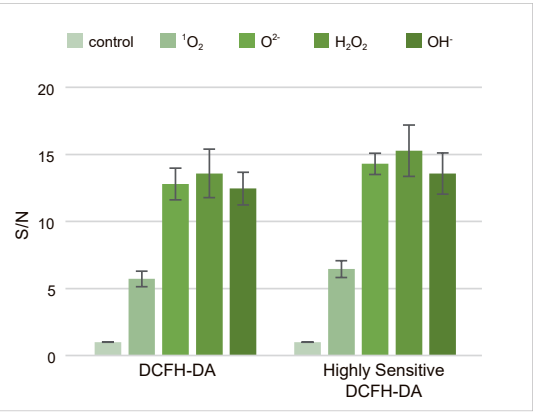
Oxidative Stress

ROS Assay Kit -Highly Sensitive DCFH-DA-

ROS Assay Kit -Highly Sensitive DCFH-DA- overcomes these limitations. The dye allows ROS detection with higher sensitivity than DCFH-DA. Moreover, the Loading Buffer included in this kit maintains cellular health during assays.

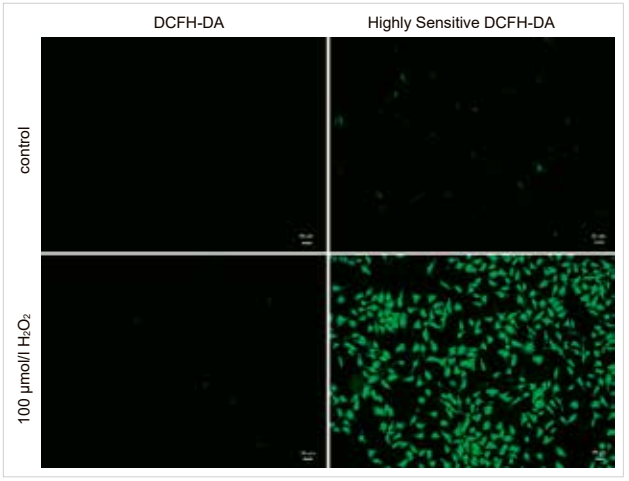
The reactivity of the Highly Sensitive DCFH-DA for ROS is similar to the reactivity of 2'-7' dichlorofluorescein diacetate (DCFH-DA). The Highly Sensitive DCFH-DA also has similar fluorescence characteristics (λ_{ex}: 505 nm, λ_{em}: 525 nm) to DCFH-DA. Therefore, ROS is detectable at the same excitation/fluorescence wavelength.

