

# Reagent for Cellular Function Analysis

## Autophagy

- Autophagic Flux Assay Kit
- DALGreen-Autophagy Detection
- DAPGreen-Autophagy Detection
- DAPRed-Autophagy Detection

## Senescence

- Cellular Senescence Detection Kit  
-SPiDER-βGal
- Cellular Senescence Plate Assay Kit  
-SPiDER-βGal

## Neurodegenerative Diseases

## Cancer

## Senescence

## Mitochondria

- Mitophagy Detection Kit
- JC-1 MitoMP Detection Kit
- MitoBright LT Series
- MT-1 MitoMP Detection Kit
- MitoBright ROS Deep Red
- Extracellular OCR Plate Assay Kit

## Cellular Metabolism

- Glycolysis/OXPHOS Assay Kit
- ATP Assay Kit-Luminescence
- Lactate Assay Kit-WST

## Ferroptosis

- FerroOrange
- Liperfluo
- Mito-FerroGreen
- MitoPeDPP
- Cystine Uptake Assay Kit
- MDA Assay Kit
- Lipid Peroxidation Probe  
-BDP 581/591 C11-

# Cancer

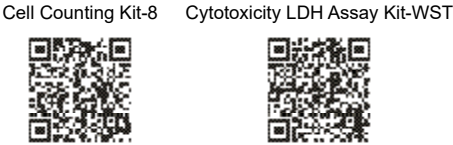
Code	Product Name	Page
MT09	JC-1 MitoMP Detection Kit	27
MT10, MT11, MT12	MitoBright LT Series	31
MT13	MT-1 MitoMP Detection Kit	27
MT16	MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	29
MD01	Mitophagy Detection Kit	28
MT02	Mtphagy Dye	28
E297	Extracellular OCR Plate Assay Kit	26
A562	Autophagic Flux Assay Kit	11
D675	DALGreen - Autophagy Detection	10
D676	DAPGreen - Autophagy Detection	10
D677	DAPRed - Autophagy Detection	10
F374	FerroOrange	16
L248	Liperfluo	14
M489	Mito-FerroGreen	30
UP05	Cystine Uptake Assay Kit	23
L267	Lipid Peroxidation Probe -BDP 581/591 C11-	15
G270	Glycolysis/OXPHOS Assay Kit	21
A550	ATP Assay Kit-Luminescence	19
L256	Lactate Assay Kit-WST	19
R252	ROS Assay Kit -Highly Sensitive DCFH-DA-	12
R253	ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	13
G257	GSSG/GSH Quantification Kit	17
G268	Glutamine Assay Kit-WST	19
G269	Glutamate Assay Kit-WST	19

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

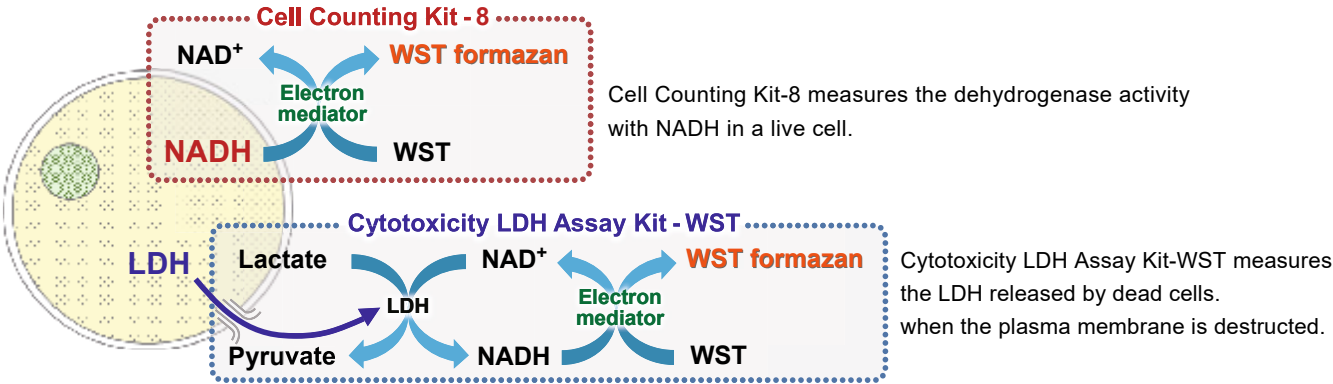
Cell Proliferation / Cytotoxicity Assay

Cell Counting Kit-8

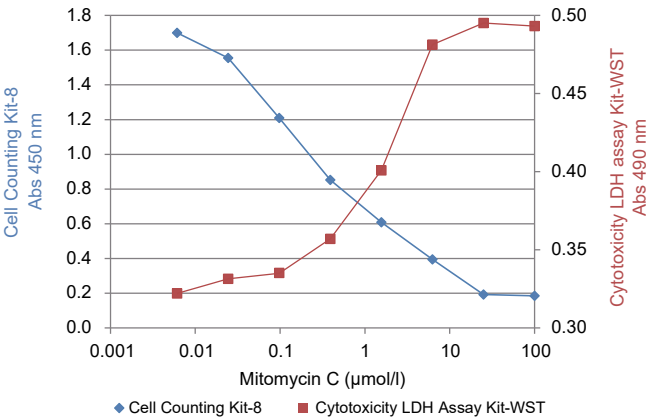
Cytotoxicity LDH Assay Kit-WST



Detection Principle



Simultaneous Usage of CCK-8 and Cytotoxicity LDH Assay Kit-WST



Drug: Mitomycin C

Cell Line: HeLa

Media: MEM, 10% FBS

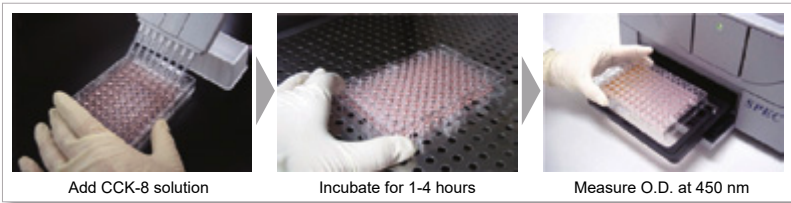
Incubation: 37°C, 5% CO<sub>2</sub> for 48 hours

Measuring Condition: Cell Counting Kit-8 (450 nm)

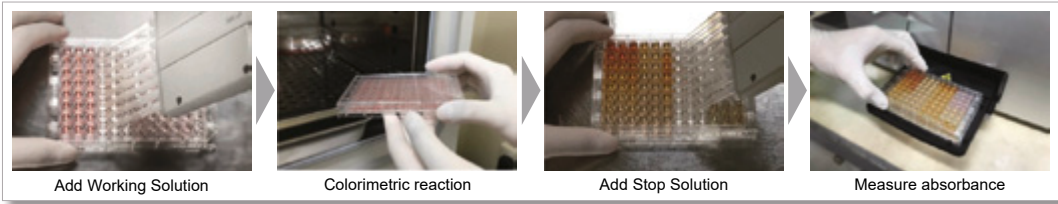
Cytotoxicity LDH Assay Kit-WST (490 nm)

# Simple Procedure

## • Cell Counting Kit-8

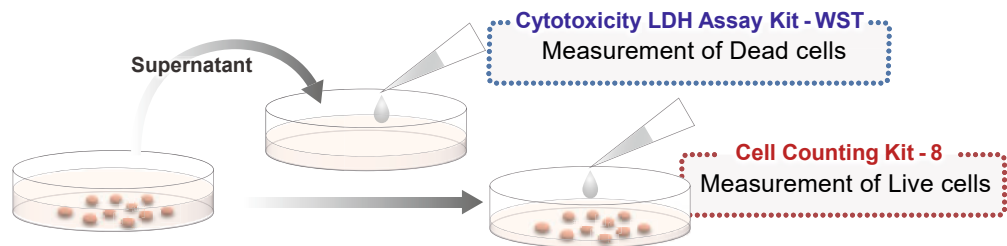


## • Cytotoxicity LDH Assay Kit-WST



## Same Samples can be used

Since same samples can be used for Cell Counting Kit-8 and Cytotoxicity LDH Assay Kit-WST, the method is convenient and time efficient.



Description	Unit	Code
Cell Counting Kit-8	500 tests	CK04-05
	1000 tests	CK04-11
	3000 tests	CK04-13
	10000 tests	CK04-20
Cytotoxicity LDH Assay Kit-WST	100 tests	CK12-01
	500 tests	CK12-05
	2000 tests	CK12-20

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

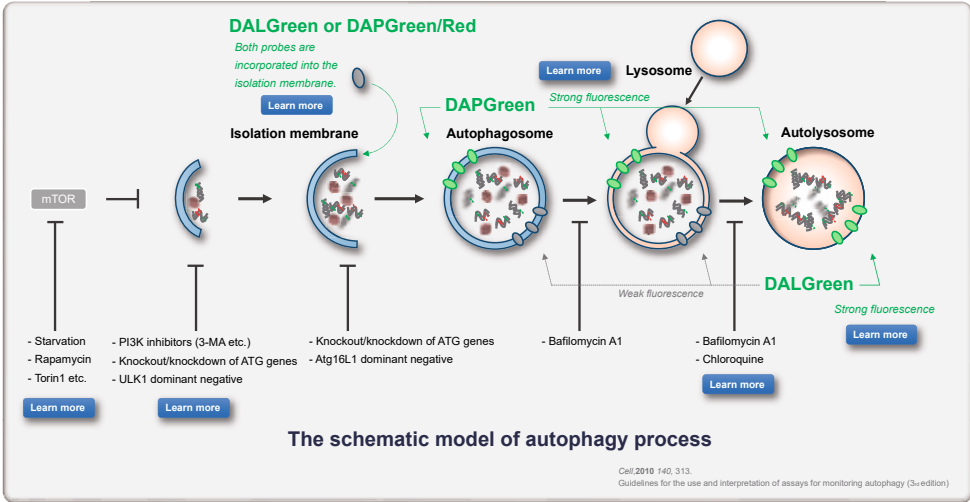
Autophagy

DAPGreen / Red - Autophagy Detection

DALGreen - Autophagy Detection



DAPGreen and DAPRed detect autophagosomes, while DALGreen detects autolysosomes. These dyes are permeable to cells and enables live cell imaging with fluorescence microscopy, and DAPGreen and DALGreen allow for quantitative assay by flow cytometry. Autophagy is an intracellular degradation system involving autophagosome formation, detected by DAPGreen and DAPRed, and lysosome fusion, detected by DALGreen, which fluoresces intensity increases in acidic conditions.



