

# Reagent for Cellular Function Analysis



5th Edition

# Reagent for Cellular Function Analysis

## Cell Proliferation/Cytotoxicity Assay

Cell Counting Kit-8  
Cytotoxicity LDH Assay Kit-WST  
Viability/Cytotoxicity Multiplex Assay  
Cell Count Normalization Kit

## Cellular Senescence

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

## Autophagy

Autophagosome Detection (DAPGreen / DAPRed)  
Autolysosome Detection (DALGreen)

## Oxidative Stress

### ROS

ROS Assay Kit -Highly Sensitive DCFH-DA-  
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-  
mtSOX Deep Red - Mitochondrial Superoxide  
Si-DMA for Mitochondrial Singlet Oxygen Imaging

### Lipid Peroxide

Liperfluor  
Lipid Peroxidation Probe -BDP 581/591 C11-  
MitoPeDPP

### Intracellular Iron Ion

FerroOrange  
Mito-FerroGreen

### Glutathione

GSSG/GSH Quantification Kit

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Glycolysis/JC-1 MitoMP Assay Kit  
Glycolysis/OXPHOS Assay Kit

### Quantification for Intracellular Metabolism

ATP Assay Kit-Luminescence  
ADP/ATP Ratio Assay Kit-Luminescence  
Glucose Assay Kit-WST  
Glutamine Assay Kit-WST  
Glutamate Assay Kit-WST  
α-Ketoglutarate Assay Kit-Fluorometric  
Lactate Assay Kit-WST  
NAD/NADH Assay Kit-WST  
NADP/NADPH Assay Kit-WST

### Uptake Assay

Glucose Uptake Assay Kit-Blue, Green, Red  
Amino Acid Uptake Assay  
Cystine Uptake Assay Kit  
Fatty Acid Uptake Assay Kit

## Mitochondria

### Metabolism

Extracellular OCR Plate Assay Kit  
Glucose Assay Kit-WST  
Lactate Assay Kit-WST

### Mitochondrial Membrane Potential

MT-1 MitoMP Detection Kit  
JC-1 MitoMP Detection Kit

### Mitophagy

Mitophagy Detection Kit  
Mtpagy Dye

### Mitochondrial Staining

MitoBright LT Green  
MitoBright LT Red  
MitoBright LT Deep Red  
MitoBright IM Red for Immunostaining

### Oxidative Stress

mtSOX Deep Red  
- Mitochondrial Superoxide Detection  
Mito-FerroGreen  
Si-DMA for Mitochondrial Singlet Oxygen Imaging  
MitoPeDPP

A large, circular, teal-colored structure, possibly a cell or a large organelle, is the background of the slide. On the left side, there is a smaller, detailed view of a cell with several red, wavy structures inside, likely representing lysosomes or other organelles. The overall theme is cellular biology and fluorescence microscopy.

## Lysosome

Lysosomal Acidic pH Detection Kit  
LysoPrime Green / Deep Red  
- High Specificity and pH Resistance  
pHLys Red - Lysosomal Acidic pH Detection

## Endocytosis

ECGreen-Endocytosis Detection  
AcidSensor Labeling Kit - Endocytic Internalization Assay

## Other Organelles

Cellular Membrane Staining Dye - PlasMem Bright  
Nucleolus Staining Dye - Nucleolus Bright

## Exosome

ExoSparkler Exosome Membrane Labeling Kit  
ExoSparkler Exosome Protein Labeling Dye  
Exo/solator Exosome Isolation Kit  
Exo/solator Isolation Filter

## Lipid Droplet

Lipid Droplet Staining Dye – Lipi Series  
Lipid Droplet Assay Kit

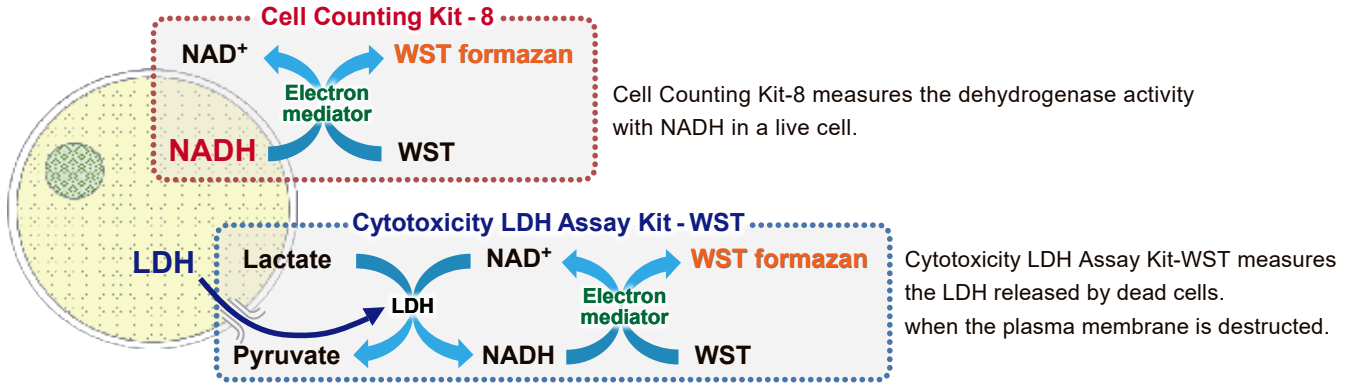


# Cell Proliferation / Cytotoxicity Assay

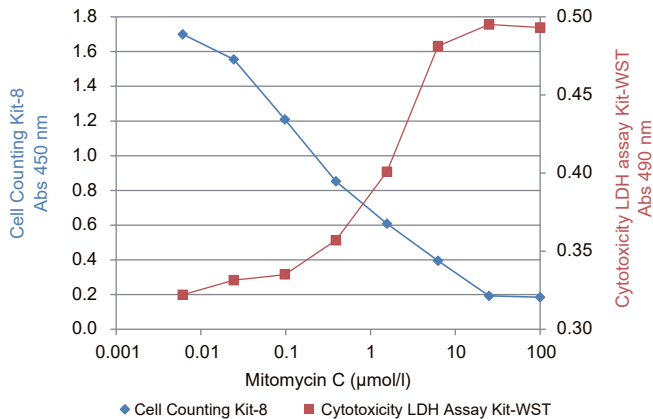
## Cell Counting Kit-8

## Cytotoxicity LDH Assay Kit-WST

### Detection Principle



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



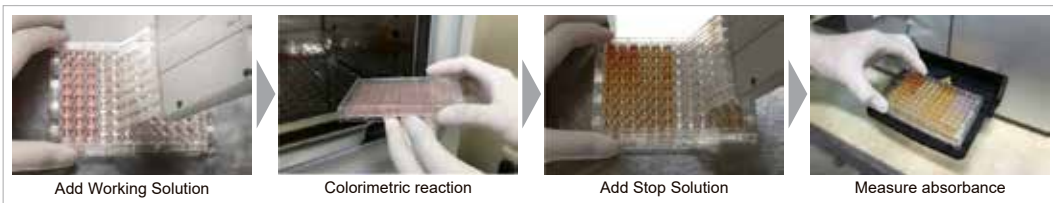
Drug: Mitomycin C  
 Cell Line: HeLa  
 Media: MEM, 10% FBS  
 Incubation: 37°C, 5% CO<sub>2</sub> for 48 hours  
 Measuring Condition: Cell Counting Kit-8 (450 nm)  
 Cytotoxicity LDH Assay Kit-WST (490 nm)

# Simple Procedure

## • Cell Counting Kit-8

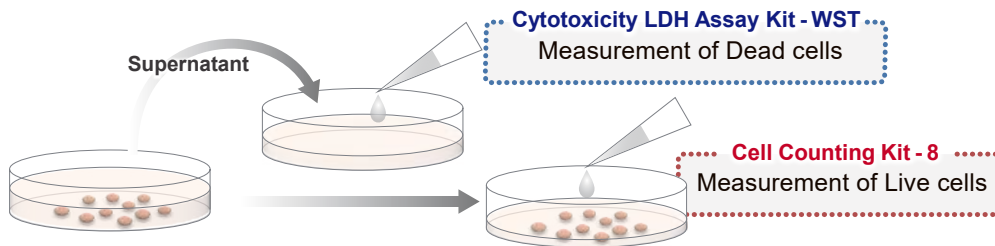


## • Cytotoxicity LDH Assay Kit-WST



### 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	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
Viability/Cytotoxicity Multiplex Assay Kit	500 tests	CK17-10

## Senescence Detection

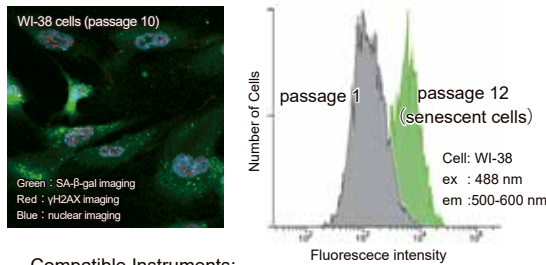
# Cellular Senescence Detection Kit - SPiDER-βGal



Cellular Senescence Detection Kit – SPiDER-βGal allows to detect SA-β-gal with high sensitivity and ease of use. SPiDER-βGal is a new reagent to detect β-galactosidase which possesses a high cell-permeability and a high retentivity inside cells. SA-β-gal are detected specifically not only in living cells but also fixed cells by using a reagent (Bafilomycin A1) to inhibit endogenous β-galactosidase activity. Therefore, SPiDER-βGal can be applied to quantitative analysis by flow cytometry.

