



Europa Bioproducts Ltd

15-17 North Street • Wicken
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ICG Labeling Kit - NH₂

Item#	Unit Size
LK31-08	1 sample (labeling 100 ug)
LK31-10	3 samples (labeling 100 ug)



Suitable wavelength for In Vivo imaging

**Quick and Easy Labelling to Antibodies:
1.5hr**

High Recovery Rate: more than 90%

Contents of the Kit

LK31-08: 1 sample

NH ₂ -reactive ICG	:1 tube	Reaction buffer	:250 µl x 1 tube
WS buffer	:1.5 ml x 1 bottle	Filtration tube	:1 tube

LK31-10: 3 samples

NH ₂ -reactive ICG	:3 tubes	Reaction buffer	:500 µl x 1 tube
WS buffer	:4 ml x 1 bottle	Filtration tube	:3 tubes

Storage condition: 0-5°C

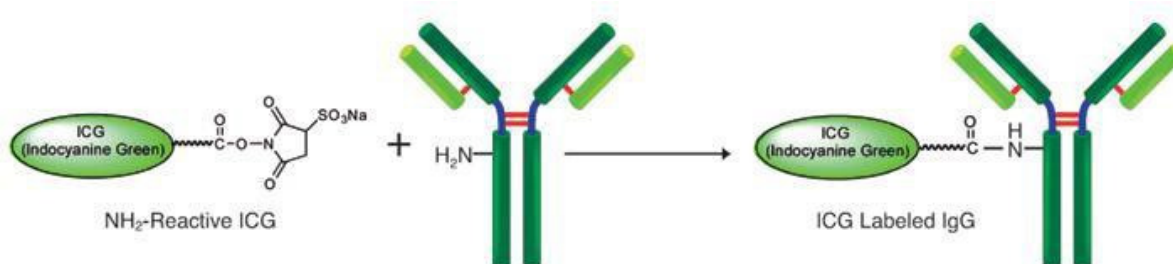
Shipping Condition: ambient temperature

Product Description

ICG Labeling Kit - NH₂ is used primarily for the preparation of ICG (Indocyanine green)-labeled antibody for near-infrared fluorescence imaging. ICG offers two remarkable properties:

- 1) ICG has a strong near-infrared fluorescence even after a few days under physiological conditions. The excitation and emission wavelength of the ICG-labeled proteins are 774 nm and 805 nm, respectively.
- 2) ICG has been used in clinical fields such as a hepatic deficiency testing. Therefore, ICG and ICG conjugates are materials suitable for in vivo imaging.

This kit contains all required components required for labeling, including storage buffer for conjugates. The labeling process is simple: Add NH₂-reactive ICG to protein solution on a filter membrane, and incubate at 37°C, for 10 minutes. A filtration tube can remove excess ICG molecules.



CERTIFICATE NO. 3222

Web site: <http://www.europa-bioproducts.com>

Registered Office: 15-17 North Street, Wicken, Ely, Cambridgeshire CB7 5XW
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Fig. 1 Fluorescein Labeling Process to IgG

Precaution

◆ If the IgG solution contains other proteins with molecular weight greater than 10,000, such as serum albumin or gelatin, purify the IgG solution before labeling fluorescein with this kit. Commercially available antibody may contain BSA or gelatin as a stabilizer. Dojindo offers IgG Purification Kit-A (AP01-10) and IgG Purification Kit-G (AP02-10) for the purification of the IgG solution

Current Publication Using This Product

Cristian Urla, Guido Seitz, et al., Successful in vivo tumor visualization using fluorescence laparoscopy in a mouse model of disseminated alveolar rhabdomyosarcoma. Surgical Endoscopy. 2014 August

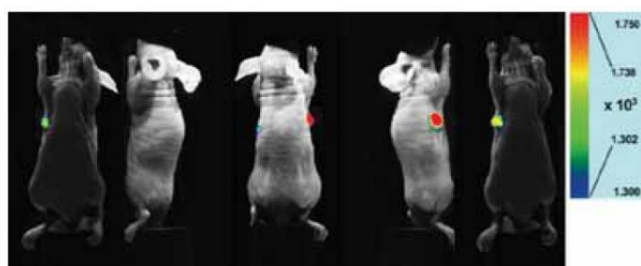


Fig. 2 Fluorescent Overlay Imaging

A mouse subcutaneous tumor model at 48 hours postinjection of ICG labeled antibody(50 µg) shows clear evidence of selective accumulation in tumor.

mouse: BALB/c nu/ nu (female, 11 weeks)
tumor cell: HeLa (4 weeks after transplant in subcutaneous right axilla)
antibody: anti-integrin α2 antibody
measurement condition: Ex 785 nm, Em 845/55 nm(center/band), exposure time: 10 sec

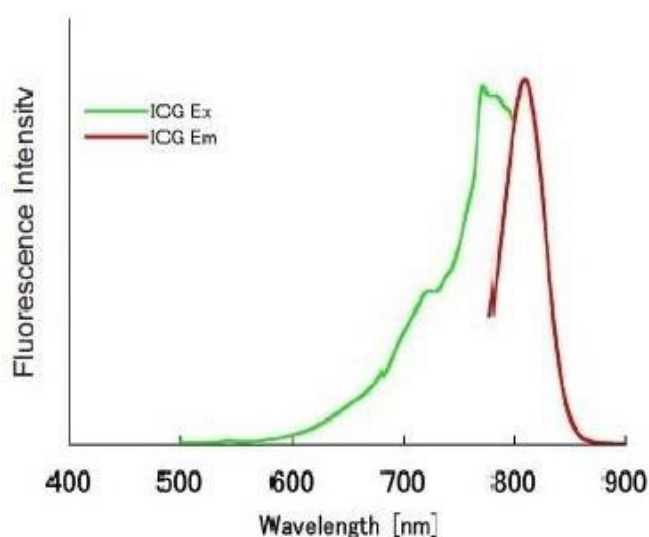


Fig. 3 Fluorescent Property of ICG Dye

Can I use this kit for F(ab')₂?

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

Can I use this kit for other proteins or peptides?

Yes, if the molecular weight of the reduced form is greater than 50,000 or less than 5,000, and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. If the molecular weight is greater than 50,000, follow the labeling

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protocol for IgG, and use 0.5-1 nmol of sample protein for LK09-10. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules. If the molecular weight is between 5,000 and 50,000, contact our customer service at info@dojindo.com or 1-877-987-2667 for more information.

Can I use this kit to label oligopeptides or oligonucleotides?

Yes, if the molecular weights of the oligonucleotide or the oligopeptide are less than 5,000 and they have at least one SH group. Follow the labeling protocol for small molecule.

What is the minimum amount of IgG that can be labeled with LK13-10?

The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. However, even 10 µg IgG can be labeled using 1/5 volume of SH-reactive peroxidase solution at Step 8.

How many alkaline phosphatase molecules per reduced IgG are introduced?

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

Do I have to use a 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 per 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 peroxidase.

Do I have to use Storage buffer included with the kit?

No, you don't have to use Storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment.

My sample contains small insoluble material. What should I do?

Spin the sample and use the supernatant for labeling.

Does unconjugated SH-reactive ALP still have a reactive maleimide after the labeling reaction to IgG? No. Nearly 100% of SH-reactive ALP is used for the IgG labeling or the small molecule labeling.

Does Storage buffer contain animal products or polymers?

No, Storage buffer does not contain any animal products, polymers, or heavy metal ions.



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