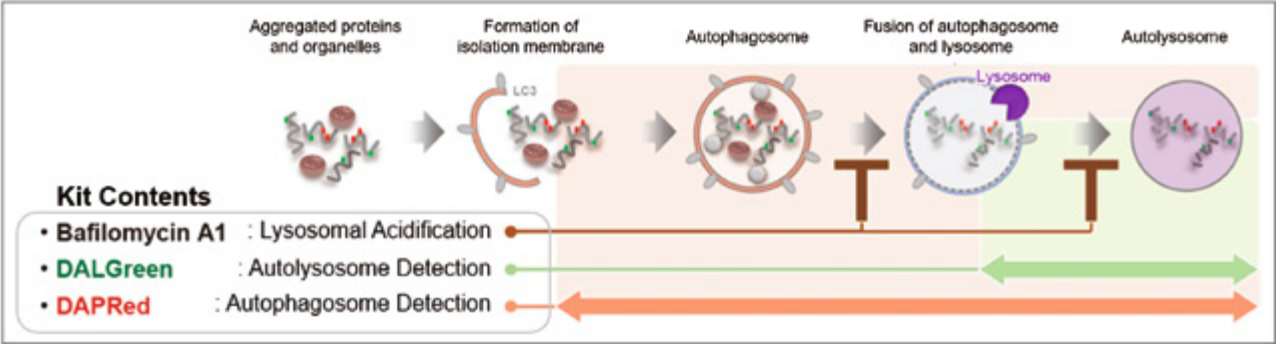
Feature of Each Dye

	Applicable instruments			Fluorescent properties	Volume / the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	✓	✓	✓	Ex = 425-475 nm Em = 500-560 nm <small>* For confocal microscope, the sample can be excited at 488 nm</small>	5 nmol x 1 / 35 mm dish: 25 (when used in 1.0 μmol/l)	LC3-GFP MDC Cyto-ID etc.
DAPRed	✓			Ex = 500-560 nm Em = 690-750 nm	5 nmol x 1 / 35 mm dish: 25 (when used in 1.0 μmol/l)	
DALGreen	✓	✓		Ex = 350-450 nm Em = 500-560 nm <small>* For confocal microscope, the sample can be excited at 488 nm</small>	20 nmol x 1 / 35 mm dish: 10 (when used in 1.0 μmol/l)	LC3-GFP-RFP etc.

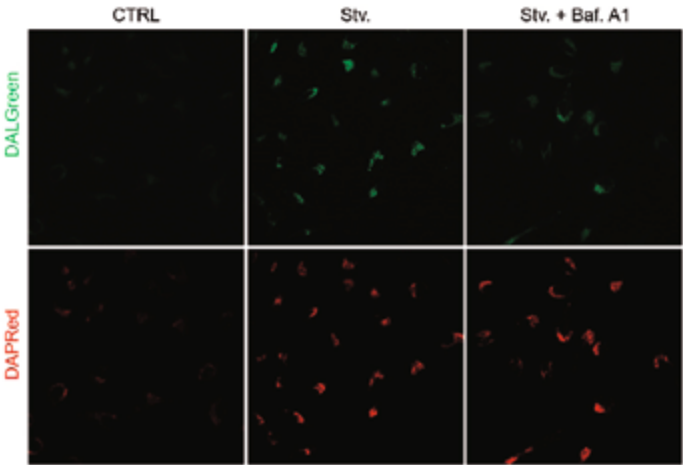
\*Double staining imaging by DAPGreen and DALGreen is not possible

Autophagy

# Autophagic Flux Assay Kit

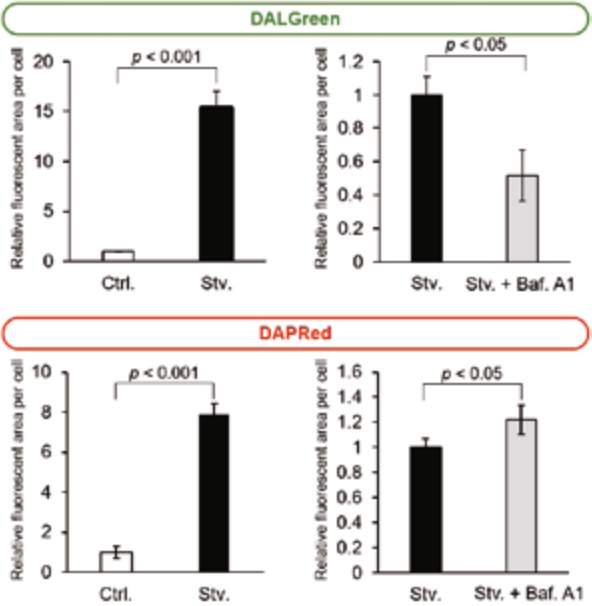


## Experimental Example: Autophagy Flux Analysis



By culturing HeLa cells in HBSS with starvation, autophagy was induced and DAPRed and DALGreen fluorescence increased. Addition of Baf. A1 decreased DALGreen fluorescence, indicating that autolysosomes were reduced and Autophagy Flux was inhibited.

Quantification method: Fluorescence values (area) were obtained in Image J and normalized by the number of cells per field of view\*. Number of samples: n=3  
\*Please obtain images with the same number of cells per field of view as possible.



Description	Unit	Code
Autophagic Flux Assay Kit	1 set*	A562-10
DALGreen - Autophagy Detection	20 nmol	D675-10
DAPGreen - Autophagy Detection	5 nmol	D676-10
DAPRed - Autophagy Detection	5 nmol	D677-10

\*Equivalent to 5 dishes (35 mm dish)

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.



Oxidative Stress

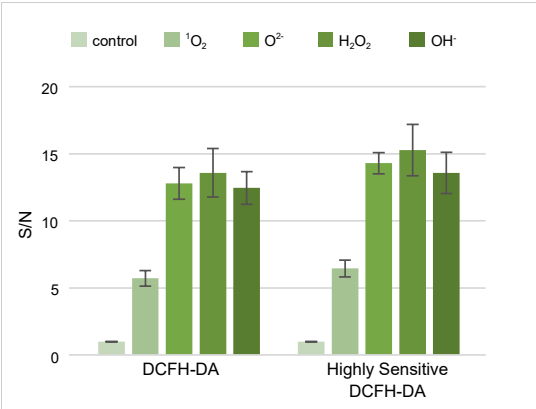
ROS Assay Kit -Highly Sensitive DCFH-DA-



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.

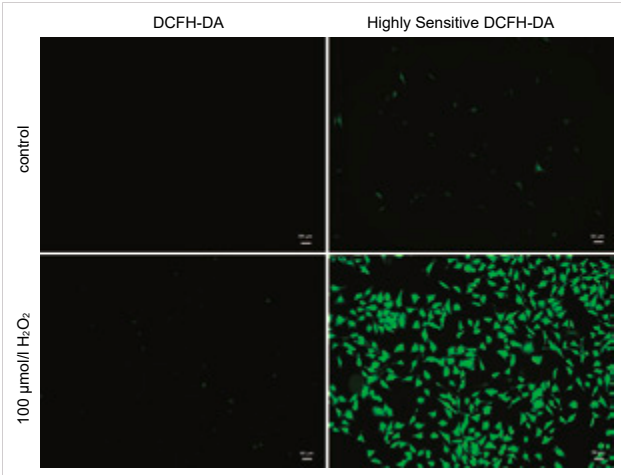
The reactivity of the Highly Sensitive DCFH-DA for ROS is similar to the reactivity of 2'-7' dichlorofluorescein diacetate (DCFH-DA). The Highly Sensitive DCFH-DA also has similar fluorescence characteristics ( $\lambda_{ex}$ : 505 nm,  $\lambda_{em}$ : 525 nm) to DCFH-DA. Therefore, ROS is detectable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

The selectivity for ROS

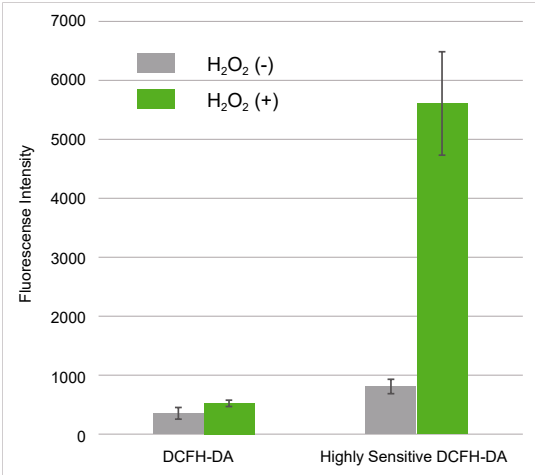


High Sensitive Detection Compared with DCFH-DA

Detection using fluorescent microscope



Detection using microplate reader



Hydrogen peroxide ( $H_2O_2$ )-treated HeLa cells ( $1 \times 10^4$  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