### SPiDER-βgal

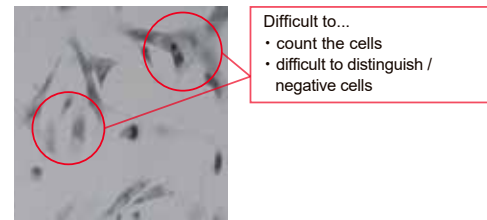
Compatible with quantitative analysis



Compatible Instruments:  
 ✓ Microscope ✓ Flow Cytometer ✓ Plate Reader

### X-Gal

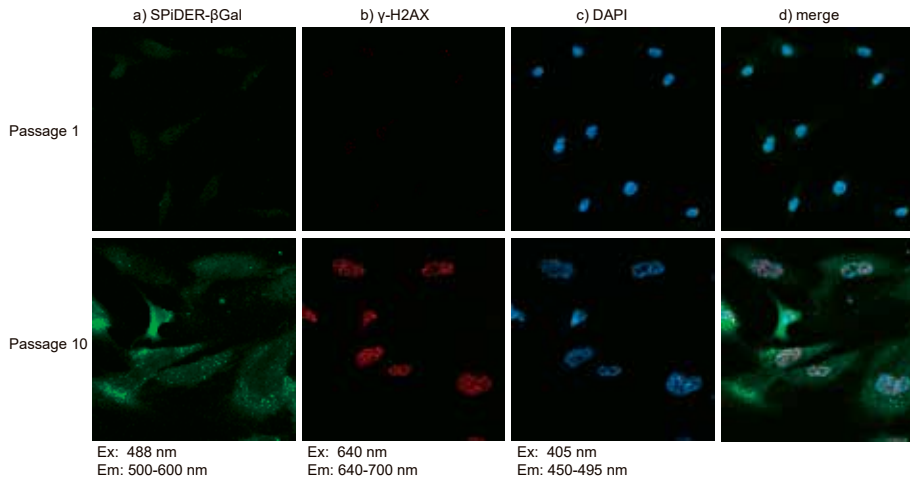
Difficult to quantify



Difficult to...  
 • count the cells  
 • difficult to distinguish / negative cells

Compatible Instruments:  
 ✓ Microscope

## Co-staining of SA- β-gal and DNA Damage marker in WI-38 cells



WI-38 cells were treated with anti- γ-H2AX antibody and observed under a confocal microscope. The procedure involved several steps, including fixing the cells, permeabilizing, blocking, adding primary and secondary antibodies, staining with DAPI, and washing the cells. The experiment aimed to detect DNA damage and study DNA repair pathways.

Description	Unit	Code
Cellular Senescence Detection Kit - SPiDER-βGal	10 assays	SG03-10



This product is a simple detection kit by plate assay for senescence-associated β-galactosidase (SA-β-gal) activity which is used as a marker for senescent cells. By simply adding SPiDER-βGal, a reagent for detection of β-galactosidase, to 96 well plates, this kit allows you to quantify SA-β-gal activity and makes it possible to evaluate multiple samples. When normalization is done by the results obtained by counting cells, quantifying nucleic acids (a relevant product), or quantifying proteins, the measured values obtained using this kit become available for evaluating SA-β-gal activity according to cell number.

## Correlation with Imaging Data

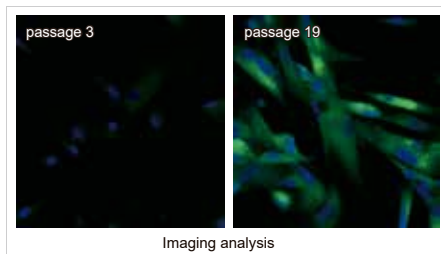
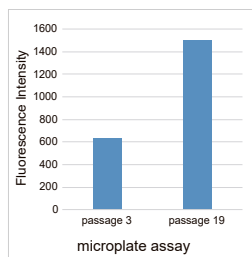


Plate Assay  
Ex. 535nm / Em. 580nm

Imaging data  
Green: Ex. 488nm / Em. 500-600nm (SA-β-Gal staining with Cellular Senescence Detection Kit – SPiDER-βGal(Code SG04))  
Blue: Ex. 405nm / Em. 450-495nm (Nuclear staining with -Cellstain- DAPI solution(Code D523))

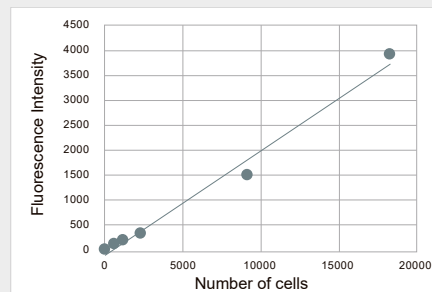
As a result, it was confirmed that in both kits, SA-β-gal staining increased in the high-passage WI-38 cells. Bear in mind that although initial cell seeding densities are the same, cell densities at the time of plate assay differ due to low proliferation rate of senescent cells at higher passage levels. Therefore, in this experiment, we used SA-β-Gal activity values normalized by the results obtained using the Cell Count Normalization Kit in which cell number is determined by a nuclear marker.

## Cell Count Normalization Kit

Cell Count Normalization Kit includes nucleic acid staining dye, Hoechst 33342 which binds to nuclear DNA to emit blue fluorescence. By measuring this blue fluorescence, correction of the measured value can easily be carried out in simple steps whereas the visual cell counting method requires complicated procedure. Moreover, unlike the correction by protein or ATP amount, the kit requires no lysis procedure. In addition, Quenching Buffer included in the kit enables a direct measuring of fluorescence signal without any background.



### Highly correlated to cell number



Description	Unit	Code
Cellular Senescence Plate Assay Kit - SPiDER-βGal	20 tests	SG05-01
	100 tests	SG05-05
	200 tests	C544-02
Cell Count Normalization Kit	1000 tests	C544-10

## Autophagy

# DAPGreen / Red - Autophagy Detection

## DALGreen - Autophagy Detection

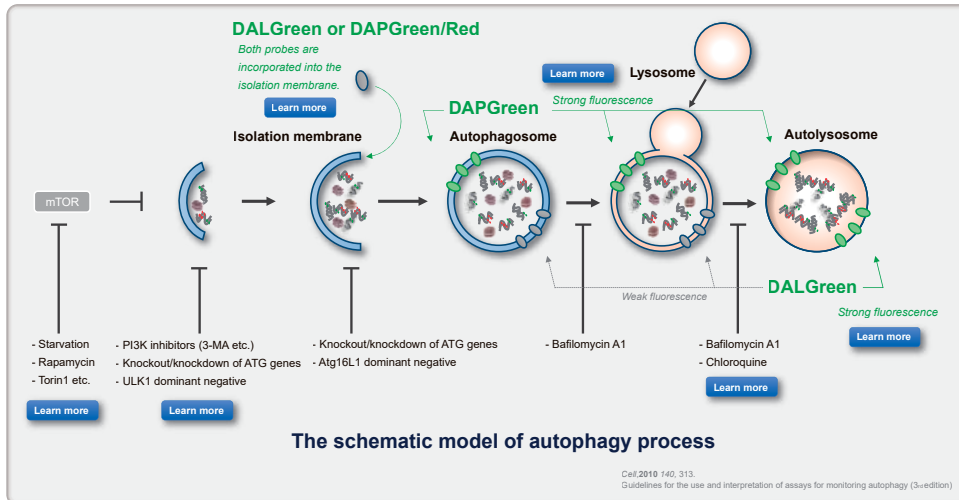
DALGreen

DAPGreen

DAPRed



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			
<b>DAPGreen</b>	○	○	○	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.
<b>DAPRed</b>	○	×	×	Ex = 500-560 nm Em = 690-750 nm	5 nmol x 1 / 35 mm dish: 25 (when used in 1.0 μmol/l)	
<b>DALGreen</b>	○	○	×	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

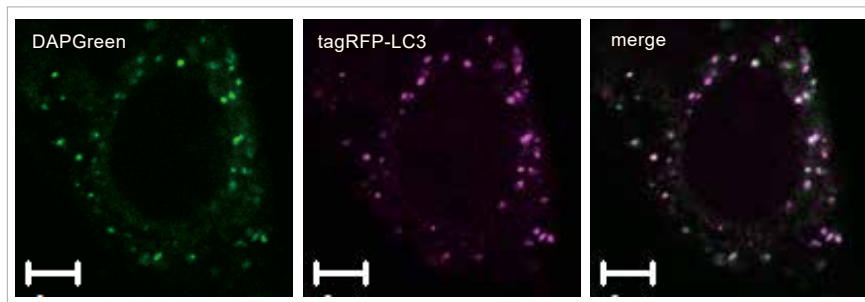


## High Correlation with LC3

DAPGreen

### The Condition of Autophagy Detection

After adding DAPGreen to the RFP-LC3 expressed HeLa cells, cells were treated with rapamycin to induce autophagy. Fluorescent imaging was conducted by confocal microscopy, 4 hours after autophagy induction.



#### Result

Almost all DAPGreen signals were colocalized with LC3.

#### Imaging Condition

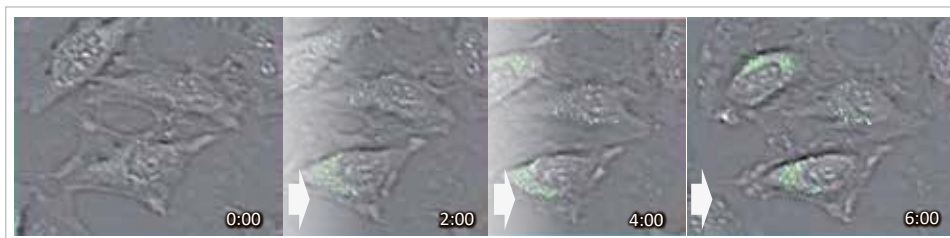
DAP Green: Ex = 488 nm, Em = 500 – 563 nm

Scale bar: 10  $\mu$ m

## Time-lapse imaging

DALGreen

The fluorescence intensity of DALGreen increased in autophagy-induced cells.



#### Detection Condition

Ex = 405 nm, Em = 500 – 550 nm

Confocal quantitative image cytometer CQ1, Yokogawa Electric Corporation

HeLa cells were stained with DALGreen, and autophagy was induced in an amino acid-free medium. Time-lapse observation was performed up to 6 hours after the induction of autophagy.