The selectivity for ROS

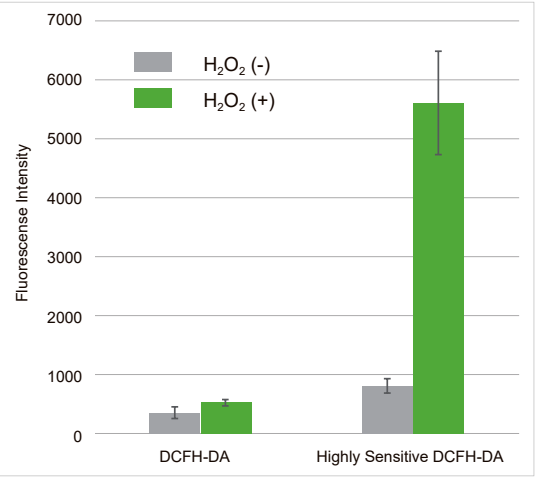


High Sensitive Detection Compared with DCFH-DA

Detection using fluorescent microscope



Detection using microplate reader



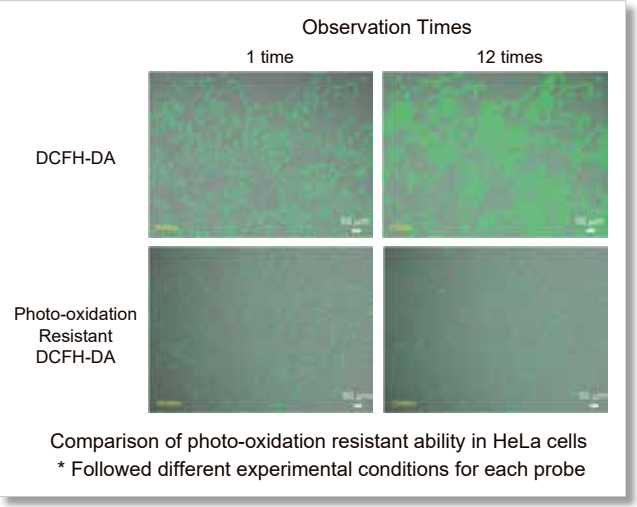
Hydrogen peroxide (H_2O_2)-treated HeLa cells (1×10^4 cells/ml) were stained with DCFH-DA or the ROS Assay Kit-Highly Sensitive DCFH-DA, and the fluorescence intensity of intracellular ROS was compared between two detection kits. As a result, the ROS Assay Kit-Highly Sensitive DCFH-DA in high-sensitivity detection of intracellular ROS was better than DCFH-DA.

Description	Unit	Code
ROS Assay Kit -Highly Sensitive DCFH-DA-	100 tests	R252-10

ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-

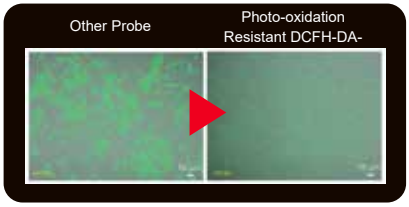
The dye that is employed in this kit allows ROS detection with higher sensitivity than DCFH-DA; It does not leak from cells because the fluorescent dye can immobilize protein via a chemical bond, and it is resistant to photo-oxidation compared with DCFH-DA. Moreover, the Loading Buffer in the kit maintains cellular health during assays.

Resistant to Photo-oxidation

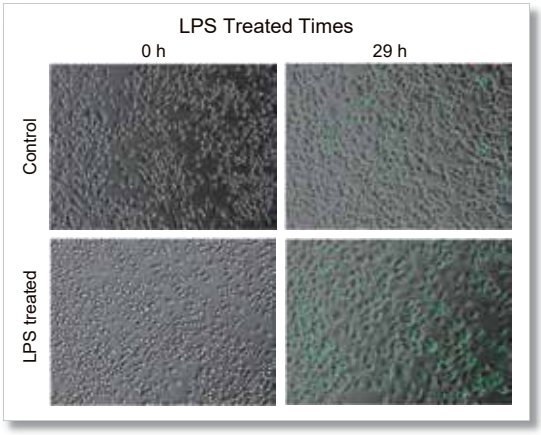


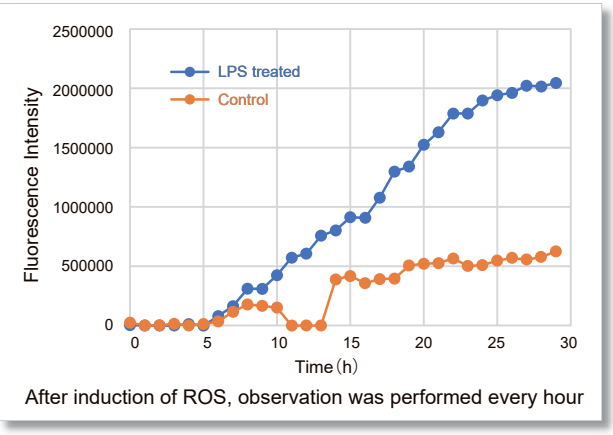
Comparison of photo-oxidation resistant ability in HeLa cells
* Followed different experimental conditions for each probe

