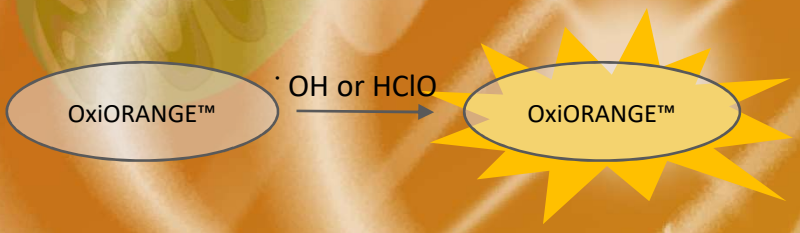
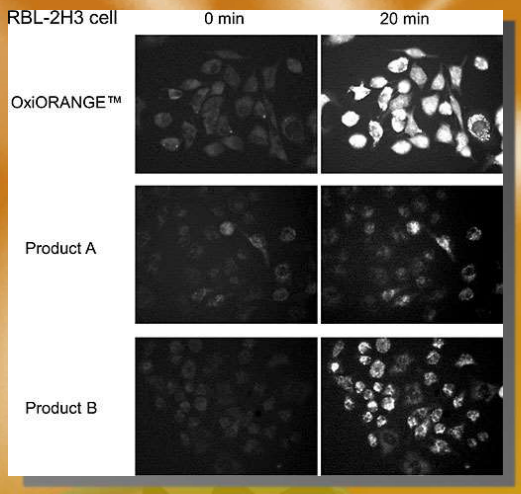
GORYO CHEMICAL

Measurement of highly reactive oxygen species (hROS) by orange fluorescence

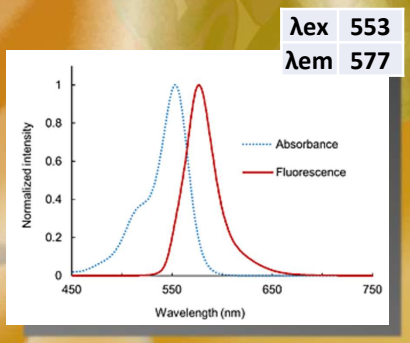
OxiORANGE™

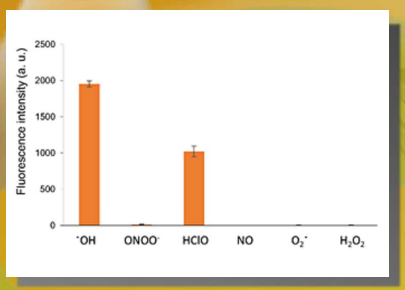
- OxiORANGE™ is an orange fluorescent probe to detect hydroxy radical (・OH) or hypochlorous acid (HClO) in live-cell imaging.
- Its nearly red fluorescence spectrum allows multicolor imaging with green (ex. GFP, FITC) and blue (ex. Hoechst 33342) fluorophores.
- Because of its positive charge, OxiORANGE™ tends to localize within mitochondria.
- It has high photostability and is suitable for time-lapse imaging of intracellular hROS generation.





RBL-2H3 cells loaded with 1 μM of OxiORANGE™ (above), product A (center), or product B (bottom) were stimulated by the addition of 0.5 μM H2O2. Photos were taken just after the addition of the probes (left) and 20 minutes later (right) in the same excitation/observation conditions.





Absorbance/fluorescence spectra (upper) and reactivity with various ROS (bottom).
About 30 times fluorescence increase after reaction with hydroxy radical (・OH) is observed.

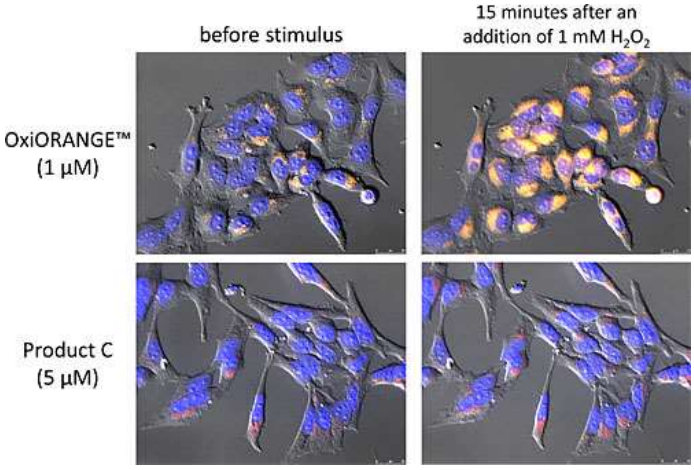
Comparison between mitochondria-localizing probes to detect oxidative stress.