Description	Unit	Code
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

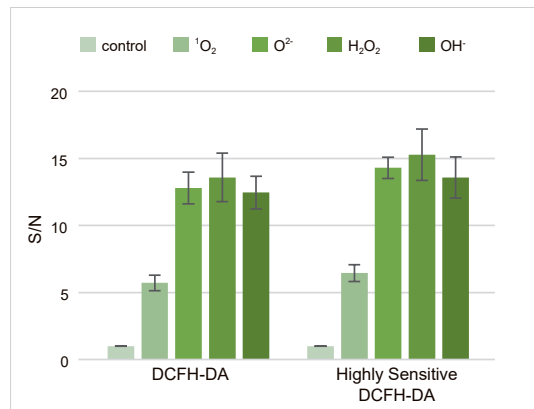
# ROS Assay Kit -Highly Sensitive DCFH-DA-



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.

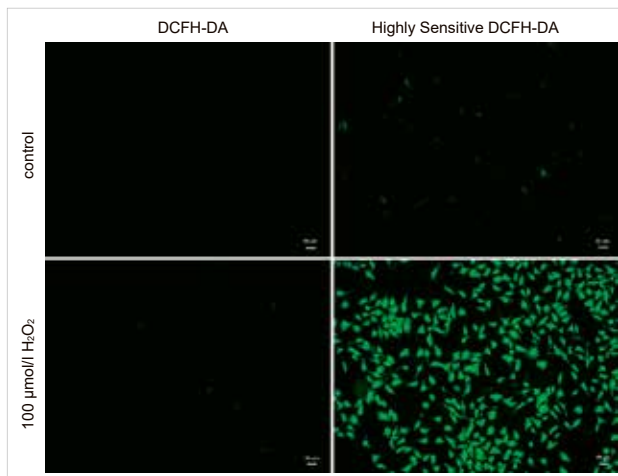
The reactivity of the Highly Sensitive DCFH-DA for ROS is similar to the reactivity of 2'-7' dichlorofluorescein diacetate (DCFH-DA). The Highly Sensitive DCFH-DA also has similar fluorescence characteristics ( $\lambda_{ex}$ : 505 nm,  $\lambda_{em}$ : 525 nm) to DCFH-DA. Therefore, ROS is detectable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

## The selectivity for ROS

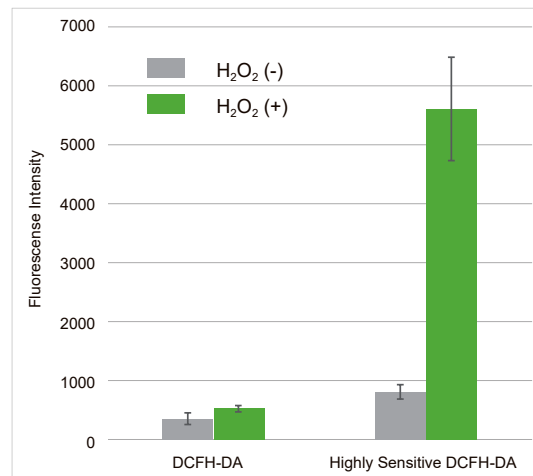


## High Sensitive Detection Compared with DCFH-DA

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

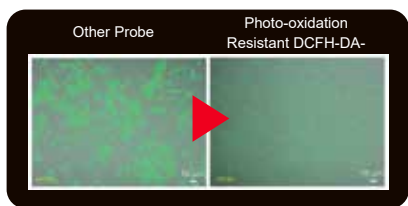
## Oxidative Stress

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

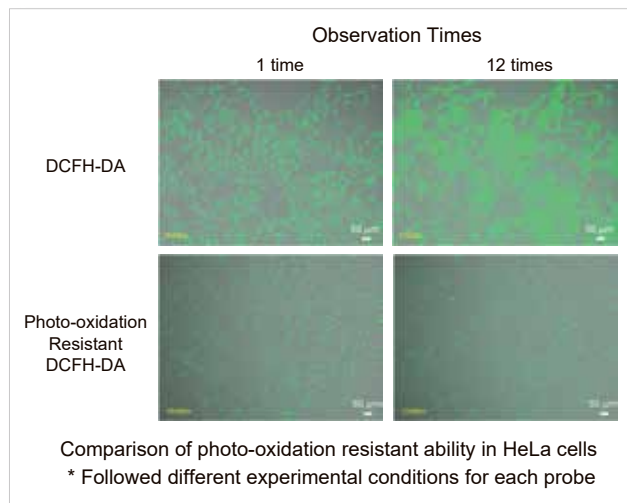


The dye that is employed in this kit allows ROS detection with higher sensitivity than DCFH-DA; It does not leak from cells because the fluorescent dye can immobilize protein via a chemical bond, and it is resistant to photo-oxidation compared with DCFH-DA. Moreover, the Loading Buffer in the kit maintains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

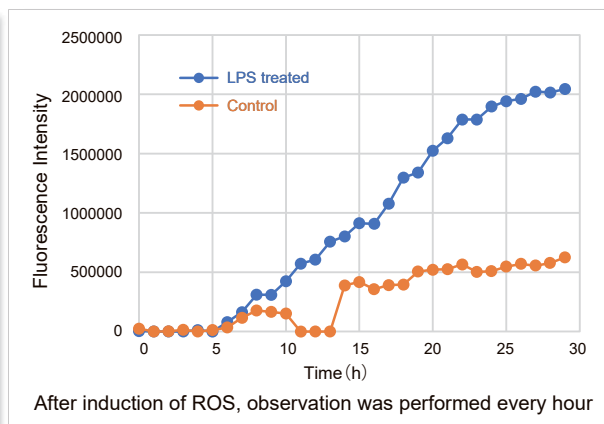
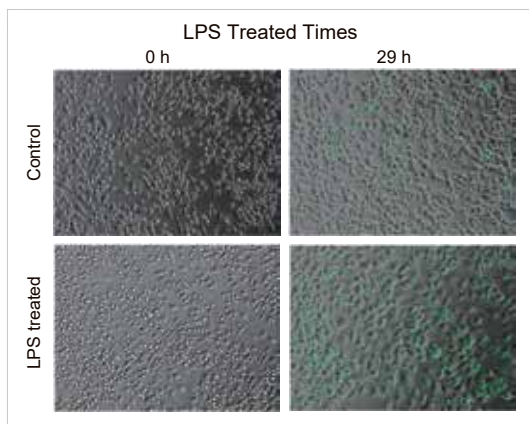
### Time-lapse imaging movie Available



### 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

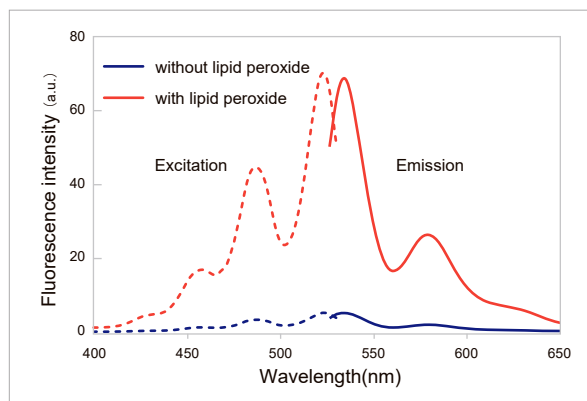
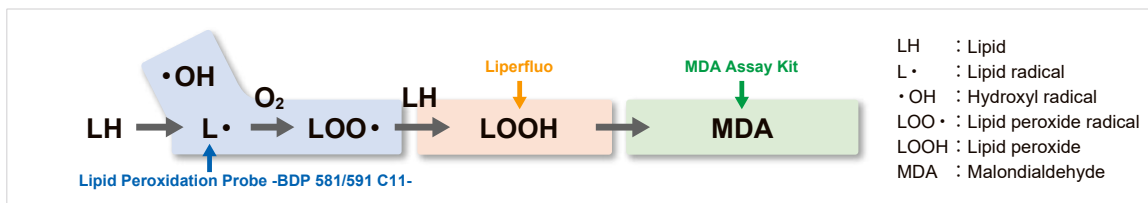
Other Organelles  
Exosome, Lipid Droplet, etc.



## Lipid Peroxide Detection

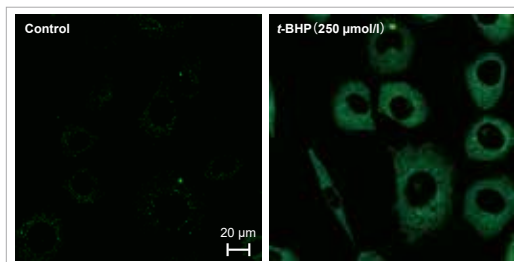
# Liperfluo

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

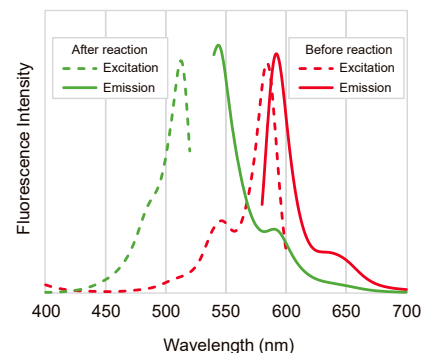
Description	Unit	Code
Liperfluo	1 set (50 μg × 5)	L248-10

## Lipid Peroxidation Detection

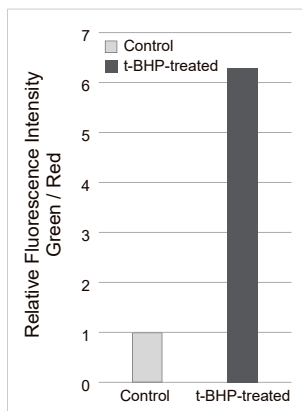
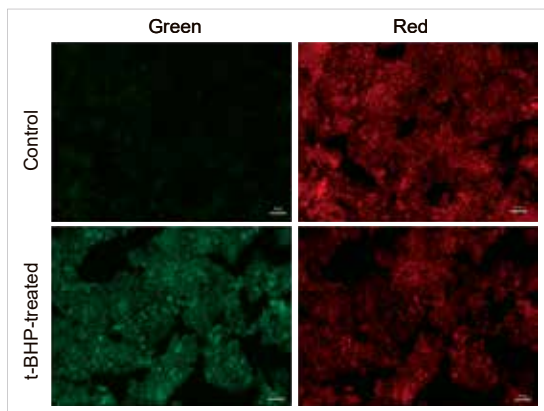
# Lipid Peroxidation Probe -BDP 581/591 C11-



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



<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  $\mu$ m

Fluorescent Plate Reader

Green: Ex = 490 nm, Em = 520-540 nm

Red: Ex = 570 nm, Em = 600-620 nm

HepG2 cells stained with this probe were stimulated with HBSS solution containing 200  $\mu$ mol/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.