Time-lapse imaging movie Available



Simultaneous Detection of ROS in LPS-treated macrophages





After induction of ROS, observation was performed every hour

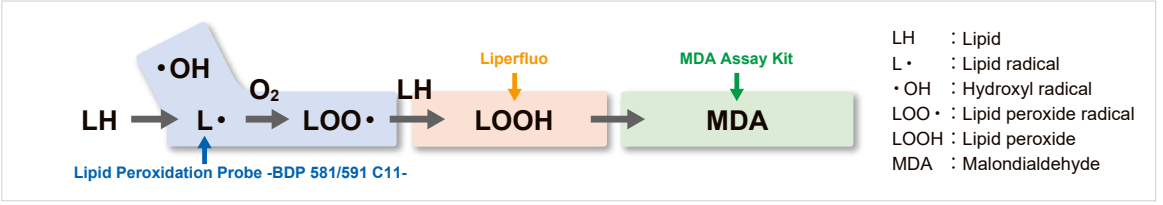
In Lipopolysaccharide (LPS) treated RAW 264.7 cells, after being stained with regular DCFH-DA, Highly Sensitive DCFH-DA, or Photo-oxidation Resistant DCFH-DA, the intracellular ROS level was compared. The results showed that the Dojindo Laboratories' probes could detect intracellular ROS with higher sensitivity.

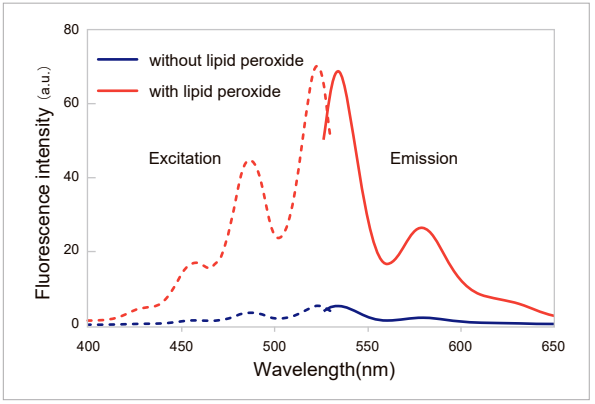
Description	Unit	Code
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	100 tests	R253-10

Lipid Peroxide Detection

Liperfluo

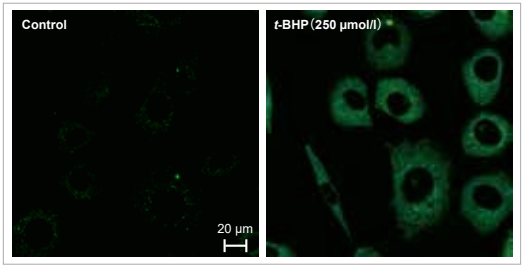
Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and makes it suitable for imaging lipid peroxides in cell membranes. It's used to monitor lipid peroxidation in ferroptosis research through fluorescence microscopy and flow cytometry.





Excitation and emission
without lipid peroxide spectra of Liperfluo with or without lipid peroxide in ethanol.

Lipid Peroxide Detection in Living Cells



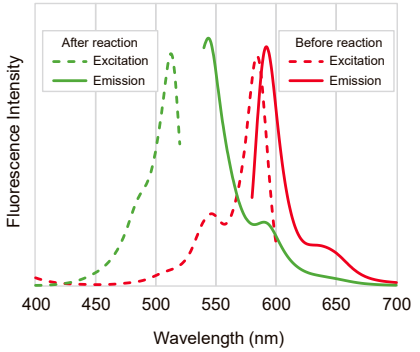
Liperfluo added to cells, t-BHP induced lipid peroxidation and cells were observed under confocal microscope to study ferroptosis.