Oxidative Stress

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



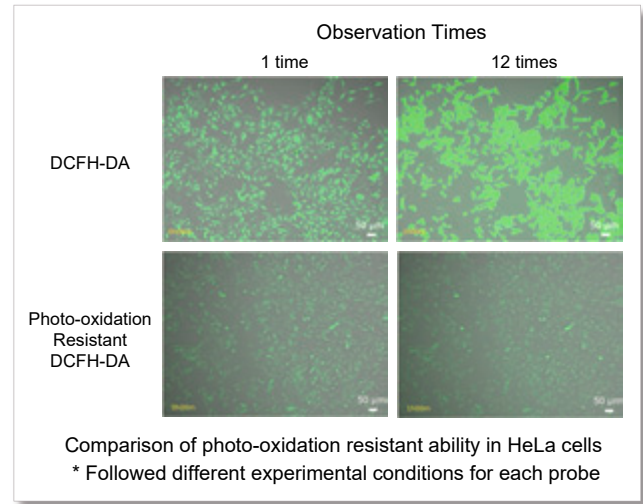
The dye that is employed in this kit allows ROS detection with higher sensitivity than DCFH-DA; It does not leak from cells because the fluorescent dye can immobilize protein via a chemical bond, and it is resistant to photo-oxidation compared with DCFH-DA. Moreover, the Loading Buffer in the kit maintains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

Time-lapse imaging movie Available

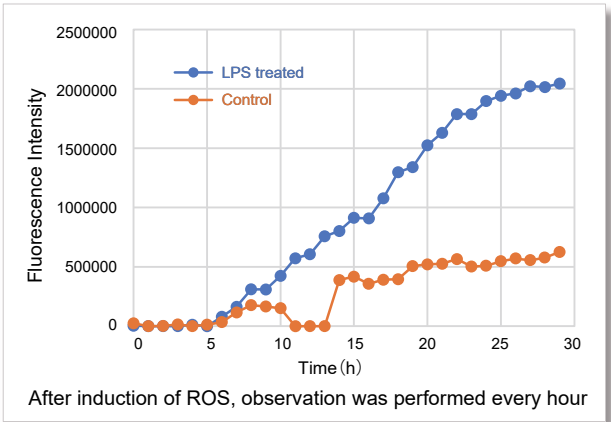
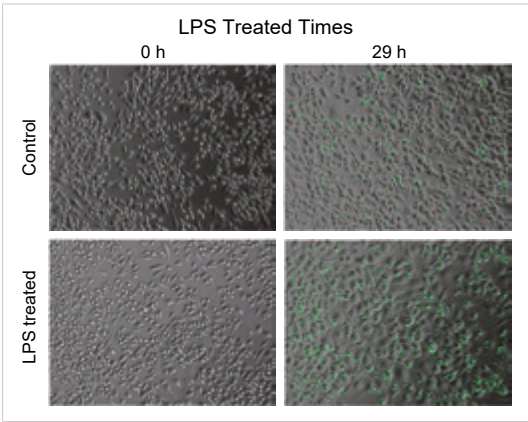
Other Probe

Photo-oxidation Resistant DCFH-DA-

Resistant to Photo-oxidation



Simultaneous Detection of ROS in LPS-treated macrophages



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

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

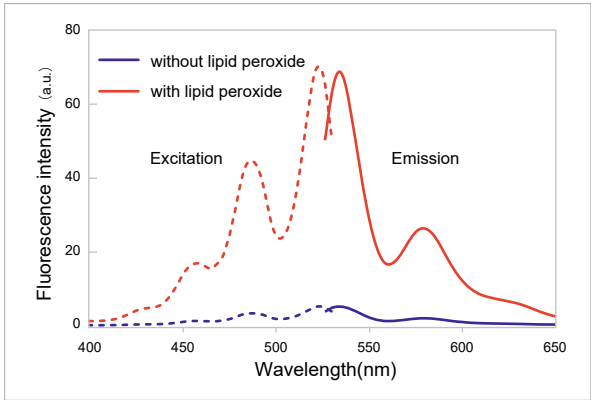
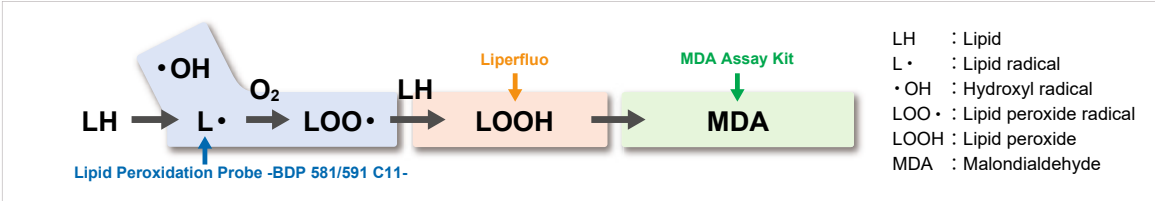


Lipid Peroxide Detection

Liperfluo

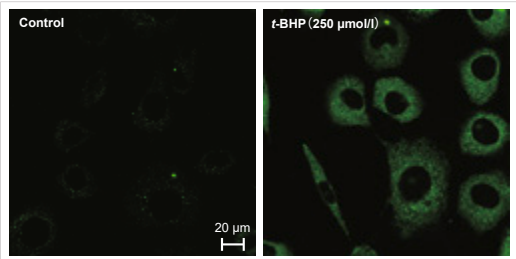


Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and 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

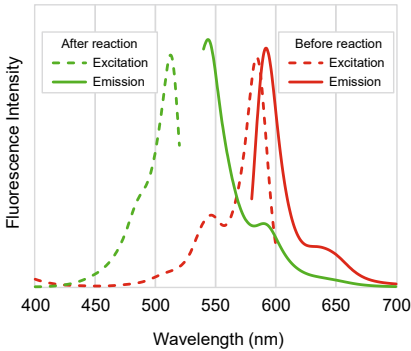
	Description	Unit	Code
Liperfluo		1 set (50 μg × 5)	L248-10

Lipid Peroxidation Detection

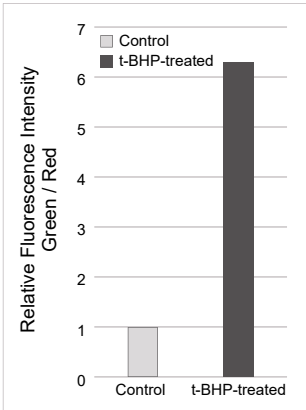
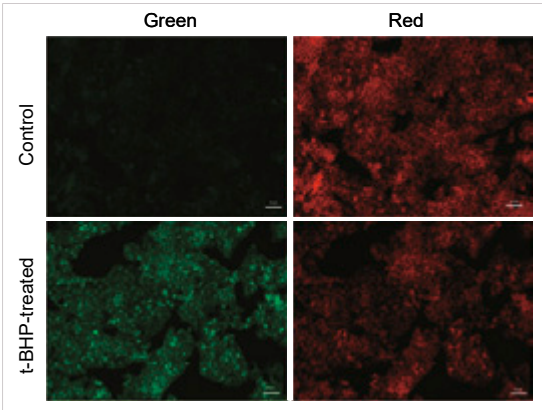
Lipid Peroxidation Probe -BDP 581/591 C11-



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

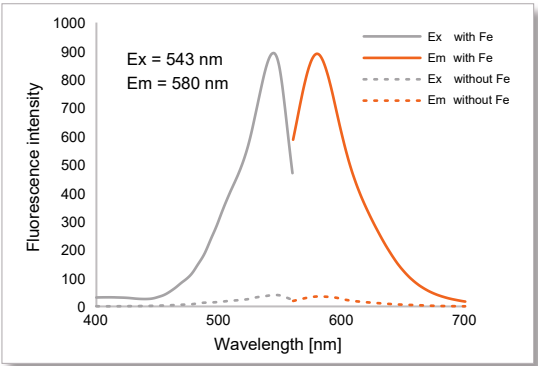
Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

# Intracellular Iron Ion Measurement

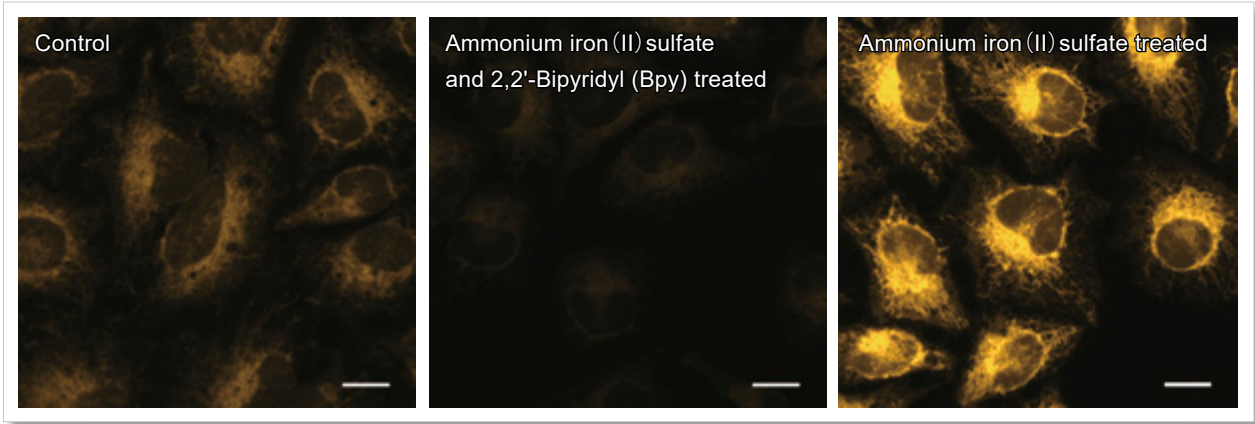
## FerroOrange



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<sup>2+</sup> in HeLa cells was detected by the FerroOrange.  
Ex = 561 nm, Em = 570-620 nm, Scale bars 20 μm

