ICG Labeling Kit - NH_2 Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk31.pdf

General Information

ICG Labeling Kit - NH_2 is primarily used for the preparation of ICG (Indocyanine green)-labeled antibody for near-infrared fluorescence imaging. ICG is approved to be used in clinical field such as a hepatic deficiency test, and has near-infrared fluorescence lasting a few days under physiological conditions. Fluorescence detection in near-infrared region is very popular due to the lower background fluorescence. Therefore, ICG and ICG conjugates are suitable materials for ***in vivo*** imaging. NH_2-Reactive ICG, a component of this kit, has a succinimidyl ester group, and can easily make a covalent bond with an amino group of the target protein without any activation process. Filtration Tube included in this kit is used for sample protein in removing small molecules such as amine compounds that interfere with a labeling reaction.The labeling process is very simple. Add the NH_2-Reactive ICG to protein solution on a filter membrane, and incubate at 37°C for 10 min. Excess ICG molecules can be removed by a filtration tube. The excitation and emission wavelengths of the ICG-labeled proteins are 774 nm and 805 nm, respectively.

Kit Contents

	【3 samples】
- NH_2-Reactive ICG	3 tubes
- WS Buffer	4 ml × 1
- Reaction Buffer	500 µl × 1
- Filtration Tube	3 tubes

Capacity

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 ICG is taken out from the seal bag, keep the unused NH_2-Reactive ICG (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)
- DMSO
- Microtubes
- PBS

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 ICG labeling, because it might interfere the 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.

General Protocol for protein labeling

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

Step 2.
Mix the solution with pipetting several times, and centrifuge at 8,000 x ***g*** for 15 min.[b]

Step 3.
Add 10 µl DMSO to NH_2-Reactive ICG, and dissolve with pipetting.[c]

Step 4.
Add 100 µl Reaction Buffer to the Filtration tube, and then add 8 µl NH_2-Reactive ICG solution[d] to the Filtration Tube and pipette to mix.

Step 5.
Incubate the tube at 37°C for 10 min.

Step 6.
Add 100 µl WS Buffer to the Filtration Tube, and centrifuge at 8,000 x ***g*** for 15 min.[b] Discard the filtrate.

Step 7.
Add 200 µl WS Buffer to the Filtration Tube, and centrifuge at 8,000 x ***g*** for 15 min.[b] Repeat this step one more time.

Step 8.
Add 200 µl PBS, and pipette about 10 times to recover the conjugate.[e] Transfer the solution to a microtube (not included in this kit), and store at 0-5°C.

a) The volume of protein solution should be less than 100 µl. If the antibody concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total protein accumulation becomes 50-200 µg.
b) If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min.
c) NH_2-Reactive ICG is on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve. NH_2-Reactive ICG can be hydrolyzed by moisture in DMSO. Proceed to Step 4 immediately after the preparation of the NH_2-Reactive ICG solution.
d) If the amount of protein is 200 µg, add entire NH_2-Reactive ICG solution.
e) You can use an appropriate buffer for the downstream experiments.

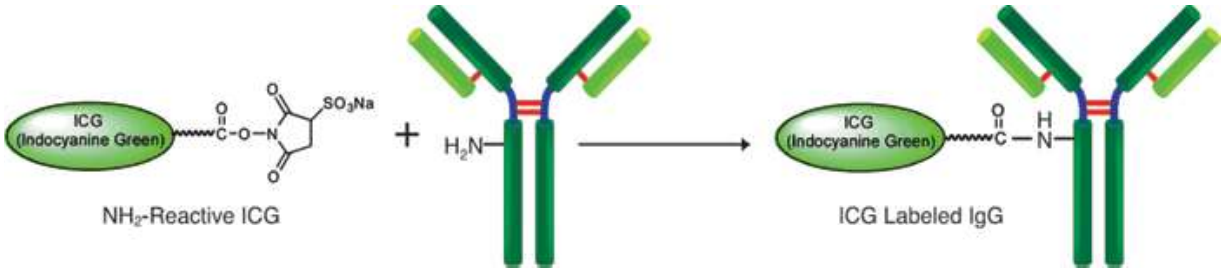
Determination of Dye / protein Ratio

Dilute the ICG-labeled protein solution with PBS or other neutral buffers to a proper volume, and measure the absorbance of the protein solution at 280 nm and 800 nm. Calculate the ratio using the following equation: When a target protein is IgG, use 216,000 as the ε of protein. Molar absorption coefficient of ICG in PBS is 147,000.

$$\text{Ratio (ICG / protein)} = \frac{A_{800} / 147{,}000}{(A_{280} - A_{800} \times 0.075)/(\varepsilon \text{ of protein})}$$

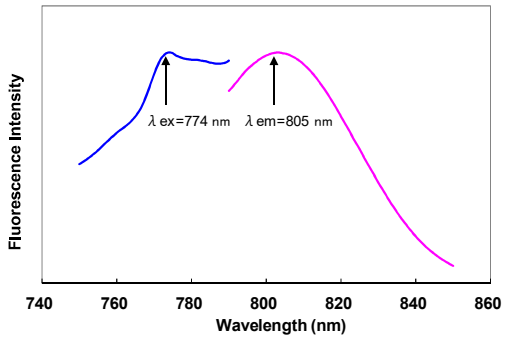
A_{800}: absorbance at 800 nm
A_{280}: absorbance at 280 nm
ε: molar absorption coefficient of protein at 280 nm

Labeling Reaction



NH_2-Reactive ICG

ICG Labeled IgG



Excitation and Emission Spectra of ICG

Q & A

◆ Can I use this kit to label antibodies which is commercially available?

Yes. However, if the antibody solution contains other proteins such as serum albumin or gelatin, labeling reaction might be interefered by that protein. Purification of the antibody solution with affinity chromatography is necessary prior to using this kit.
Contact us for the purification procedure, if you need.

◆ How long is the ICG-labeled protein stable?

Stability of conjugate depends on the protein itself. Please use the conjugate as soon as possible. For long term storage, aliquot and store the sample solution at -20°C.

◆ How many ICG molecules are introduced to protein?

The number of conjugated ICG depends on the protein. In the case of rabbit IgG, one molecule of ICG conjugate to each protein molecule.

◆ Can I use this kit to label oligonucleotides or oligopeptides?

No. Oligonucleotides and oligopeptides may be too small to retain on the membrane filter of Filtration Tube.

If you require assistance, please contact Dojindo cuctomer service.

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