Cell line: L929
Microscope: Zeiss LSM510META
Filter type: FITC (GFP, Alexa488) wide filter
HFT UV/488
NFT490
BP505-550

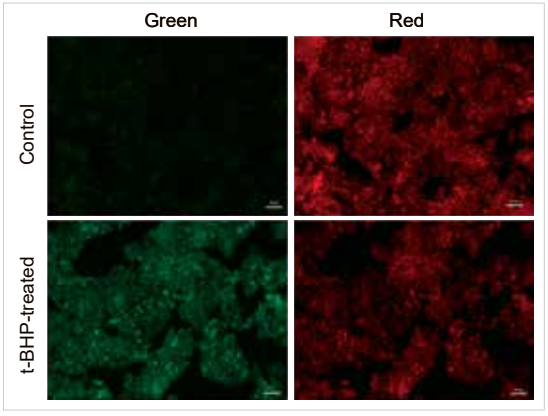
Description	Unit	Code
Liperfluo	1 set (50 μg × 5)	L248-10

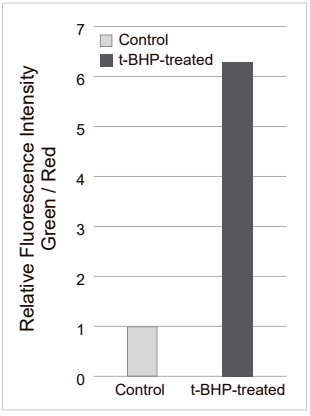
Lipid Peroxidation Probe -BDP 581/591 C11-

Lipid Peroxidation Probe -BDP 581/591 C11- is a fluorescent probe for detecting lipid peroxidation. This fluorescent probe does not react with lipid peroxides but reacts with lipid radicals generated when lipids are peroxidized, resulting in the detection of lipid peroxidation. The unreacted probe emits red fluorescence, but after reacting with radicals around lipids, it changes its fluorescence from red to green. Thus, lipid peroxidation can be detected with high sensitivity because it is detected by the ratio of red to green fluorescence intensity.



Lipid Peroxidation Assay





HepG2 cells stained with this probe were stimulated with HBSS solution containing 200 µmol/l *t*-BHP for 2 hours, and the fluorescence intensity was compared with control cells. As a result, a decrease in red fluorescence and an increase in green fluorescence were observed with high sensitivity in *t*-BHP-treated cells compared to untreated cells. The cells were detected using a plate reader, and the values obtained were calculated as the intensity ratio of green/red fluorescence, which allowed quantified lipid peroxidation. Furthermore, an increase in the histogram of green fluorescence was observed when the cells were detected using a flow cytometer. Which improves that this dye is three different instruments.

<Experimental Conditions>
Fluorescent Microscope
Green: GFP filter (Ex = 450-490 nm, Em = 500-550 nm)
Red: TexasRed filter (Ex = 540-580 nm, Em = 600-660 nm)
Scale bar: 50 µm

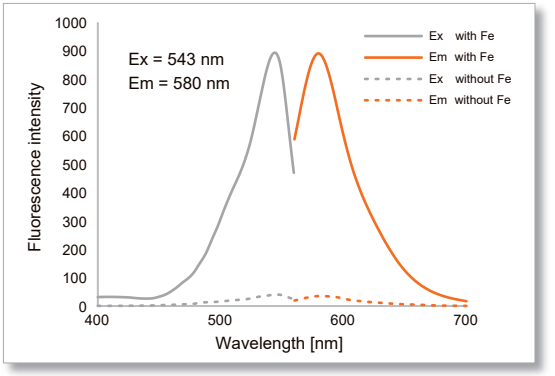
Fluorescent Plate Reader
Green: Ex = 490 nm, Em = 520-540 nm
Red: Ex = 570 nm, Em = 600-620 nm

Description	Unit	Code
Lipid Peroxidation Probe -BDP 581/591 C11-	200 tests	L267-10

Intracellular Iron Ion Measurement

FerroOrange

Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and makes it suitable for imaging lipid peroxides in cell membranes. It's used to monitor lipid peroxidation in ferroptosis research through fluorescence microscopy and flow cytometry.