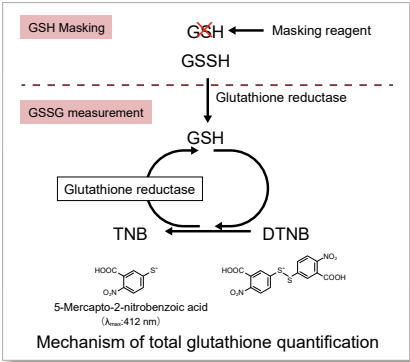
Description	Unit	Code
FerroOrange	1 tube	F374-10
	3 tube	F374-12

# 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 ( $\lambda_{\text{max}} = 412 \text{ nm}$ ) of DTNB (5,5-dithio-bis- (2-nitrobenzoic acid)). The quantity of GSH can also be determined, by subtracting GSSG from the total amount of glutathione. With this kit, GSH/ GSSG concentrations from  $0.5 \text{ }\mu\text{mol/l}$  to  $50 \text{ }\mu\text{mol/l}$  and GSSG concentrations from  $0.5 \text{ }\mu\text{mol/l}$  to  $25 \text{ }\mu\text{mol/l}$  can be quantified.



### Assay Procedure

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

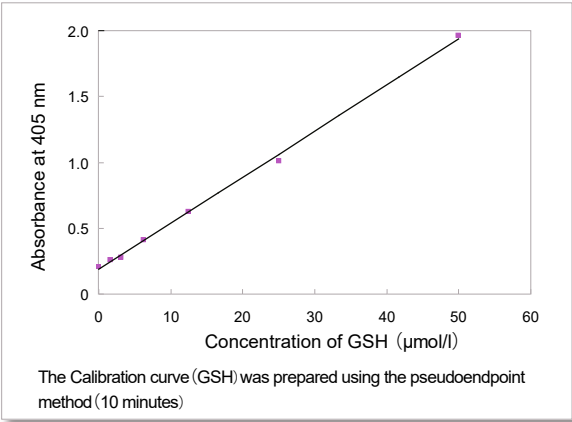
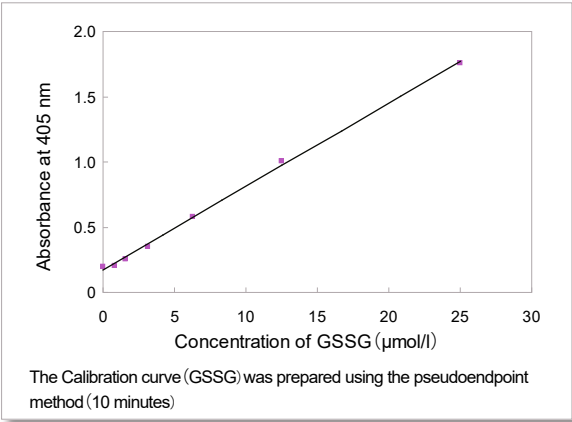
2) Add Buffer solution to each well

3) Incubate at  $37^{\circ}\text{C}$  for 1 h.

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

6)-7) After incubating at  $37^{\circ}\text{C}$  for 10 minutes, measure the absorbance of each well with a microplate.

### Calibration Curve



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

Proliferation
Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles
Exosome, Lipid Droplet, etc.

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.

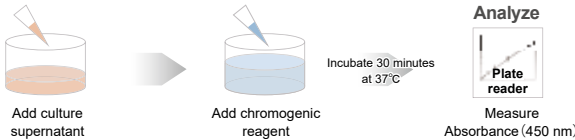
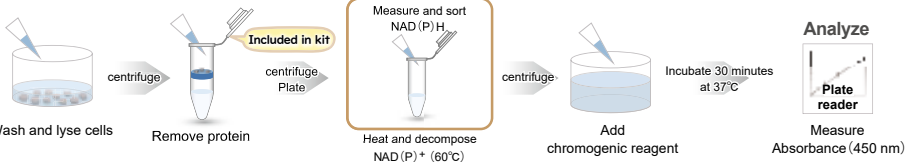
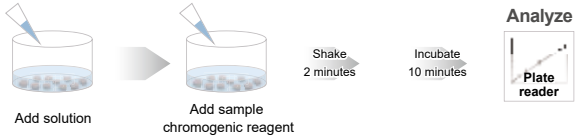
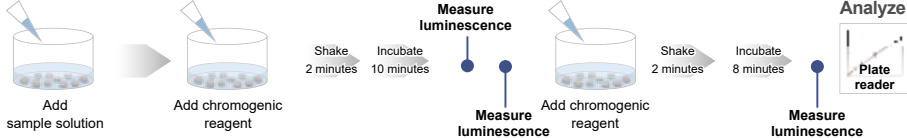
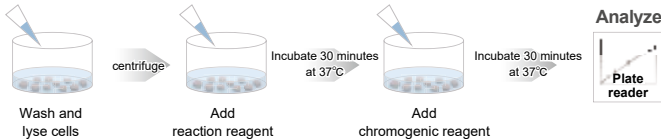
# Measurements of Intracellular Metabolism



Description	Unit	Code
Starter Kit		
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10
Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272-10
Quantification for Intracellular Metabolism		
ATP Assay Kit-Luminescence	50 tests	A550-10
	200 tests	A550-12
ADP/ATP Ratio Assay Kit-Luminescence	100 tests	A552-10
Glucose Assay Kit-WST	50 tests	G264-05
	200 tests	G264-20
Glutamine Assay Kit-WST	100 tests	G268-10
Glutamate Assay Kit-WST	100 tests	G269-10
α-Ketoglutarate Assay Kit-Fluorometric	100 tests	K261-10
	50 tests	L256-10
Lactate Assay Kit-WST	200 tests	L256-20
	100 tests	N509-10
NADP/NADPH Assay Kit-WST	100 tests	N510-10
Uptake Assay Kit		
Glucose Uptake Assay Kit-Blue	1 set	UP01-10
Glucose Uptake Assay Kit-Green	1 set	UP02-10
Glucose Uptake Assay Kit-Red	1 set	UP03-10
Amino Acid Uptake Assay	20 tests	UP04-10
	100 tests	UP04-12
Cystine Uptake Assay Kit	20 tests	UP05-10
	100 tests	UP05-12
Fatty Acid Uptake Assay Kit	100 tests	UP07-10

# Simple Procedure for First Time User

For a first-time user, the kit includes the reagents and components necessary for measuring samples. You'll soon realize how easy it is to use.

Determination index	Detection	Operation
<div>Glucose</div> <div>Lactate</div> <div>Glutamine</div> <div>Glutamate</div> <div>NAD/NADH</div> <div>NADP/NADPH</div>	Colorimetric	<p>Simply transfer the culture supernatant to a plate and mix it with the chromogenic reagent</p>  <p>Wash and lyse cells    Add culture supernatant    Add chromogenic reagent    Incubate 30 minutes at 37°C    Analyze (Plate reader)    Measure Absorbance (450 nm)</p>
		 <p>Wash and lyse cells    Remove protein    Included in kit    Measure and sort NAD(P)H    Heat and decompose NAD(P)<sup>+</sup> (60°C)    Add chromogenic reagent    Incubate 30 minutes at 37°C    Analyze (Plate reader)    Measure Absorbance (450 nm)</p>
<div>ATP</div> <div>ADP/ATP</div>	Luminescent	<p>Kit includes ATP standard - very easy to use</p>  <p>Add solution    Add sample    Shake 2 minutes    Incubate 10 minutes    Analyze (Plate reader)    Measure luminescence</p>
		 <p>Add sample solution    Add chromogenic reagent    Shake 2 minutes    Incubate 10 minutes    Measure luminescence    Add chromogenic reagent    Shake 2 minutes    Incubate 8 minutes    Analyze (Plate reader)    Measure luminescence</p>
<div><math>\alpha</math>-ketoglutaric acid</div>	Fluorescent	<p>Less variable results than existing assays</p>  <p>Wash and lyse cells    Add reaction reagent    Incubate 30 minutes at 37°C    Add chromogenic reagent    Incubate 30 minutes at 37°C    Analyze (Plate reader)</p>

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.





