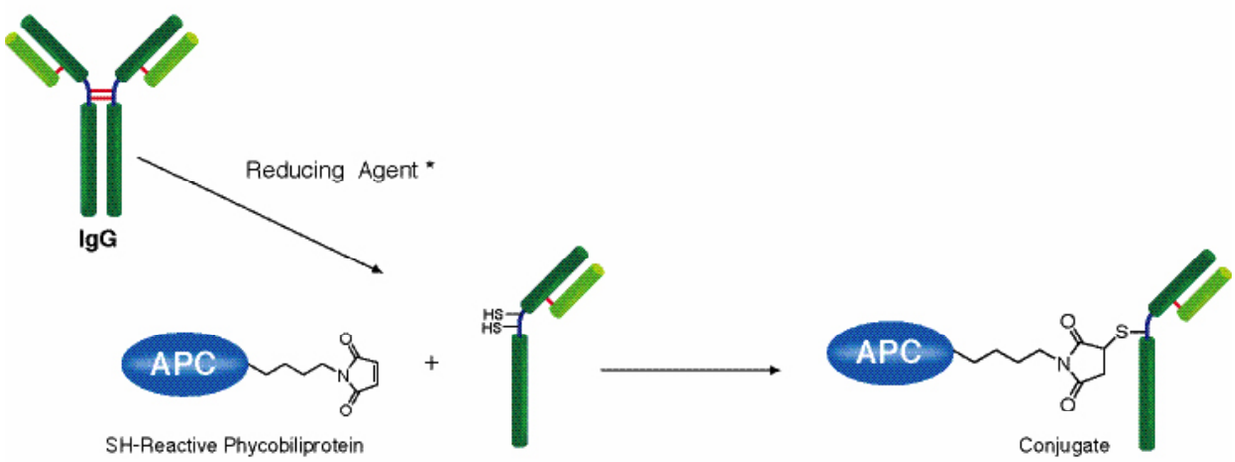
After a SH-Reactive Allophycocyanin is taken out from the seal bag, keep the unused SH-Reactive Allophycocyanin (s) in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

Required Equipment

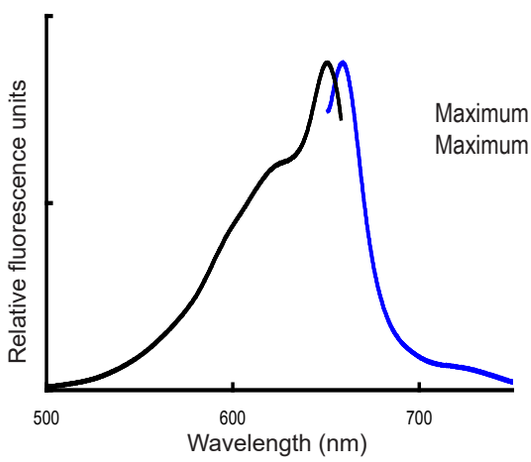
- 10 µl, 200 µl adjustable pipettes
- Microcentrifuge
- Incubator (37°C)
- Microtubes

Precaution

- If the target protein has sulfhydryl groups, skip the reducing procedure(Step 3-6).
- 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 allophycocyanin 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 amount of Reducing Agent is optimized for the preparation of the reduced IgG. Please examine the necessary amount of Reducing Agent for the



* Disulfides that are not in the hinge region may be reduced.

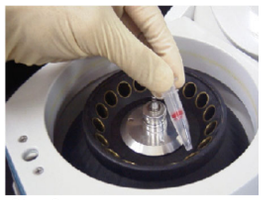


Maximum excitation wavelength: 650 nm
Maximum emission wavelength : 660 nm

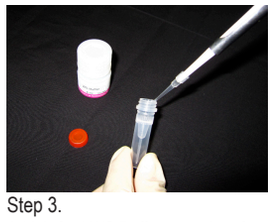
Excitation and emission spectra of Allophycocyanin-labeled protein

- Labeling for IgG -

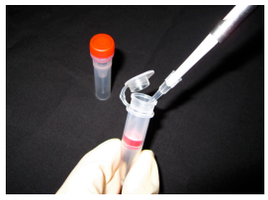
Step 1.
Add 100 µl WS Buffer and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.



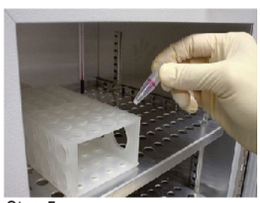
Step 2.
Pipette to mix and centrifuge at 8,000 x ***g*** for 10 min.[b]



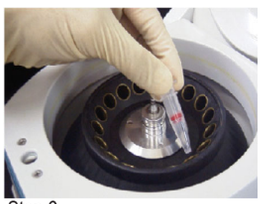
Step 3.
Add 150 µl WS Buffer to a tube of Reducing Agent, and dissolve with pipetting.



Step 4.
Transfer 100 µl of the Reducing Agent solution onto the membrane of Filtration Tube, and pipette to dissolve the IgG.

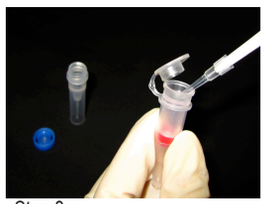


Step 5.
Incubate the tube at 37°C for 30 min. Add 100 µl RA Solution, and centrifuge at 8,000 x ***g*** for 10 min.[b]

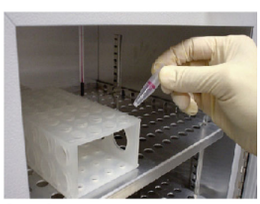


Step 6.
After discard the filtrate, add 200 µl RA Solution, and centrifuge at 8,000 x ***g*** for 10 min again.[b]

Step 7.
Add 50 µl Reaction Buffer to SH-Reactive Allophycocyanin, and dissolve it with pipetting.[c]



Step 8.
Transfer SH-Reactive Allophycocyanin solution to the Filtration Tube, and pipette to mix.



Step 9.
Incubate the tube at 37°C for 1 h.

Step 10.
Add 150 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) SH-Reactive Allophycocyanin is unstable in Reaction Buffer. Proceed to Step 8 immediately after the preparation of the SH-Reactive Allophycocyanin solution.
d) One to two allophycocyanin should be introduced into one reduced IgG molecule. Unconjugated allophycocyanin 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 storage the conjugate. You can choose any kinds of buffers appropriate for your experiment.

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.
- **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.
- **Can I use the Allophycocyanin 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 Allophycocyanin 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 need more information, please contact Dojindo technical service.

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