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

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

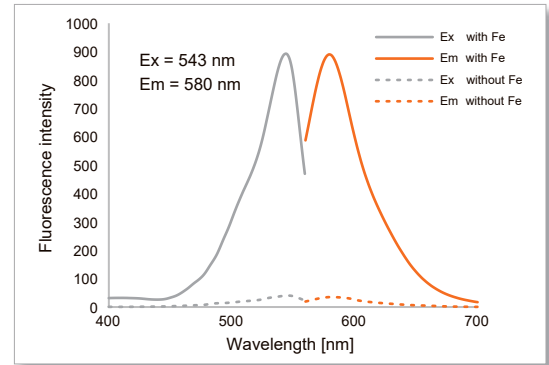
Other Organelles  
Exosome, Lipid Droplet, etc.

## Intracellular Iron Ion Measurement

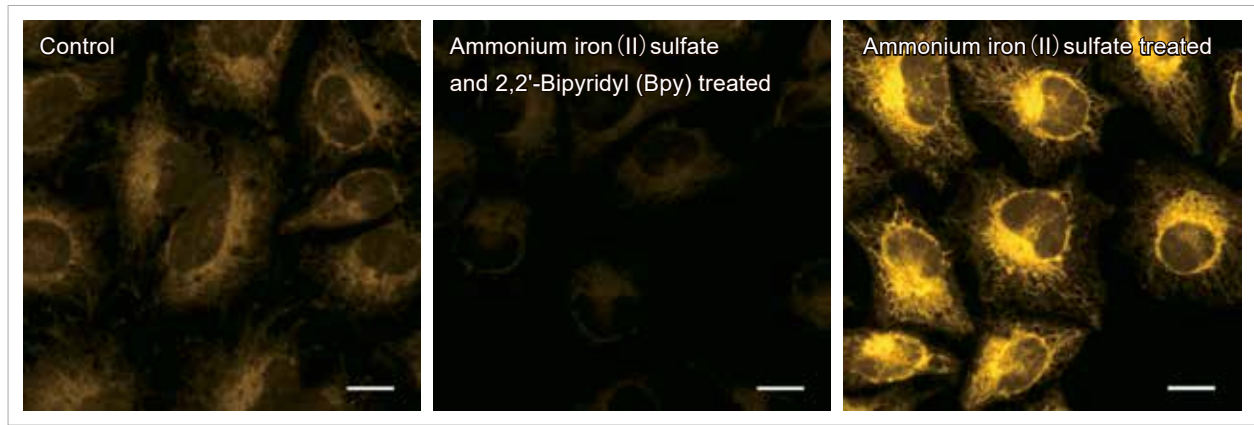
# FerroOrange



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  $\mu\text{mol/l}$ ) or Ammonium iron (II) sulfate (100  $\mu\text{mol/l}$ ) were prepared. The change of intracellular  $\text{Fe}^{2+}$  in HeLa cells was detected by the FerroOrange.

Ex = 561 nm, Em = 570-620 nm, Scale bars 20  $\mu\text{m}$

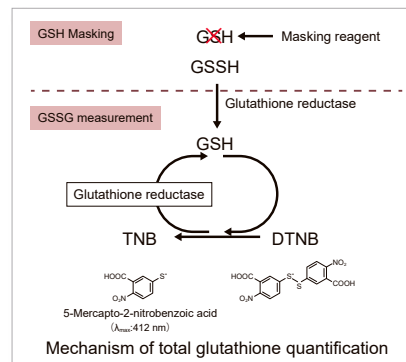
	Description	Unit	Code
Other Organelles Exosome, Lipid Droplet, etc.	FerroOrange	1 tube	F374-10
		3 tube	F374-12

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

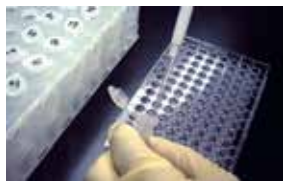
## GSSG/GSH Quantification Kit



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 ( $\lambda_{max} = 412 \text{ nm}$ ) 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  $0.5 \mu\text{mol/l}$  to  $50 \mu\text{mol/l}$  and GSSG concentrations from  $0.5 \mu\text{mol/l}$  to  $25 \mu\text{mol/l}$  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.

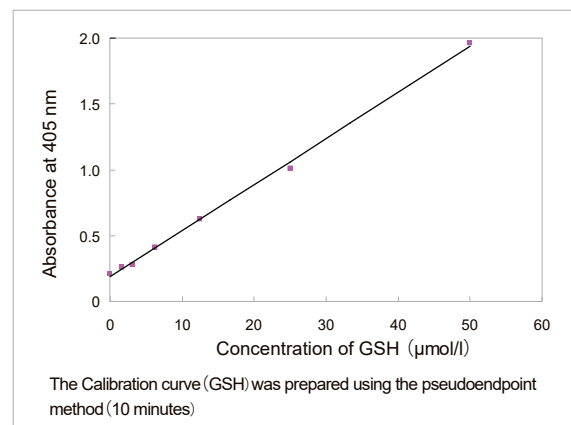
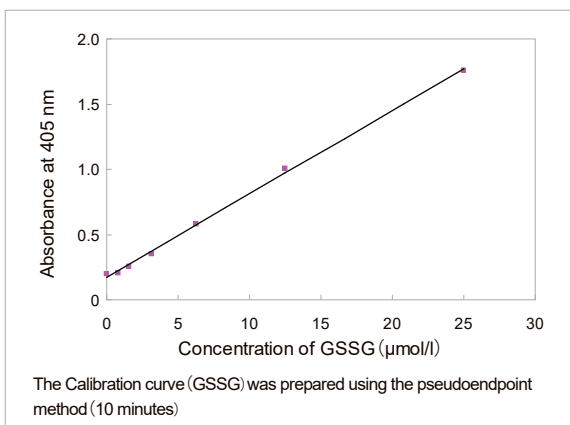


4)-5) Add substrate working solution and Enzyme/ Coenzyme working solution to each well.



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.

# Measurements of Intracellular Metabolism



	Description	Unit	Code
	<b>Starter Kit</b>		
	Glycolysis/OXPHOS Assay Kit	50 tests	G270-10
	Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272-10
	<b>Quantification for Intracellular Metabolism</b>		
		50 tests	A550-10
	ATP Assay Kit-Luminescence	200 tests	A550-12
	ADP/ATP Ratio Assay Kit-Luminescence	100 tests	A552-10
		50 tests	G264-05
	Glucose Assay Kit-WST	200 tests	G264-20
	Glutamine Assay Kit-WST	100 tests	G268-10
	Glutamate Assay Kit-WST	100 tests	G269-10
	$\alpha$ -Ketoglutarate Assay Kit-Fluorometric	100 tests	K261-10
		50 tests	L256-10
	Lactate Assay Kit-WST	200 tests	L256-20
	NAD/NADH Assay Kit-WST	100 tests	N509-10
	NADP/NADPH Assay Kit-WST	100 tests	N510-10
	<b>Uptake Assay Kit</b>		
	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
		20 tests	UP04-10
	Amino Acid Uptake Assay	100 tests	UP04-12
		20 tests	UP05-10
	Cystine Uptake Assay Kit	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
<p><b>Glucose</b></p> <p><b>Lactate</b></p> <p><b>Glutamine</b></p> <p><b>Glutamate</b></p> <p><b>NAD/NADH</b></p> <p><b>NADP/NADPH</b></p>	Colorimetric	<p><b>Simply transfer the culture supernatant to a plate and mix it with the chromogenic reagent</b></p> <p>Incubate 30 minutes at 37°C</p> <p>Analyze Plate reader Measure Absorbance (450 nm)</p>
		<p>Wash and lyse cells</p> <p>centrifuge</p> <p>Remove protein</p> <p>Included in kit</p> <p>centrifuge</p> <p>Plate</p> <p>Heat and decompose NAD(P)<sup>+</sup> (60°C)</p> <p>centrifuge</p> <p>Add chromogenic reagent</p> <p>Incubate 30 minutes at 37°C</p> <p>Analyze Plate reader Measure Absorbance (450 nm)</p>
<p><b>ATP</b></p> <p><b>ADP/ATP</b></p>	Luminescent	<p><b>Kit includes ATP standard - very easy to use</b></p> <p>Add solution</p> <p>Add sample chromogenic reagent</p> <p>Shake 2 minutes</p> <p>Incubate 10 minutes</p> <p>Analyze Plate reader</p>
		<p>Add sample solution</p> <p>Add chromogenic reagent</p> <p>Shake 2 minutes</p> <p>Incubate 10 minutes</p> <p>Measure luminescence</p> <p>Add chromogenic reagent</p> <p>Shake 2 minutes</p> <p>Incubate 8 minutes</p> <p>Measure luminescence</p> <p>Analyze Plate reader</p>
<p><b><math>\alpha</math>-ketoglutaric acid</b></p>	Fluorescent	<p><b>Less variable results than existing assays</b></p> <p>Wash and lyse cells</p> <p>centrifuge</p> <p>Add reaction reagent</p> <p>Incubate 30 minutes at 37°C</p> <p>Add chromogenic reagent</p> <p>Incubate 30 minutes at 37°C</p> <p>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