## Intracellular Metabolism

# Glycolysis/JC-1 MitoMP Assay Kit

- Two indicators can be measured in one sample  
(Lactate production and mitochondrial membrane potential)
- Easy-to-understand detailed protocol

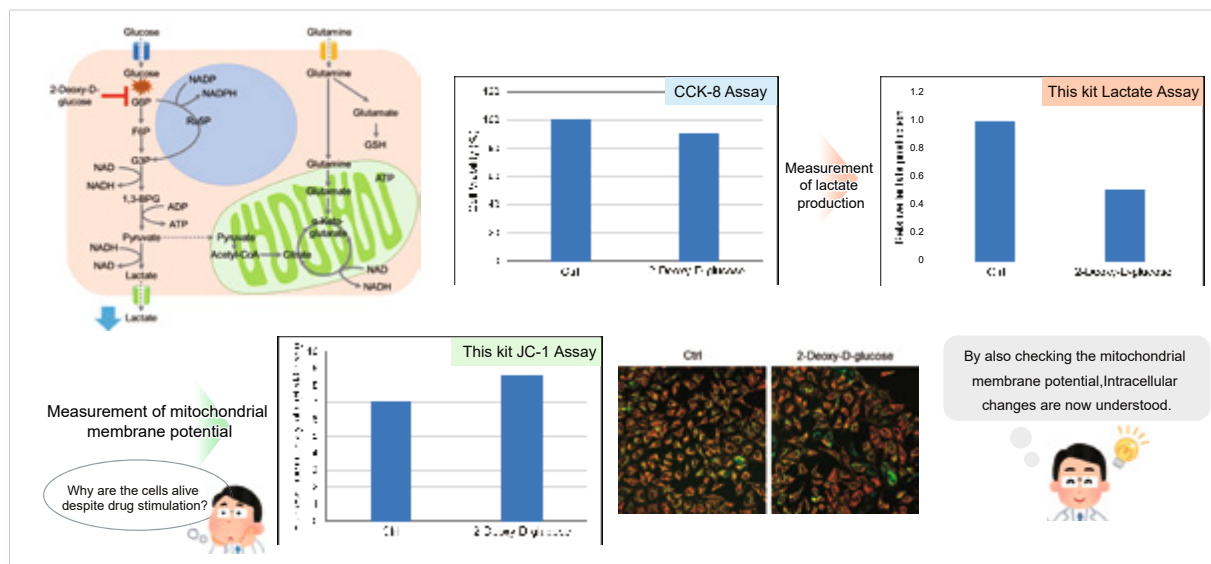
Intracellular metabolic changes caused by any stimulations can be detected by measuring lactate production and mitochondrial membrane potential. In certain instances, cells manage to survive despite sustaining damage to their glycolytic system or mitochondrial function, the principal pathways for energy production. It is understood that this occurs as cells strive to persist and prevent cell death by augmenting glycolysis even when mitochondrial function is compromised, or by activating mitochondrial function when glycolysis is impaired.

### Experimental Example:

#### Intracellular metabolic changes in HeLa cells treated with the glycolytic inhibitor 2-Deoxy-D-glucose

When we evaluated cell viability in 2-DG-treated HeLa cells using the CCK-8\* assay, we observed minimal changes in viability. However, given the observed decrease in lactate production, it prompted us to question how cell viability was maintained in spite of glycolytic system inhibition. To answer this, we examined the mitochondrial membrane potential using the JC-1 Assay. The results from this investigation suggest that HeLa cells preserve their survival by boosting mitochondrial function when the glycolytic system is inhibited by 2-DG.

\* Cell Counting Kit-8 (product code: CK04) is not included in this kit.



Description	Unit	Code
Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272-10

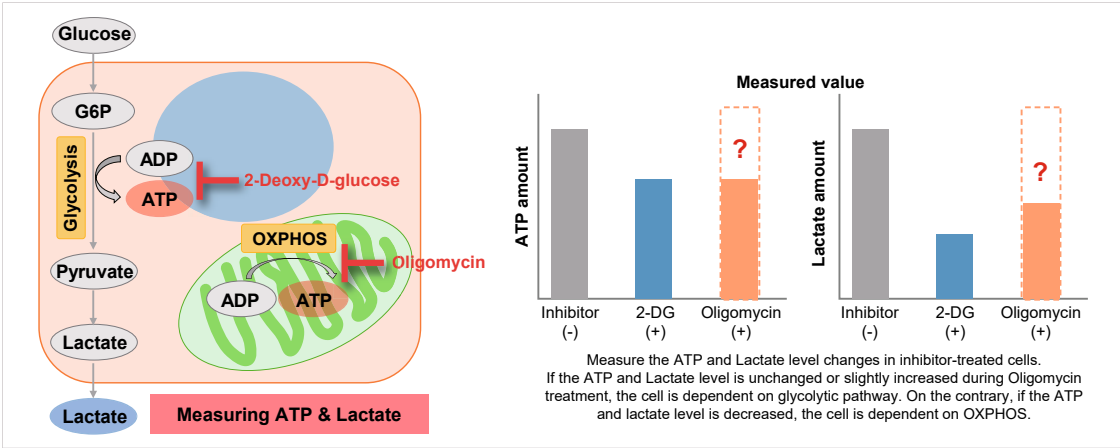
Intracellular Metabolism

# Glycolysis/OXPHOS Assay Kit



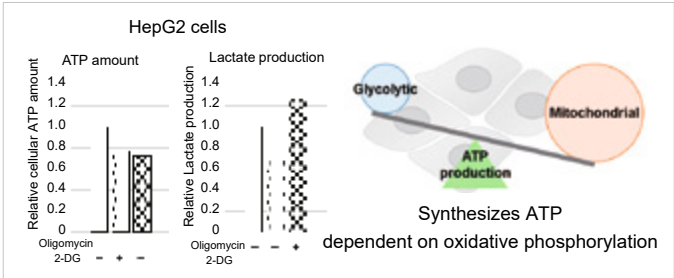
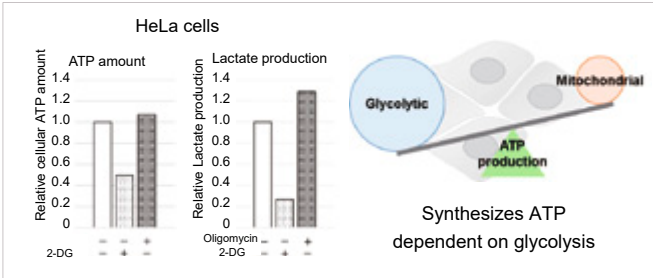
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

Combining methods (1) and (2) can be used to measure the metabolic pathway dependency of cells. Cells are treated with oligomycin or 2-DG to inhibit OXPHOS or ATP synthesis in the glycolytic pathway, and the amounts of ATP and lactate production are measured, respectively. Changes in the amount of ATP can be used to determine the efficiency of energy production, and changes in the amount of lactate produced can be used to determine changes in glycolytic capacity and evaluate whether cells are dependent on glycolysis or OXPHOS.



## Experimental Example:

### Comparison of metabolic pathway dependence in different cell line



Description	Unit	Code
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.

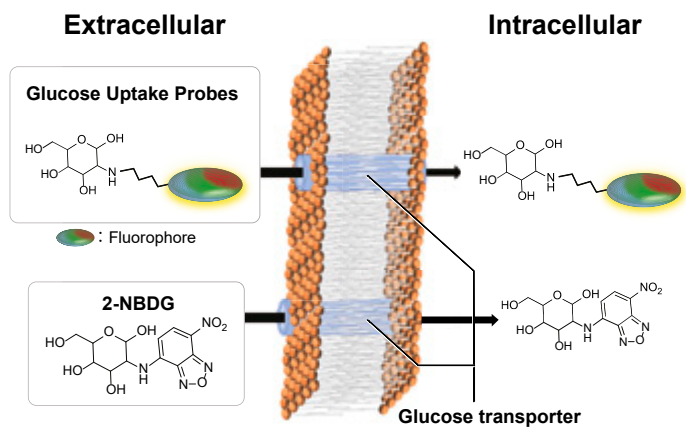
Proliferation  
Cytotoxicity  
Senescence  
Autophagy  
Oxidative  
Stress  
Metabolism  
Mitochondria  
Lysosome  
Endocytosis  
Other Organelles  
Exosome, Lipid Droplet, etc.

Intracellular Metabolism

# Glucose Uptake Assay Kit



- Highly sensitive and simple measurement of glucose uptake capacity
- Applicable for microscopy & FCM
- Reduces dye leakage after staining



Glucose Uptake Probe allowing highly sensitive detection of cellular glucose uptake by fluorescence imaging or flow cytometry. The WI Solution in this kit can enhance cellular retention to provide more reliable experimental data. Also, compare with the existing method (2-NBDG), the measurement time can be significantly reduced.

## Comparison with Existing Method

The comparison of the Glucose Uptake Probe Series and the existing method(2-NBDG) is as below.

product name	Fluorescence microscope	Plate reader detection	FCM detection	Retention ability	Fluorescence characteristics
Glucose Uptake Assay Kit-Blue	○	×	○	1 hour *	$\lambda_{ex}$ :386 nm $\lambda_{em}$ :474 nm
Glucose Uptake Assay Kit-Green	○	○	○	1 hour *	$\lambda_{ex}$ :507 nm $\lambda_{em}$ :518 nm
Glucose Uptake Assay Kit-Red	○	○	○	1 hour *	$\lambda_{ex}$ :560 nm $\lambda_{em}$ :572 nm
2-NBDG	○	×	○	30 minutes or less *	$\lambda_{ex}$ :465 nm $\lambda_{em}$ :540 nm

\*Result of A549 cells, the retention time for other cell lines may be different.

