GORYO CHEMICAL

For research use only

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# OxiORANGE™

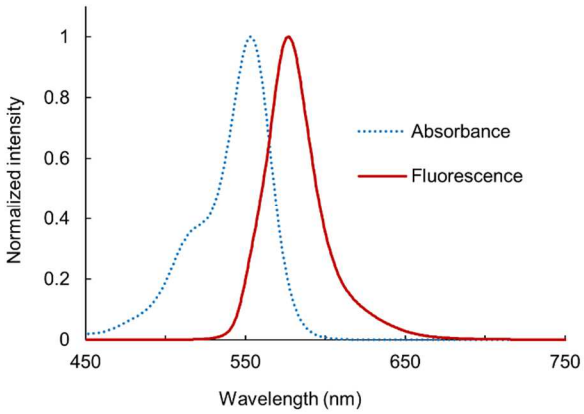
Table 1. Product information

| Catalog no. | Product name | Amount | Storage upon receipt | Stability |
|---|---|---|---|---|
| GC3004-01 | OxiORANGE™ | 100 nmol × 5 | ≤−20°C, keep desiccated and protected from light. | 1 year (when unopened and stored as described.) |

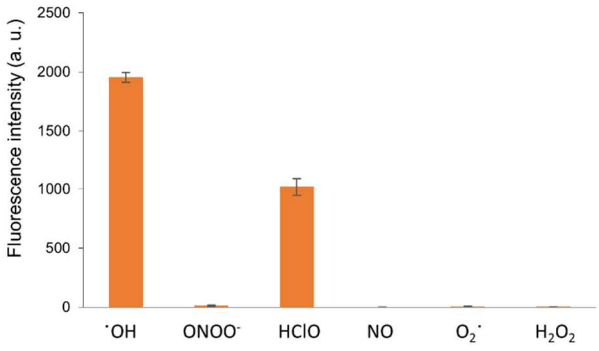
## 1. About OxiORANGE™

OxiORANGE™ is an orange fluorescent probe to detect highly reactive oxygen species (hROS). It has inherently no fluorescence but fluoresces upon reaction with hydroxy radical ( ˙OH) or with hypoclhlorous acid (HClO). It tends to localize in mitochondria because of its positive charges. Cell permeable OxiORANGE™ is suitable for live-cell imaging. It is also suitable for time-lapse imaging because of its high photostability. Further, you can image its fluorescence after fixation with 3-4% paraformaldehyde for 20 minutes.

Exitation/emission spectra




ROS selectivity




### ■ Storage

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

## 2. An example of live cell imaging

### ■ Materials required but not provided

- N,N-dimethylformamide (DMF)
- Observation buffer (1 × PBS pH 7.4, HBSS, Krebs-Ringer phosphate (KRP) buffer, etc.)

## ■ Procedure

**Timelapse imaging of hROS production in HeLa cells**

1. Dissolve OxiORANGE™ 1 vial (100 nmol) in 100 µL DMF to prepare 1 mM solution.
2. Dilute the 1 mM OxiORANGE™ solution with observation buffer or culture media to 1 µM (cell staining solution).
   ※ Optimization of the dye concentration and the incubation time is required. In GORYO Chemical, Inc., incubation in 1 µM dye at 37°C for 20 min gave good results for HeLa cells (human cervical cancer cell line), RAW264.7 cells (mouse macrophage-like cell lines) and HL-60 (Human promyelocytic leukemia cell line).
3. Remove the culture medium from the glass bottom dish and wash cells once with the observation buffer or with the culture medium.
4. Add the cell staining solution to the dish and incubate at 37°C for 20 min.
5. After the staining, wash cells 2 times with the observation buffer.
6. Add new observation buffer.
   ※ We recommend using cell culture medium without phenol red instead of using buffers, because starved HeLa cells are known to produce ROS.
7. Induce the production of hROS by the addition of 500 µM $H_2O_2$ (final conc.) and start observation under microscope.
   ※ We detected the fluorescence signal, 15 min after the stimulation.

## ■ Fluorescence observation

Use 532 nm or 543 nm light source for excitation. Maximum emission is observed at 577 nm. In fluorescence microscopy, use green excitaton filter cube such as Cy3, G-2A, G-2E/C, TRITC (Nikon) or U-FGW, U-FGWA, U-FRFP, U-FGNA, U-MWIGA3, U-MNIGA3 (Olympus).

Table 2. Related Products

| Catalog no. | Product name | Major applicationds |
|---|---|---|
| SK3001-01 | HPF | Specific fluorescence probe to detect hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$). |
| SK3002-01 | APF | Specific fluorescence probe to detect hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^-$) and hypochlorous acid ($ClO^-$). |
| SK3003-01 | NiSPY-3 | Specific fluorescence probe to detect only peroxynitrite ($ONOO^-$). |
| GC3006-01 | HySOx | Specific fluorescence probe to detect only hypochlorous acid (HClO) |
| GC3007-01 | HYDROP | Specific fluorescence probe to detect only hydrogen peroxide ($H_2O_2$). |
| GC301 | AcidiFluor™ ORANGE | A fluorescence probe to detect acidic organelles in living cells |
| GC901 | FeRhoNox™-1 | A fluorescence probe to detect ferrous ion ($Fe^{2+}$) in Golgi. |

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