GORYO CHEMICAL

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CopperGREEN™

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC902	CopperGREEN™	50 nmol × 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About CopperGREEN™

CopperGREEN™ is a fluorescent probe that specifically detects Cu^{I} ion. Copper is one of the trace metals required by living organisms, and is an essential cofactor for enzyme activities of cytochrome c oxidase, superoxide dismutase, and tyrosinase. On the other hand, abnormal homeostasis of copper leads to severe disorders such as Menkes syndrome and Wilson's disease, or may be related to Alzheimer's disease and cancers. Among two redox states of copper, Cu^{I} and Cu^{II}, Cu^{I} is dominant in the intracellular reducing environment. Cell permeable CopperGREEN™ which has almost no fluorescence, fluoresces upon reaction with the intracellular Cu^{I}. Therefore it is suitable for live cell imaging of Cu^{I}.

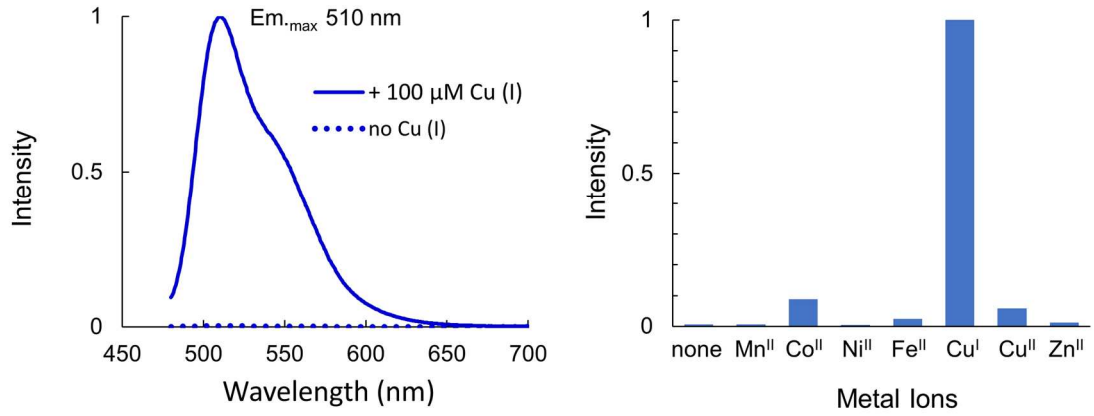

Figure 1. (*left*) Fluorescence emission spectra before and after the reaction to Cu^{I} ions. The graph shows spectra of 5 µM CopperGREEN™ in 50 mM Hepes, 2 mM glutathione (pH7.2). Fluorescence increases >100-fold upon reaction with Cu^{I}. (*right*) Metal ion selectivity of CopperGREEN™ in 50 mM Hepes buffer (pH 7.2). Significant increase of fluorescence is observed only in the presence of Cu^{I} ions.

■ Storage

This product is shipped at room temperature. Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Storing as a solution is not recommended.

2. An example of live cell imaging

■ Materials required but not provided

・Dimethyl sulfoxide (DMSO)

・Observation buffer (1×PBS pH 7.4, Hank's Balanced Salt Solution (HBSS), etc.). It should be a solution without phenol red.

■ Preparation of reagent

1. CopperGREEN™ is a colorless solid. Before opening the vial, spin down the solid to the bottom by a microcentrifuge.
2. Warm the vial to the room temperature and add 50 µl of DMSO to one vial to make 1 mM stock solution. Dissolve the crystal completely by careful pipetting. Solution will be also colorless liquid. Use neutral buffer solution for the dilution of the stock solution. CopperGREEN™ could be oxidized in acidic solutions.

■ Example cell imaging procedure

3. Prepare HeLa cells seeded on a glass-bottom dish and cultured for >12 hours at 37ºC, 5% CO_2. Add 100 µM $CuCl_2$ to the cell culture medium (ex. DMEM + 8% FBS + P.S.) and culture for >12 hours. Then gently rinse cells 2 times with PBS containing 200 µM EDTA to remove extracellular Cu^{2+}.
4. Dilute the 1 mM CopperGREEN™ solution with the culture medium to 5 µM (Cell staining solution).
5. Replace the cell culture medium with the cell staining solution and incubate at 37ºC, 5% CO_2 for 3 hours.
6. Optimizations of the dye concentration and the incubation time may be required. In GORYO Chemical, Inc., fluorescent signal appears after 2 hours incubation with 5 µM dye at 37°C. Incubation for 3 hours gave good results.
7. After the staining, rinse 2 times with the observation buffer.
8. Observe the cells with a fluorescent microscope.

※ Nonspecific fluorescence may be observed from acidic organelles. Addition of 10 mM NH_4Cl or 100 nM bafilomycin A1 to the medium, 30 minutes before the addition of CopperGREEN™ suppresses acidification of lysosomes and reduces nonspecific fluorescence signals. Please keep the NH_4Cl concentration during the staining and the observation.

■ Fluorescence observation

CopperGREEN™ reacted with Cu^{I} is exited with lights around 440—490 nm wavelength, and emits fluorescence with a peak at 510 nm. Use 488 nm laser or blue excitation filter cubes for GFP or FITC for fluorescent microscopes.

Table 2. Related Products

Catalog no.	Product name	Major applications
GC901	FeRhoNox™-1	Detection of ferrous ions (Fe^{2+}) in Golgi.
SK2001-01	ZnAF-2	Detection of Zn^{2+} ions.
SK2002-01	ZnAF-2DA	For live cell imaging of Zn^{2+} ions.
GC3006-01	HySOx	Detection and live cell imaging of hypochlorous acid (HOCl).
GC3007-01	HYDROP™	Detection of intracellular hydrogen peroxide (H_2O_2).
SK3001-01	HPF	Detection of hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$).
SK3002-01	APF	Detection of hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl).
SK3003-01	NiSPY-3	Detection of peroxynitrite ($ONOO^-$).

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