Description	Unit	Code
Glucose Uptake Assay Kit-Blue	1 set	UP01-10
Glucose Uptake Assay Kit-Green	1 set	UP02-10
Glucose Uptake Assay Kit-Red	1 set	UP03-10

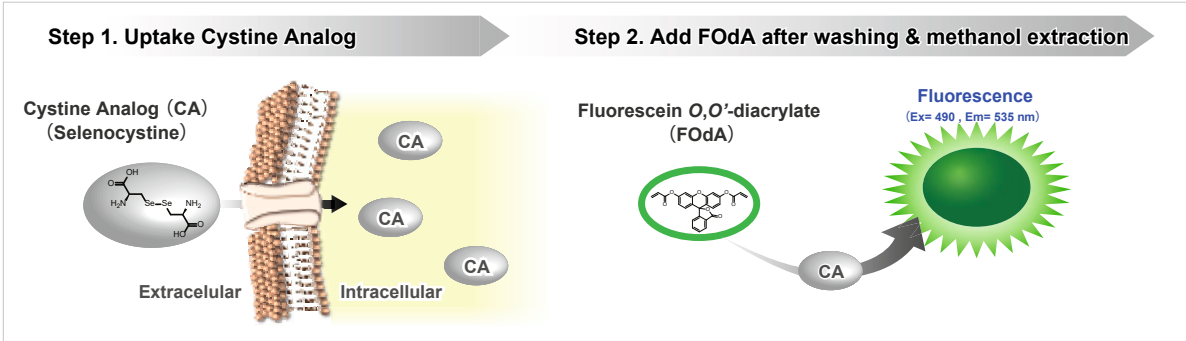
Intracellular Metabolism

# Cystine Uptake Assay Kit



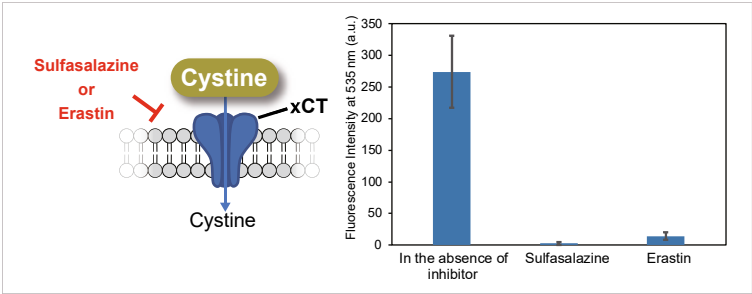
- Easier way to cystine uptake assay
- Applied for plate assay

The Cystine Analog (CA) in this kit can be taken up into cells via xCT, and the incorporated CA can be specifically detected using the Fluorescent Probe and Reducing Agent. Thus, the xCT activity can be measured easily.[Patent applied]



## Evaluation of xCT inhibitor Sulfasalazine or Erastin

Using this kit, we measured the inhibitory effect of sulfasalazine and erastin on cystine uptake by HeLa cells. The fluorescence intensity of the sulfasalazine and elastin groups decreased significantly, indicating that both reagents inhibit cystine uptake.



Experiment Condiitons

Cell Line: HeLa cells

Pretreatment: DMEM (cystine-free, serum-free), 37°C, 5 min

Uptake conditions: 0.5 mmol/l sulfasalazine or 2 µmol/l erastin / Cystine Analog / DMEM (cystine-free, serum-free), 37°C, 30 min

Instrument: Fluorescent Plate Reader

Filter: Ex=485 nm, Em=535 nm

Description	Unit	Code
Cystine Uptake Assay Kit	20 tests	UP05-10
	100 tests	UP05-12

Proliferation
Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

# Mitochondrial Research



Proliferation  
Cytotoxicity  
Senescence  
Autophagy  
Oxidative Stress  
Metabolism  
Mitochondria  
Lysosome  
Endocytosis  
Other Organelles  
Exosome, Lipid Droplet, etc.

## Mitochondrial Superoxide Detection

MitoBright ROS Deep Red - Mitochondrial Superoxide Detection

Allow to detecting mitochondrial superoxide with a long wavelength (Deep Red)

## Singlet Oxygen Detection

Si-DMA for Mitochondrial Singlet Oxygen Imaging

Real-time visualization of  $^1O_2$  generation

## Lipophilic Peroxide Detection

MitoPeDPP

Live-cell fluorescent imaging of lipophilic peroxide

## Ferrous Ion Detection

Mito-FerroGreen

Live-cell fluorescent imaging of intracellular  $Fe^{2+}$

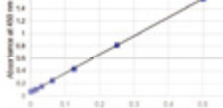
## Mitophagy Detection

Mitophagy Detection Kit

Live-cell fluorescent imaging of mitophagy without transfection

## Measurement of Glucose

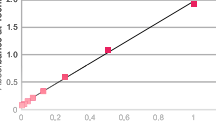
Glucose Assay Kit-WST



Measurement of intracellular glucose concentrations via fluorescence

## Measurement of Lactate

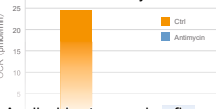
Lactate Assay Kit-WST



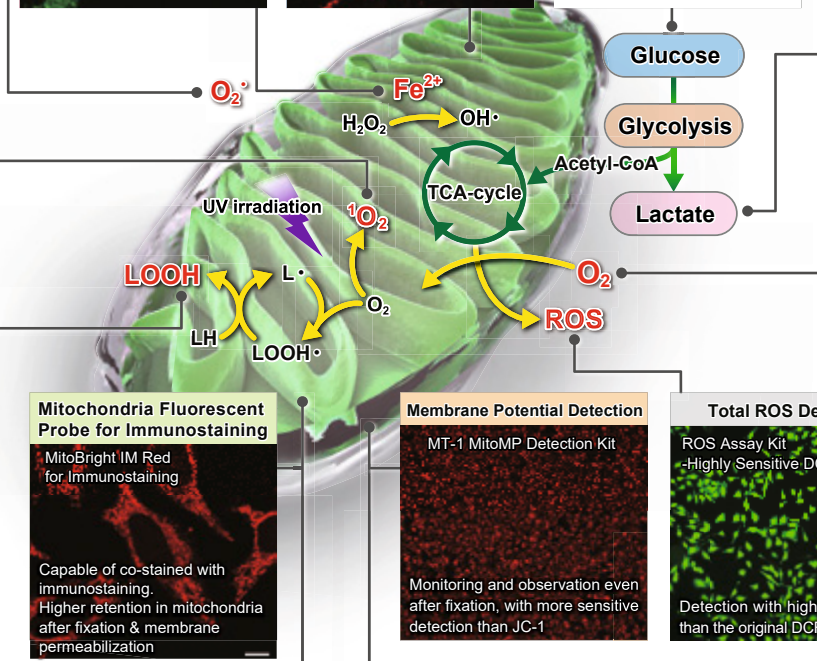
Measuring lactate to infer glycolytic activity

## Oxygen consumption rate (OCR) Detection

Extracellular OCR Plate Assay Kit



Applicable to regular fluorescent plate reader with temperature-controlled incubation



## Mitochondria Fluorescent Probe for Immunostaining

MitoBright IM Red for Immunostaining

Capable of co-stained with immunostaining. Higher retention in mitochondria after fixation & membrane permeabilization

## Membrane Potential Detection

MT-1 MitoMP Detection Kit

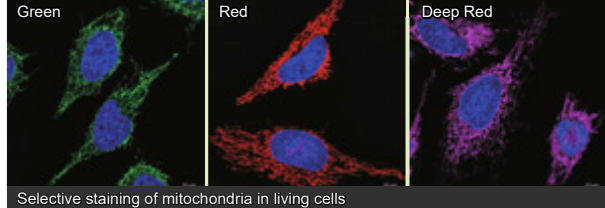
Monitoring and observation even after fixation, with more sensitive detection than JC-1

## Total ROS Detection

ROS Assay Kit -Highly Sensitive DCFH-DA-

Detection with higher sensitivity than the original DCFH-DA

## Mitochondrial Staining MitoBright LT Series (Green / Red / DeepRed)



Selective staining of mitochondria in living cells

## Membrane Potential Detection

JC-1 MitoMP Detection Kit

Analysis of mitochondrial membrane potential through fluorescence color ratios via microscopy, FCM, or microplate reader

Description	Unit	Code
Metabolism		
Extracellular OCR Plate Assay Kit	100 tests	E297-10
Glucose Assay Kit-WST	50 tests	G264-05
	200 tests	G264-20
Lactate Assay Kit-WST	50 tests	L256-10
	200 tests	L256-20
Mitochondrial Membrane Potential		
MT-1 MitoMP Detection Kit	1 set	MT13-10
JC-1 MitoMP Detection Kit	1 set	MT09-10
Mitophagy		
Mitophagy Detection Kit	1 set	MD01-10
Mtphagy Dye	5 $\mu\text{g} \times 3$	MT02-10
Mitochondrial Staining		
MitoBright LT Green	400 $\mu\text{l}$	MT10-12
MitoBright LT Red	400 $\mu\text{l}$	MT11-12
MitoBright LT Deep Red	400 $\mu\text{l}$	MT12-12
MitoBright IM Red for Immunostaining	20 $\mu\text{l} \times 1$	MT15-10
	20 $\mu\text{l} \times 3$	MT15-12
Oxidative Stress		
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	100 nmol $\times 1$	MT16-10
	100 nmol $\times 3$	MT16-12
Mito-FerroGreen	1 set (50 $\mu\text{g} \times 2$ )	M489-10
Si-DMA for Mitochondrial Singlet Oxygen Imaging	2 $\mu\text{g}$	MT05-10
MitoPeDPP	5 $\mu\text{g} \times 3$	M466-10

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.