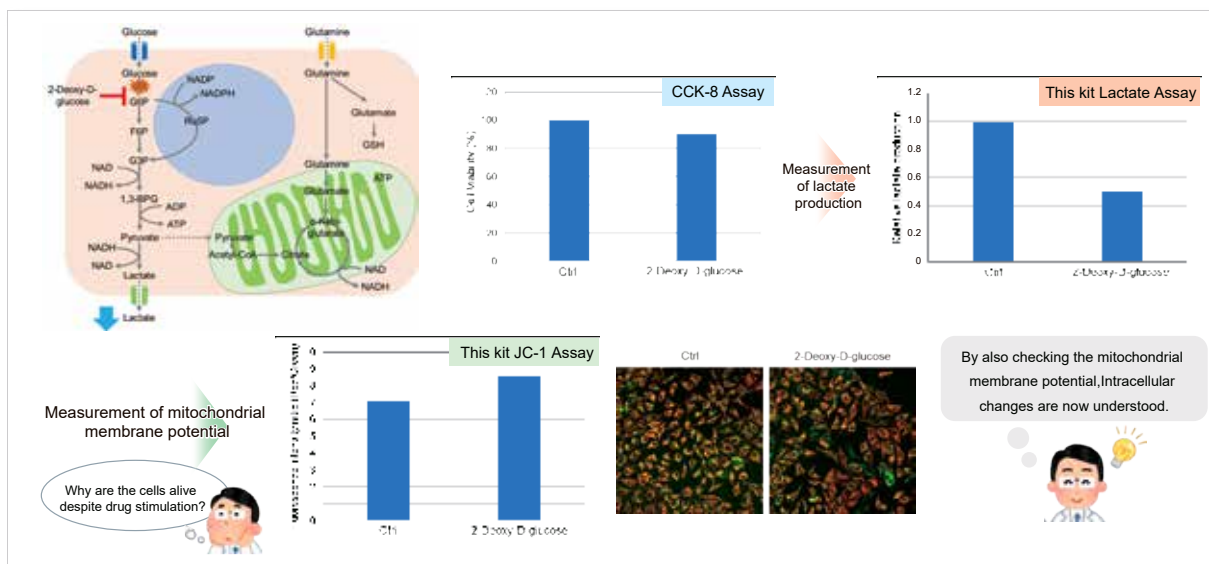
Intracellular metabolic changes caused by any stimulations can be detected by measuring lactate production and mitochondrial membrane potential. In certain instances, cells manage to survive despite sustaining damage to their glycolytic system or mitochondrial function, the principal pathways for energy 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.



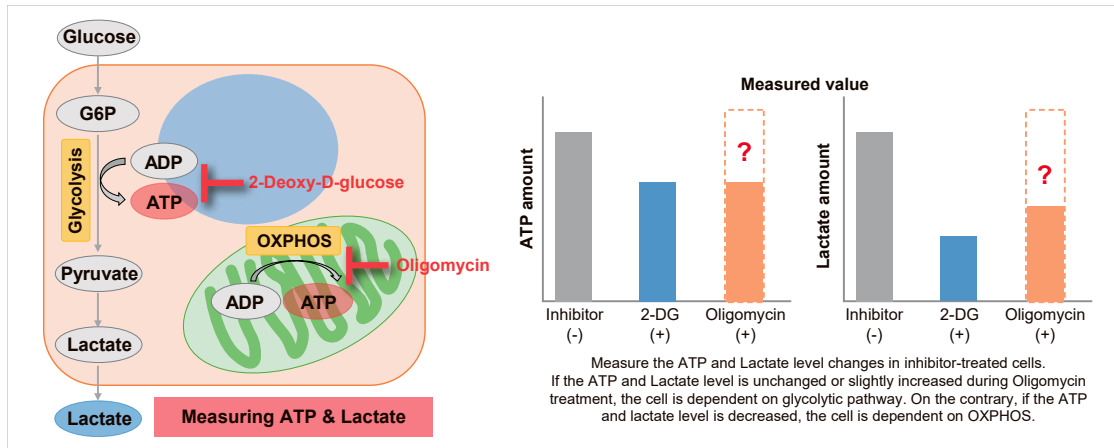
Description	Unit	Code
Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272-10

# Glycolysis/OXPPOS Assay Kit



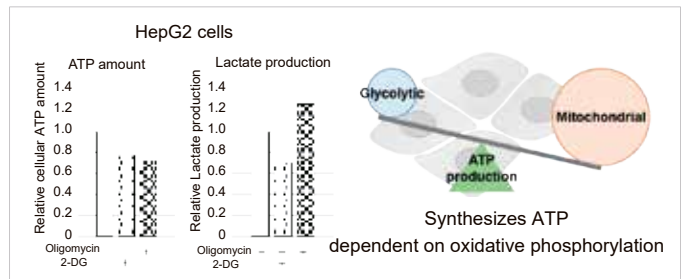
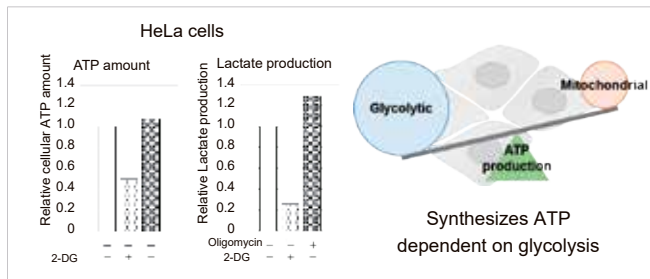
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

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 OXPPOS 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 OXPPOS.



## Experimental Example:

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



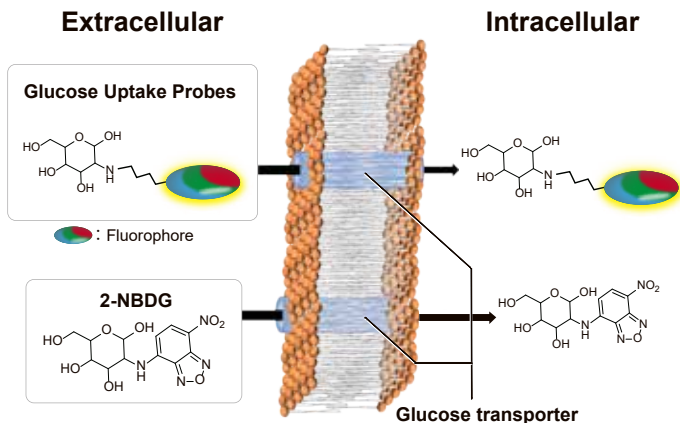
Description	Unit	Code
Glycolysis/OXPPOS Assay Kit	50 tests	G270-10

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

# 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
<b>Glucose Uptake Assay Kit-Blue</b>	○	×	○	1 hour *	$\lambda_{ex}$ :386 nm $\lambda_{em}$ :474 nm
<b>Glucose Uptake Assay Kit-Green</b>	○	○	○	1 hour *	$\lambda_{ex}$ :507 nm $\lambda_{em}$ :518 nm
<b>Glucose Uptake Assay Kit-Red</b>	○	○	○	1 hour *	$\lambda_{ex}$ :560 nm $\lambda_{em}$ :572 nm
<b>2-NBDG</b>	○	×	○	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

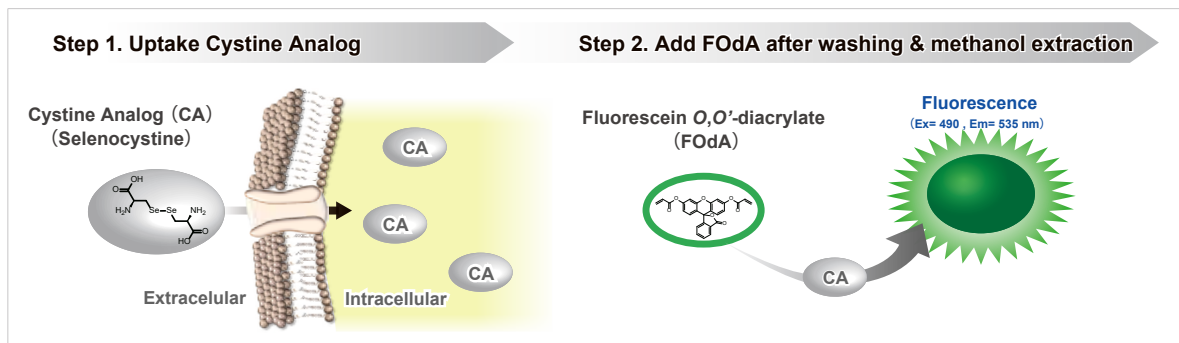
# Intracellular Metabolism

## Cystine Uptake Assay Kit



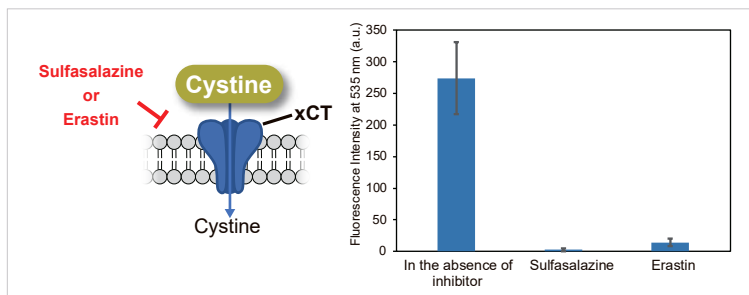
- Easier way to cystine uptake assay
- Applied for plate assay

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 erastin groups decreased significantly, indicating that both reagents inhibit cystine uptake.