Experimental Example

HeLa cells treated with chelator of iron 2,2′-bipyridyl (Bpy) (100 μmol/l) or Ammonium iron (II) sulfate (100 μmol/l) were prepared. The change of intracellular Fe^{2+} in HeLa cells was detected by the FerroOrange.

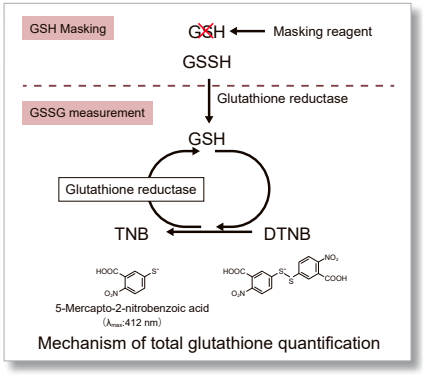
Ex = 561 nm, Em = 570-620 nm, Scale bars 20 μm

Description	Unit	Code
FerroOrange	1 tube	F374-10
	3 tube	F374-12

Quantification of Reduced (GSH) and Oxidized (GSSG) Glutathione

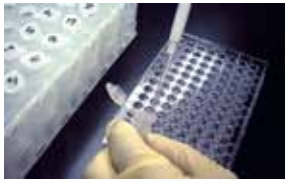
GSSG/GSH Quantification Kit

The GSSG/GSH Quantification kit contains Masking Reagent of GSH. GSH will be deactivated in the sample by simply adding the Masking Reagent. Then, using the enzymatic recycling system, only the GSSG will be detected by measuring the absorbance (λmax = 412 nm) of DTNB (5,5-dithio-bis- (2-nitrobenzoic acid). The quantity of GSH can also be determined, by substracting GSSG from the total amount of glutathione. With this kit, GSH/ GSSG concentrations from 0.5 µmol/l to 50 µmol/l and GSSG concentrations from 0.5 µmol/l to 25 µmol/l can be quantified.



Mechanism of total glutathione quantification

Assay Procedure

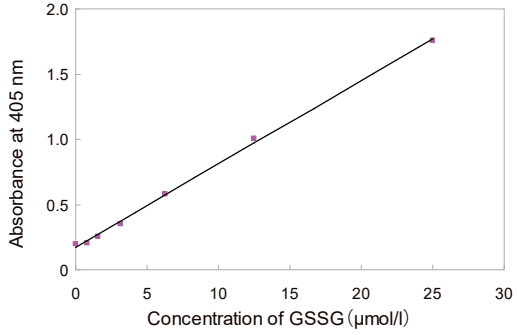

1) GSSG/GSH Standard Solution and add Sample A or Sample B to each well.
2) Add Buffer solution to each well


3) Incubate at 37℃ for 1 h.

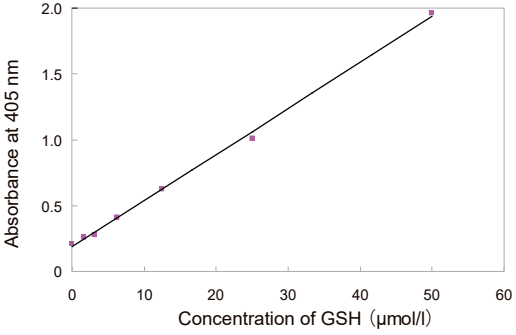

4)-5) Add substrate working solution and Enzyme/ Coenzyme working solution to each well.


6)-7) After incubating at 37℃ for 10 minutes, measure the absorbane of each well with a microplate.

Calibration Curve



The Calibration curve (GSSG) was prepared using the pseudoendpoint method (10 minutes)



The Calibration curve (GSH) was prepared using the pseudoendpoint method (10 minutes)

Description	Unit	Code
GSSG/GSH Quantification Kit	200 tests	G257-10