Mitochondrial Research

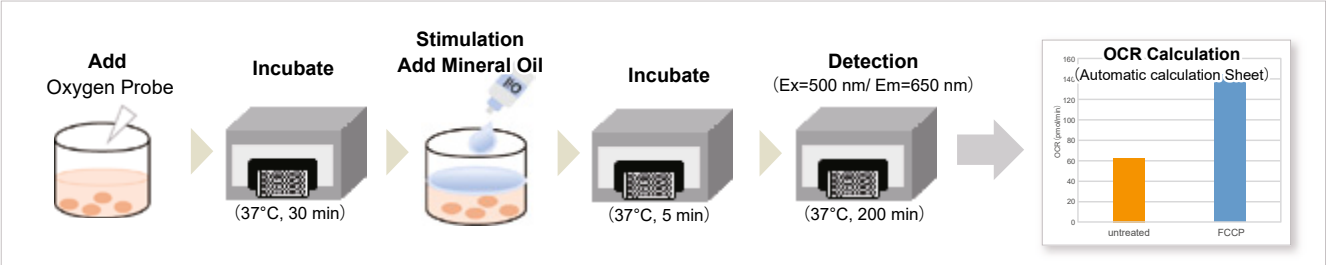
Extracellular OCR Plate Assay Kit



- Applicable to regular fluorescent plate reader with temperature-controlled incubation
- No need for an expensive instrument, special medium, and plates
- All-in-One Kit with OCR calculation Sheets



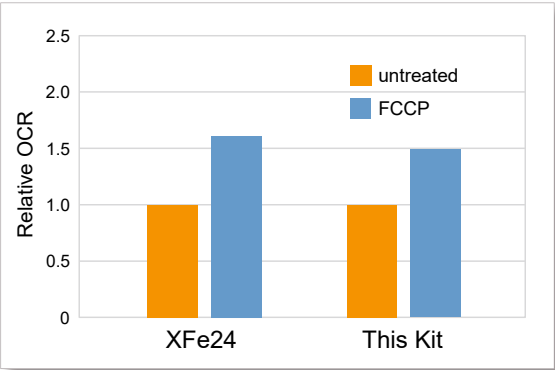
Procedure



Comparison with Flux Analyzer

Flux Analyzer (XFe24) and this kit were measured on the same day under the same conditions (cell type, cell number, and FCCP concentration). As a result, correlated data of oxygen consumption rate changes were obtained for XFe24 and this kit.

Cells: HepG2  
 Cell Number:  $5 \times 10^4$  cells/well  
 Stimulation: FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazine)  
 FCCP Concentration: 2  $\mu\text{mol/l}$



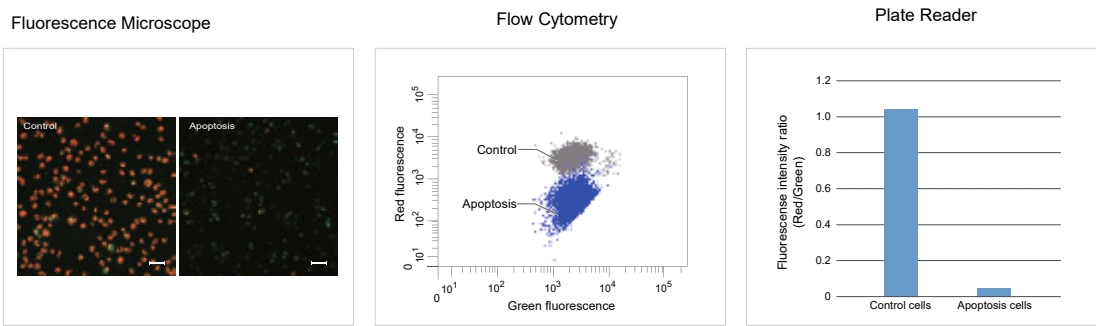
Description	Unit	Code
Extracellular OCR Plate Assay Kit	100 tests	E297-10

Mitochondrial Membrane Potential Detection

JC-1 MitoMP Detection Kit



JC-1 forms aggregate (in healthy mitochondria) with red fluorescence. As membrane potential decreases, JC-1 becomes monomers, which shows in green fluorescence. The change in ratio of red to green fluorescence is used as an indicator of mitochondrial condition.



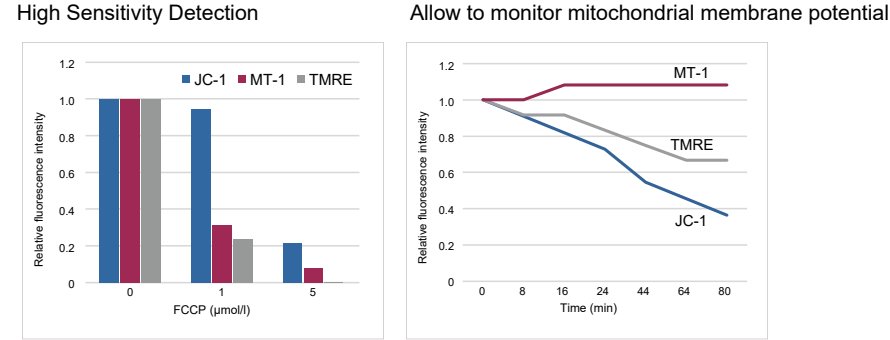
Description	Unit	Code
JC-1 MitoMP Detection Kit	1 set	MT09-10

Mitochondrial Membrane Potential Detection

MT-1 MitoMP Detection Kit



JC-1 dye, TMRE, and TMRM are widely used to monitor MMP, however, these dyes have some limitations, such as low photostability and poor retention after aldehyde fixation. These limitations result in poor reproducibility of experiments. Dojindo's MT-1 MitoMP Detection Kit overcomes these limitations. In addition, the Imaging Buffer included in this kit minimizes background fluorescence and maintains cell vitality while the assay is being performed.



Description	Unit	Code
MT-1 MitoMP Detection Kit	1 set	MT13-10

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

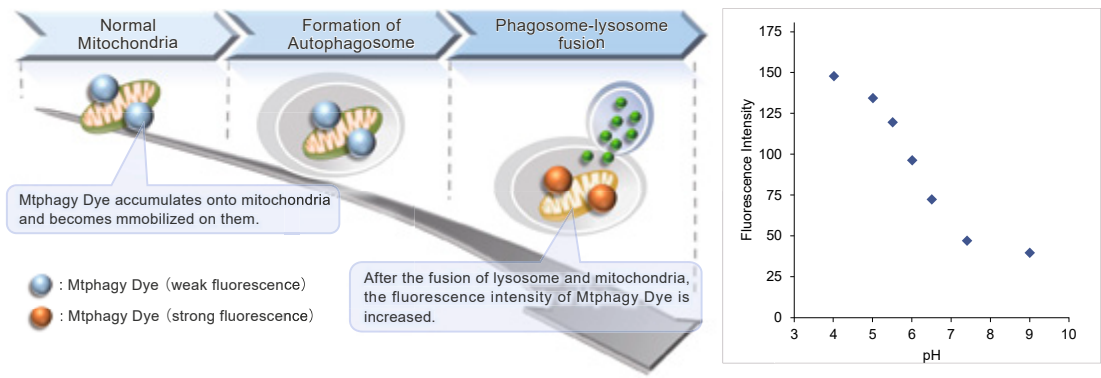
Other Organelles  
Exosome, Lipid Droplet, etc.

# Mitochondrial Research

## Mitophagy Detection Kit

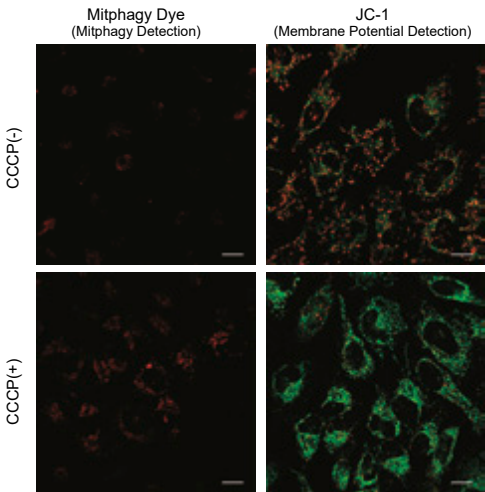


This kit is composed of Mtphagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtphagy Dye accumulates in intact mitochondria, is immobilized on it with chemical bond and exhibits a weak fluorescence from the influence of surrounding condition. When Mitophagy is induced, the damaged mitochondria fuses to lysosome and then Mtphagy Dye emits a high fluorescence. To confirm the fusion of Mtphagy Dye–labeled mitochondria and lysosome, Lyso Dye included in this kit can be used.



The fluorescent intensity of Mtphagy Dye is increased at pH 4-5.

## Mitophagy Induction and Mitochondrial Membrane Potential Changes



Mitochondrial condition in the carbonyl cyanide m-chlorophenyl hydrazine (CCCP) treated Parkin-expressing HeLa cells was compared with untreated cells using Mitophagy Detection Kit (MD01, MT02) and JC-1 MitoMP Detection Kit (MT09).

Result:

As a result, mitophagy was hardly detected in the CCCP-untreated cells, and mitochondrial membrane potential was maintained normally. On the other hand, in CCCP-treated cells, we observed a decrease in mitochondrial membrane potential (decrease in red fluorescence of JC-1) and induction of mitophagy (increase in fluorescence of Mtphagy Dye).