Experiment Conditions

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

# Mitochondrial Research



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

### Mitochondrial Superoxide Detection

mtSOX Deep Red - Mitochondrial Superoxide Detection

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

### Ferrous Ion Detection

Mito-FerroGreen

Live-cell fluorescent imaging of intracellular Fe<sup>2+</sup>

### 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

### Singlet Oxygen Detection

Si-DMA for Mitochondrial Singlet Oxygen Imaging

Real-time visualization of <sup>1</sup>O<sub>2</sub> generation

### Lipophilic Peroxide Detection

MitoPeDPP

Live-cell fluorescent imaging of lipophilic peroxide

### 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

### Oxygen consumption rate (OCR) Detection

Extracellular OCR Plate Assay Kit

Applicable to regular fluorescent plate reader with temperature-controlled incubation

### 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	
<b>Metabolism</b>			Proliferation Cytotoxicity
Extracellular OCR Plate Assay Kit	100 tests	E297-10	
Glucose Assay Kit-WST	50 tests	G264-05	Senescence
	200 tests	G264-20	
Lactate Assay Kit-WST	50 tests	L256-10	Autophagy
	200 tests	L256-20	
<b>Mitochondrial Membrane Potential</b>			
MT-1 MitoMP Detection Kit	1 set	MT13-10	Oxidative Stress
JC-1 MitoMP Detection Kit	1 set	MT09-10	
<b>Mitophagy</b>			
Mitophagy Detection Kit	1 set	MD01-10	Metabolism
Mtphagy Dye	5 µg × 3	MT02-10	
<b>Mitochondrial Staining</b>			Mitochondria
MitoBright LT Green	400 µl	MT10-12	
MitoBright LT Red	400 µl	MT11-12	Lysosome
MitoBright LT Deep Red	400 µl	MT12-12	
MitoBright IM Red for Immunostaining	20 µl × 1	MT15-10	
	20 µl × 3	MT15-12	
<b>Oxidative Stress</b>			Endocytosis
mtSOX Deep Red - Mitochondrial Superoxide Detection	100 nmol × 1	MT14-10	
		100 nmol × 3	MT14-12
Mito-FerroGreen	1 set (50 µg × 2)	M489-10	Other Organelles Exosome, Lipid Droplet, etc.
Si-DMA for Mitochondrial Singlet Oxygen Imaging	2 µg	MT05-10	
MitoPeDPP	5 µg × 3	M466-10	

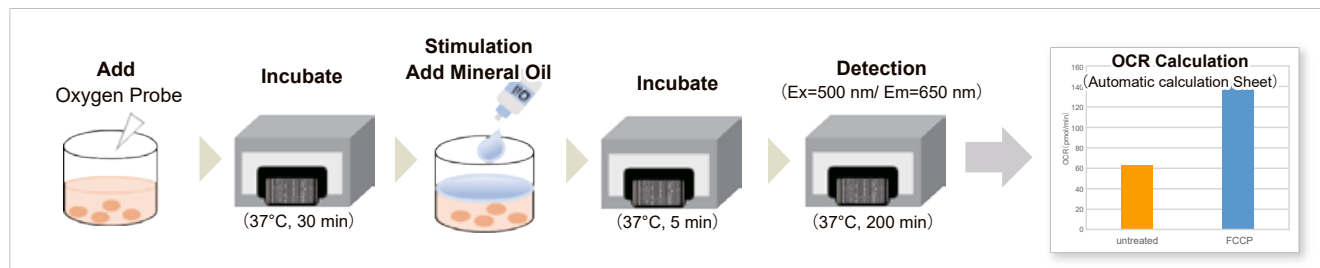
# Extracellular OCR Plate Assay Kit



- 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

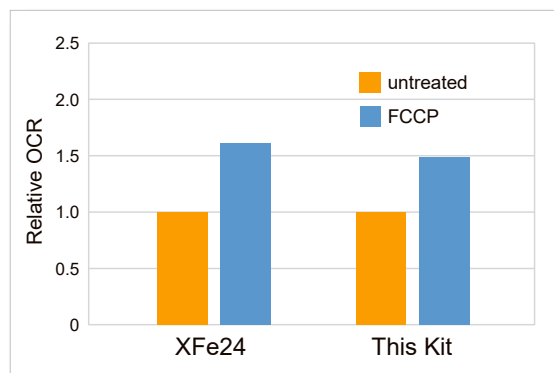


## Comparison with Flux Analyzer

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) phenylhydrazone)  
 FCCP Concentration: 2 µmol/l



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

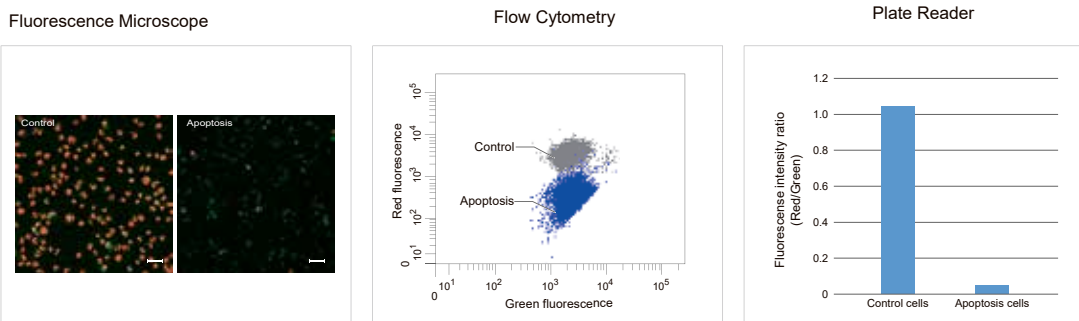
Proliferation  
Cytotoxicity  
Senescence  
Autophagy  
Oxidative Stress  
Metabolism  
Mitochondria  
Lysosome  
Endocytosis  
Other Organelles  
Exosome, Lipid Droplet, etc.



## Mitochondrial Membrane Potential Detection JC-1 MitMP 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.



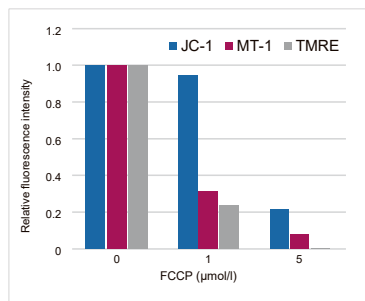
Description	Unit	Code
JC-1 MitMP Detection Kit	1 set	MT09-10

## Mitochondrial Membrane Potential Detection MT-1 MitMP Detection Kit

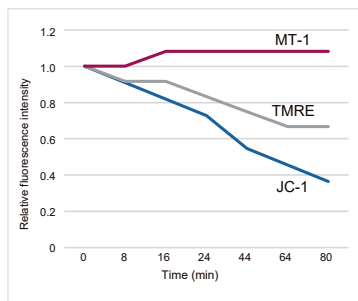


JC-1 dye, TMRE, and TMRM are widely used to monitor MMP, however, these dyes have some limitations, such as low photostability and poor retention after aldehyde fixation. These limitations result in poor reproducibility of experiments. Dojindo's MT-1 MitMP Detection Kit overcomes these limitations. In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

High Sensitivity Detection



Allow to monitor mitochondrial membrane potential



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

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

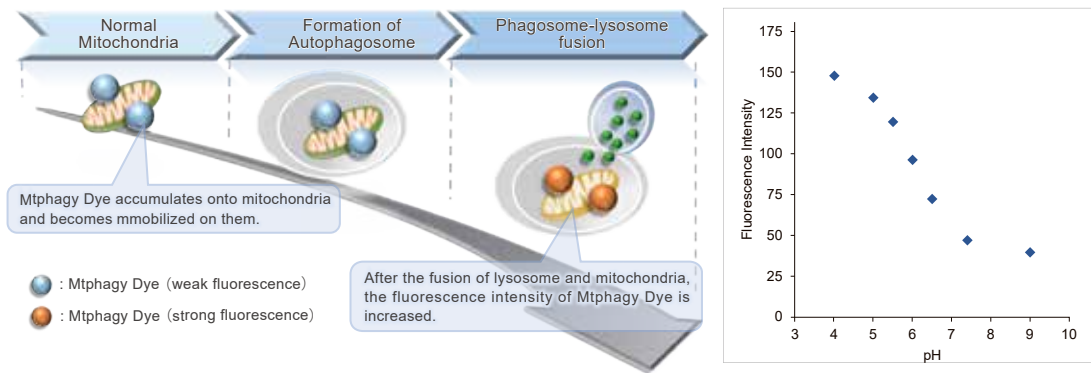
Lysosome

Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.

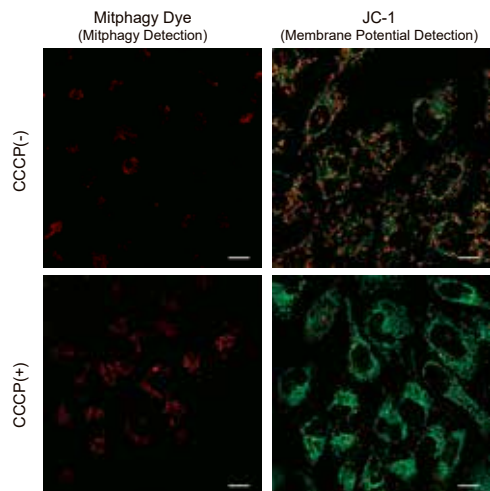


This kit is composed of Mtpagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtpagy Dye accumulates in intact mitochondria, is immobilized on it with chemical bond and exhibits a weak fluorescence from the influence of surrounding condition. When Mitophagy is induced, the damaged mitochondria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



The fluorescent intensity of Mtpagy 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 Mtpagy Dye).