OxiORANGE™ shows the brightest fluorescence among these products. Product B migrated into nucleus. In contrast, localization of OxiORANGE™ was stable.

Code no.	Product Name	Target	Size
GC3004-01	OxiORANGE™	hydroxy radical (・OH) and hypochlorous acid (HClO)	100 nmol ×5

GORYO CHEMICAL OxiORANGE™

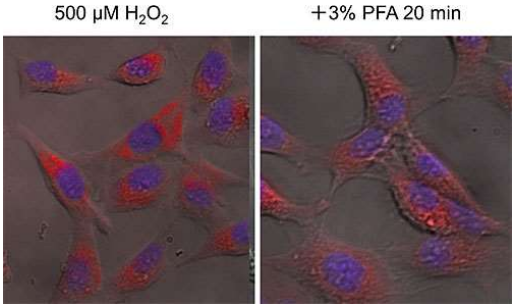
Bright and stable fluorescence



Comparison with a ROS-detecting probe.

OxiORANGE™ (1 μM, top, orange) or other product C (5 μM, bottom, deep red) was added to the medium and incubated for 30 minutes. After the medium was exchanged to HBSS, 1 mM H_2O_2 was added to stimulate ROS production. Bright signal from OxiORANGE™ was detected. DIC image (gray),Hoechst 33342 (blue), and OxiORANGE™ (orange), or Product C (red) is overlaid.

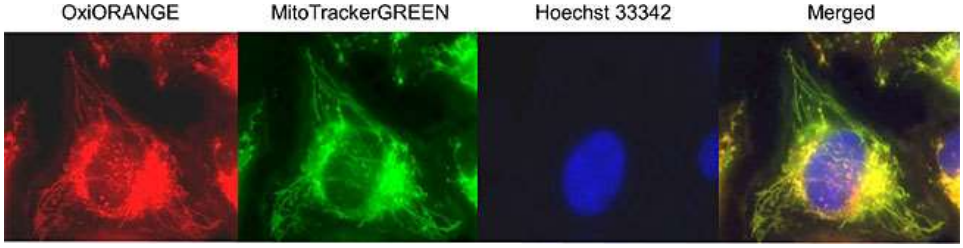
Fluorescence can be observed after mild fixation.



OxiORANGE™ fluoresces after reaction with ROS. The reaction is irreversible and the fluorescence remains after mild fixation with 3-4% PFA for 5-20 minutes.

Fluorescence of OxiORANGE™ before and after the fixation. HeLa cells were cultured for 30 minutes in the presence of 1 μM of OxiORANGE™ and 0.2 μg/mL Hoechst 33342. Cells were rinsed with HBSS two times, stimulated with 500 μM of H_2O_2, then ROS generation was observed after 30 minutes (left). Next, cells were fixed with 3% PFA containing PBS (pH 7.4) at 4℃ for 20 minutes. Cells were observed in the same condition. Images of red: OxiORANGE™, blue: Hoechist33342, and gray: DIC are overlaid. (Please test the fixation conditions prior to your experiments in case.)

Localization of OxiORANGE™ within cells



HeLa cells were stained with 0.5 μM of OxiORANGE™, 0.25 μM of MitoTrackerGREEN, and 0.2 μg/mL of Hoechst33342 for 30 minutes. Stimulated with 100 μM of hydrogen peroxide, for 30 min. Observed by fluorescence microscopy.

OxiORANGE tends to localize within mitochondria.

Localization of OxiORANGE depends on the membrane potential of mitochondria. Excess amount of OxiORANGE or other mitochondria-localizing reagents may interfere the distribution of OxiORANGE. Please evaluate the appropriate concentration in your condition, if you need it.

Goryo Chemical ROSFluor™ Series

Code no.	Product Name	Target	Size
GC3007-01	HYDROP	hydrogen peroxide (H2O2)	30 nmol × 3
SK3001-01	HPF	hydroxyl radical (・OH) and peroxynitrite ($ONOO^-$) *	1 mg
SK3002-01	APF	hydroxyl radical (・OH) and peroxynitrite ($ONOO^-$) *	1 mg
SK3003-01	NiSPY-3	peroxynitrite ($ONOO^-$)	1 mg
GC3006-01	HySOx	hypochlorous acid (HOCl)	20 μg × 5

* : The combination use of HPF and APF enables us to detect hypochlorite (OCl^-).

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