Description	Unit	Code
Mitophagy Detection Kit	1 set	MD01-10
Mtphagy Dye	5 µg × 3	MT02-10

Mitochondrial Superoxide Detection

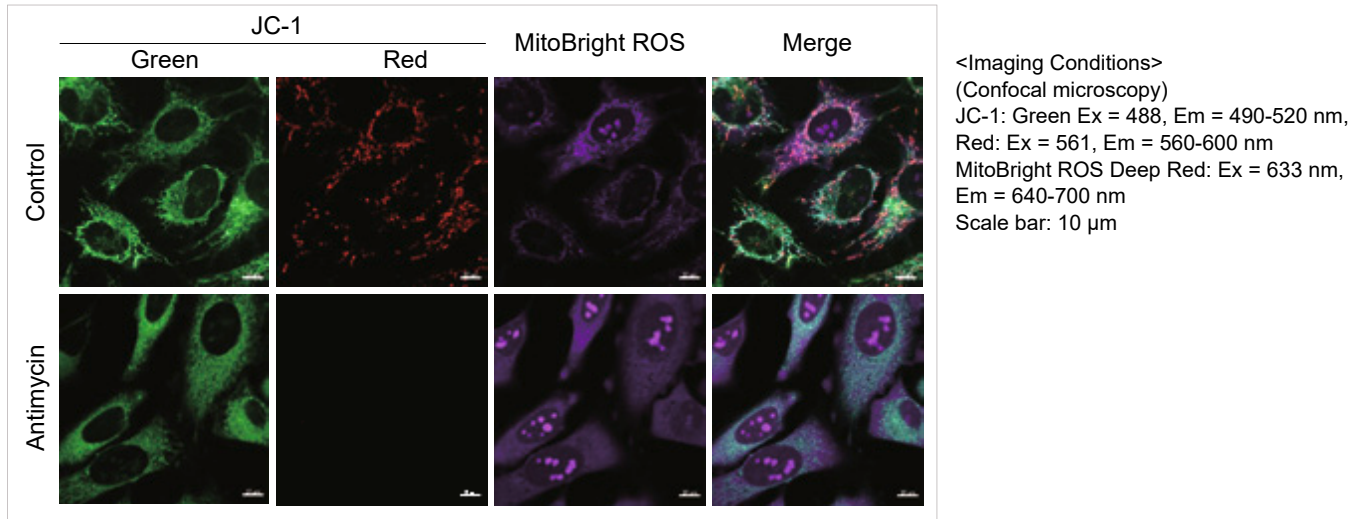
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection



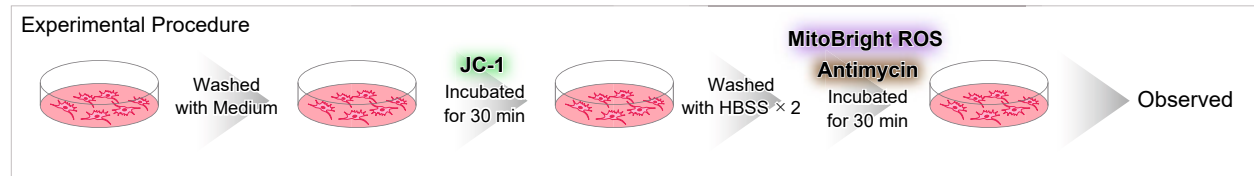
This dye emits deep red fluorescence; its fluorescence does not overlap with emission wavelengths that other red fluorescent markers use. Furthermore, the MitoBright ROS Deep Red is better able to selectively detect superoxide, compared to Company T's product Red.

Experimental Example

Simultaneously Evaluation of Mitochondrial Superoxide and Membrane Potential



After HeLa cells were washed with HBSS, co-stained with MitoBright ROS Deep Red and mitochondrial membrane potential staining dye (JC-1: code MT09), and the generated mitochondrial ROS and membrane potential were observed simultaneously. As a result, the decrease in mitochondrial membrane potential and the generation of mitochondrial ROS are simultaneously observed.



Description	Unit	Code
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	100 nmol × 1	MT16-10
	100 nmol × 3	MT16-12

Proliferation Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.

Proliferation  
Cytotoxicity  
Senescence  
Autophagy  
Oxidative  
Stress  
Metabolism  
Mitochondria  
Lysosome  
Endocytosis  
Other Organelles  
Exosome, Lipid Droplet, etc.

# Mitochondrial Superoxide Detection

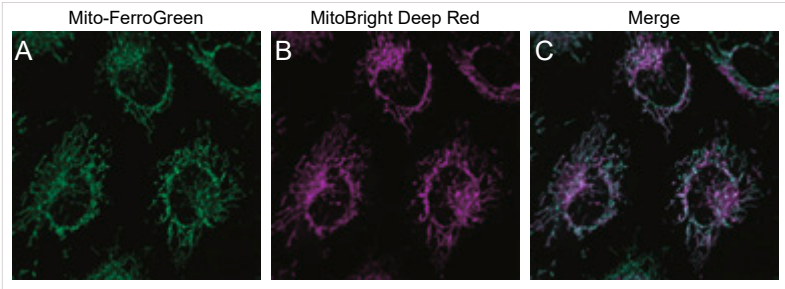
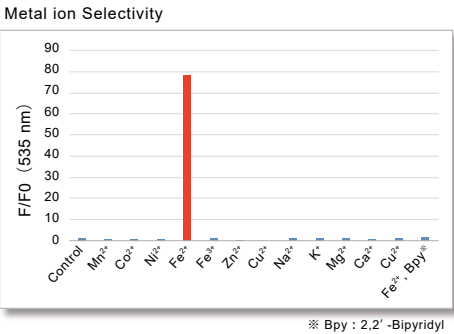
## Mito-FerroGreen



Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion ( $\text{Fe}^{2+}$ ) in mitochondria where Fe-S clusters and heme proteins are synthesized, and enables live cell fluorescent imaging of intracellular  $\text{Fe}^{2+}$ . Mito-FerroGreen has no chelating ability. Mito-FerroGreen and  $\text{Fe}^{2+}$  react irreversibly, which is different from the detection principle of calcium-iron probes such as Fluo-3.

### Double staining with mitochondrial staining probe

HeLa cells incubated with Mito-FerroGreen and MitoBright Deep Red, treated with ammonium iron(II) sulfate, were observed by fluorescence microscopy.



Double staining with mitochondrial staining probe  
Mito-FerroGreen (5  $\mu\text{mol/l}$ ) Ex/Em = 488 nm/ 500-550 nm  
MitoBright Deep Red (200 nmol/l) Ex/Em = 640 nm/ 656-700 nm  
A Mito-FerroGreen  
B MitoBright Deep Red  
C Merge

### Iron Detection Dyes

	Mito-FerroGreen (M489)	FerroOrange (F374)
Localization	Mitochondria	Intracellular
Fluorescent Property	$\lambda_{\text{ex}}$ 505 nm, $\lambda_{\text{em}}$ 535 nm	$\lambda_{\text{ex}}$ 543 nm, $\lambda_{\text{em}}$ 580 nm
Instrument (filter)	Fluorescence microscope (FITC, GFP)	Fluorescence microscope, plate reader (Cy3)
Sample	Live Cell	Live cell
The number of assays	1 set (50 $\mu\text{g} \times 2$ ) 10 assays at 35 mm dish (final concentration 5 $\mu\text{mol/l}$ )	1 tube (24 $\mu\text{g}$ ) 17 assays at 35 mm dish (final concentration 1 $\mu\text{mol/l}$ )

Description	Unit	Code
Mito-FerroGreen	1 set (50 $\mu\text{g} \times 2$ )	M489-10
FerroOrange	1 tube	F374-10
	3 tube	F374-12

# Mitochondrial Staining

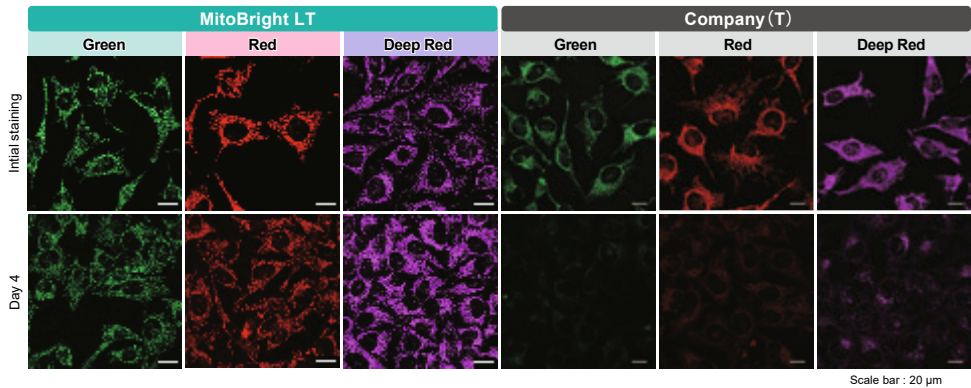
## MitoBright LT Series



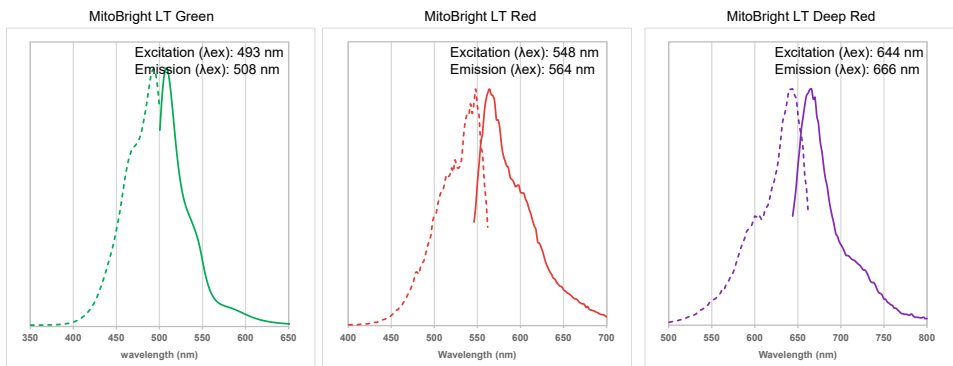
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color options (Green, Red and Deep Red), and are provided as a ready-to-use DMSO solution. A working solution can easily be prepared in a single dilution step with growth medium or HBSS.

### Stained in serum-contained media

HeLa cells were stained with MitoBright LTs or an existing reagent and observed after 4 days. MitoBright LT remained unchanged and observable even after 7 days, while the existing reagent's intensity decreased.



### Fluorescence Properties



Description	Unit	Code
MitoBright LT Green	400 μl	MT10-12
MitoBright LT Red	400 μl	MT11-12
MitoBright LT Deep Red	400 μl	MT12-12

Proliferation
Cytotoxicity
Senescence
Autophagy
Oxidative Stress
Metabolism
Mitochondria
Lysosome
Endocytosis
Other Organelles Exosome, Lipid Droplet, etc.



Follow us on **LinkedIn**