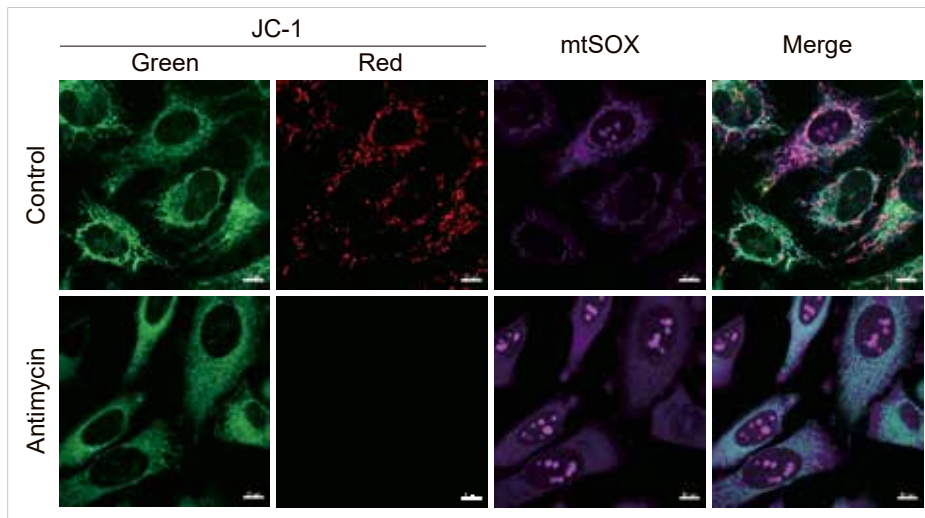
Description	Unit	Code
Mitophagy Detection Kit	1 set	MD01-10
Mtpagy Dye	5 µg × 3	MT02-10

# Mitochondrial Superoxide Detection mtSOX Deep Red



Dojindo's mtSOX Deep Red overcomes these limitations. This dye emits deep red fluorescence; its fluorescence does not overlap with emission wavelengths that other red fluorescent markers use. Furthermore, the mtSOX Deep Red is better able to selectively detect superoxide, compared to Company T' product Red. Altogether, mtSOX Deep Red is a powerful tool for researchers with a limited number of cells and can provide an understanding of how mitochondria are altered during different treatments and physiological or pathological states.

## Simultaneously Evaluation of Mitochondrial Superoxide and Membrane Potential



<Imaging Conditions>  
(Confocal microscopy)  
JC-1: Green Ex = 488, Em = 490-520 nm,  
Red: Ex = 561, Em = 560-600 nm  
mtSOX: Ex = 633 nm, Em = 640-700 nm  
Scale bar: 10  $\mu$ m

After HeLa cells were washed with HBSS, co-stained with mtSOX Deep Red and mitochondrial membrane potential staining dye (JC-1: code MT09 or MT-1: code MT13), 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
mtSOX Deep Red - Mitochondrial Superoxide Detection		100 nmol $\times$ 1	MT14-10
		100 nmol $\times$ 3	MT14-12

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

## Mitochondrial Superoxide Detection

# Mito-FerroGreen

Mito-FerroGreen

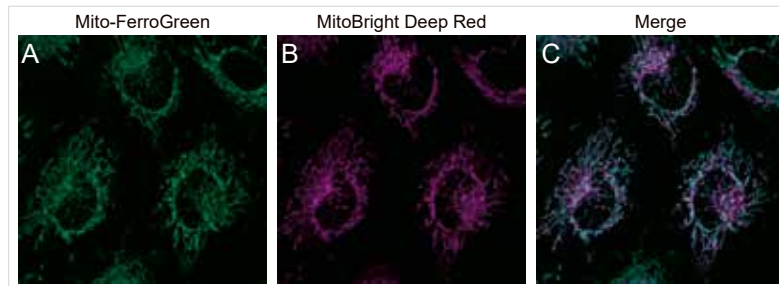
FerroOrange



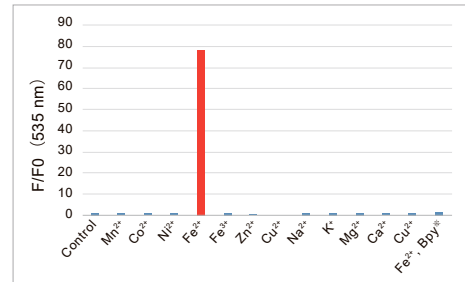
Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion ( $\text{Fe}^{2+}$ ) in mitochondria where Fe-S clusters and heme proteins are synthesized, and enables live cell fluorescent imaging of intracellular  $\text{Fe}^{2+}$ . Mito-FerroGreen has no chelating ability. Mito-FerroGreen 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.



Metal ion Selectivity



※ Bpy : 2,2'-Bipyridyl

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 $\mu\text{g}$ x 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 $\mu\text{g}$ x 2)	M489-10
FerroOrange	1 tube	F374-10
	3 tube	F374-12

# Mitochondrial Staining

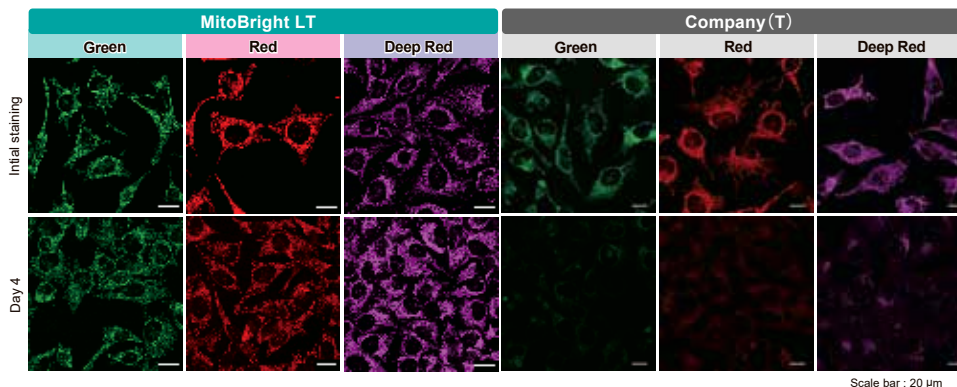
## MitoBright LT Series



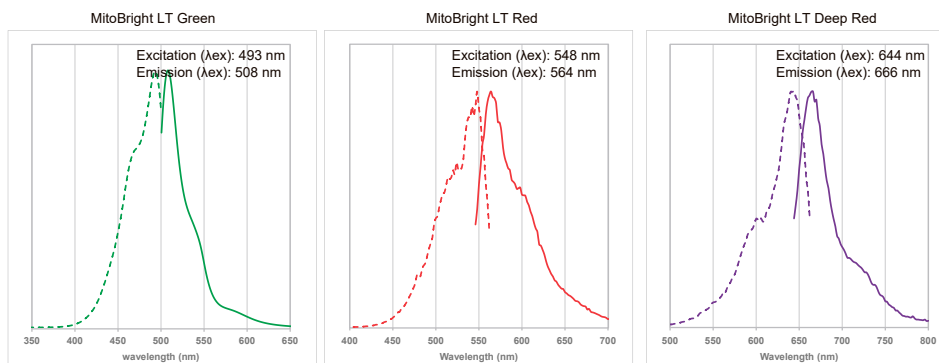
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color 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



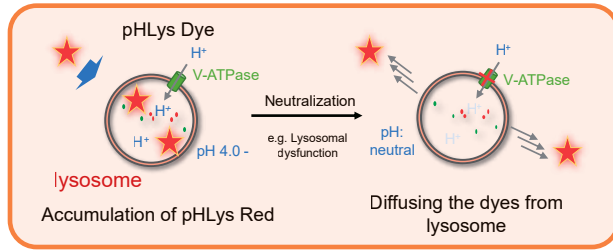
Description	Unit	Code
MitoBright LT Green	400 $\mu$ l	MT10-12
MitoBright LT Red	400 $\mu$ l	MT11-12
MitoBright LT Deep Red	400 $\mu$ l	MT12-12



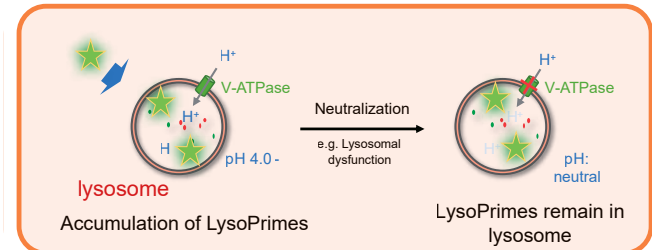
## Lysosomal Analysis

# LysoPrime Green / Deep Red - High Specificity and pH Resistance pHLys Red - Lysosomal Acidic pH Detection

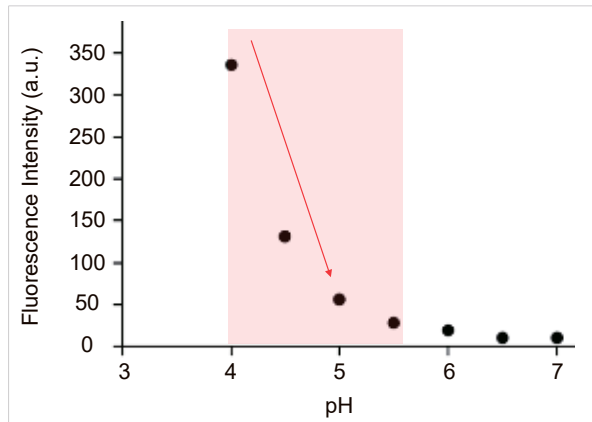
### Lysosomal pH-dependent Fluorescent Probe



### Lysosomal pH-independent Fluorescent Probe

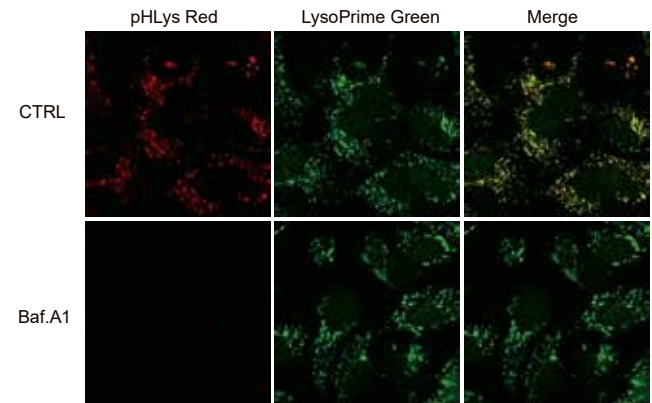


### pH dependence of pHLys Red



The fluorescence intensity of pHLys Red at each pH was confirmed in vitro, and it was confirmed that the fluorescence intensity changed sensitively within the range of lysosomal pH (pH 4.0-5.5).

### Resistance to pH changes in lysosomes



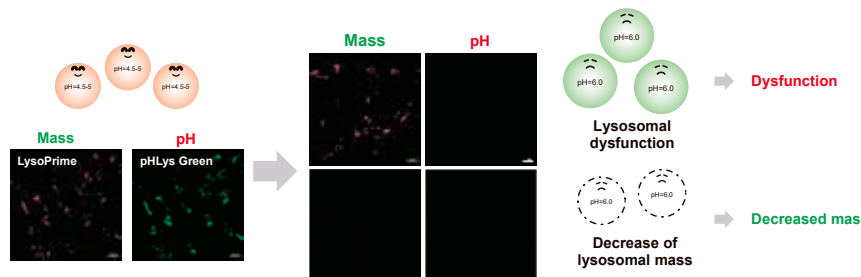
LysoPrime Green and existing dyes accumulate in acidic lysosomes, but when treated with Bafilomycin A1, a lysosomal acidity inhibitor, the existing dyes leave the lysosomes when the lysosomes are changed from acidic to neutral, resulting in a significant decrease in the fluorescence signal. On the other hand, LysoPrime Green is easily retained in the lysosome, so the decrease in the fluorescence signal is suppressed and the observation results are clearer than those of existing reagents.

## Lysosomal Analysis

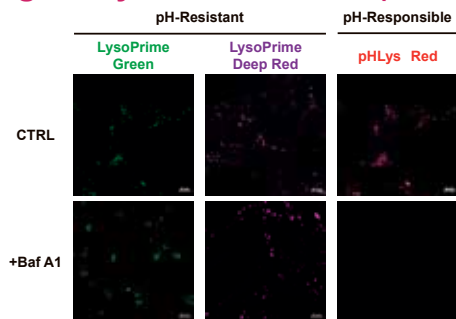
# Lysosomal Acidic pH Detection Kit



The kit includes lysosome staining dyes, pHLys Red/Green (pH dependent), and LysoPrime Green/Deep Red (pH-independent). The pHLys and LysoPrime dyes accumulate in the intact lysosomes. The fluorescence intensity of pHLys dyes are enhanced as the acidity increases, and weak fluorescence is observed when lysosomes are neutralized due to the lysosomal dysfunction. On the other hand, LysoPrime dyes gives stable emissions even lysosomes are neutralized. Lysosomal pH and lysosomal mass can be measured by combining these pHLys and LysoPrime dyes.



## Imaging Analysis: Green/Red (#L266-10)

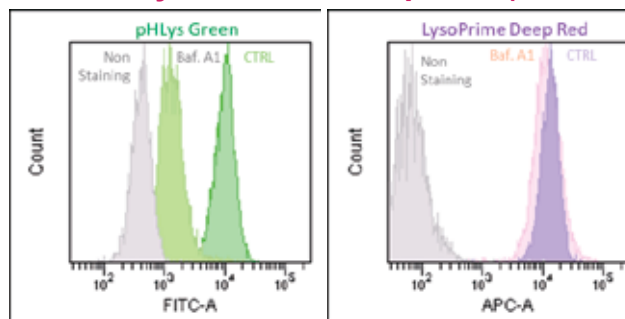


<Experimental Conditions>

LysoPrime Green: Ex = 488 nm, Em = 490 – 550 nm

pHLys Red: Ex = 561 nm, Em = 560 – 620 nm

## FCM Analysis: Green/Deep Red (#L268-10)



<Experimental Conditions>

pHLys Green: FITC Filter (Ex = 488 nm, Em = 515 – 545 nm)

LysoPrime Deep Red: APC Filter (Ex = 640 nm, Em = 650 – 670 nm)

Description	Unit	Code
Lysosomal Acidic pH Detection Kit – Green/Red *1	1 set	L266-10
Lysosomal Acidic pH Detection Kit – Green/Deep Red *2	1 set	L268-10
LysoPrime Green – High Specificity and pH Resistance	10 µl × 1	L261-10
	10 µl × 3	L261-12
LysoPrime Deep Red - High Specificity and pH Resistance	1 tube	L264-10
	3 tube	L264-12
pHLys Red - Lysosomal Acidic pH Detection	1 tube	L265-10
	3 tube	L265-12

\*1 Green/Red: combination of LysoPrime Green and pHLys Red, \*2 Green/Deep Red: combination of pHLys Green and LysoPrime Deep Red

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.

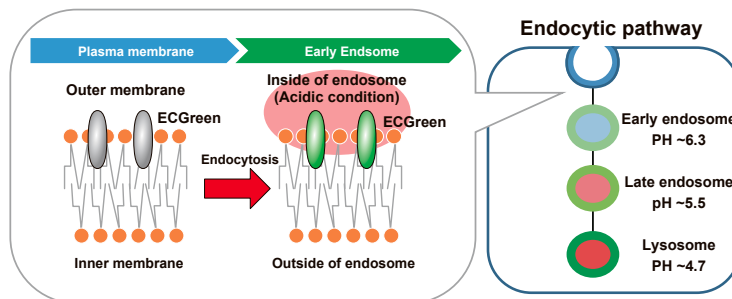


## Endocytosis

# ECGreen-Endocytosis Detection

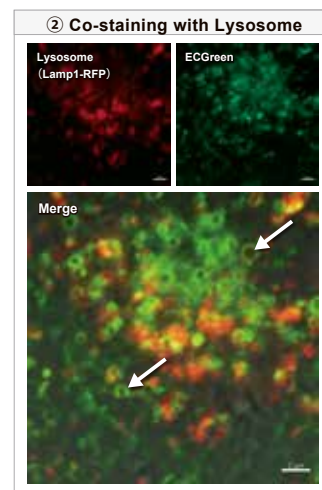
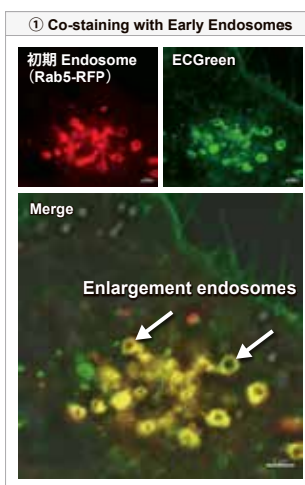
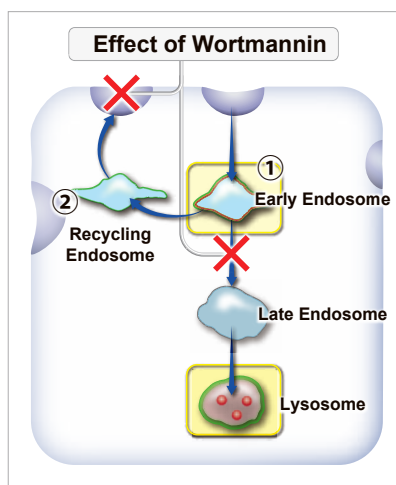
ECGreen-Endocytosis Detection is a pH dependent fluorescence dye that localizes to vesicle membrane. The visualization of endocytosis using the ECGreen is a more direct method than fluorescent analogs and allows visualization endocytosis from the stage of early endosomes.

### The detection mechanism of endocytosis



### Clear visualization of intracellular vesicular trafficking

It has been known that Wortmannin inhibits the recycling of endosomes or transition to lysosomes and causes enlargement of endosomes. To evaluate these changes caused by Wortmannin, early endosomes were co-stained by ECGreen and Rab5-RFP (marker protein of early endosomes), and lysosomes were co-stained by ECGreen and lysosome staining reagent. In adding Wortmannin, ECGreen was colocalized with enlarged endosomes (Rab5-RFP). On the other hand, ECGreen wasn't colocalized with lysosomes.



	Description	Unit	Code
	ECGreen-Endocytosis Detection	40 $\mu$ l	E296-10



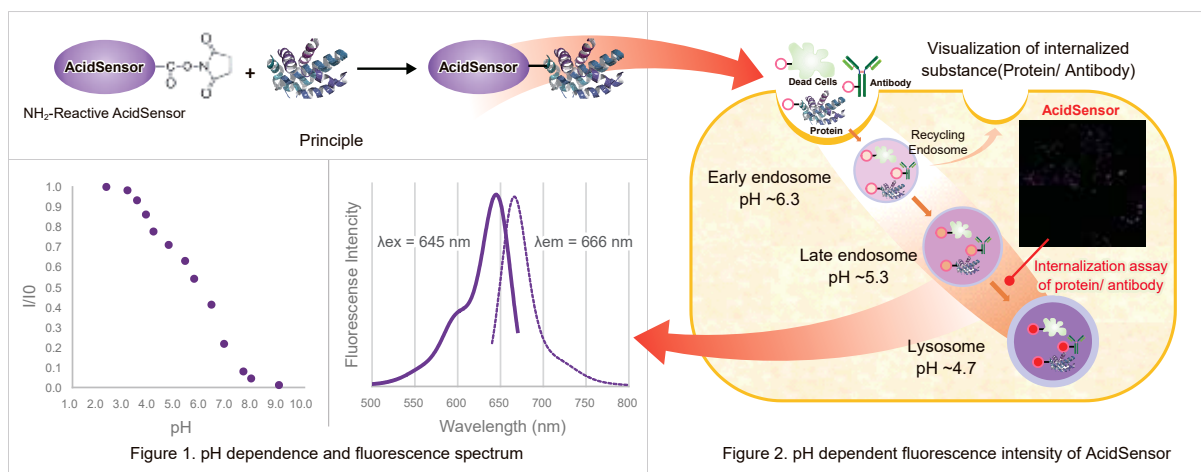
## Endocytosis

# AcidSensor Labeling Kit – Endocytic Internalization Assay

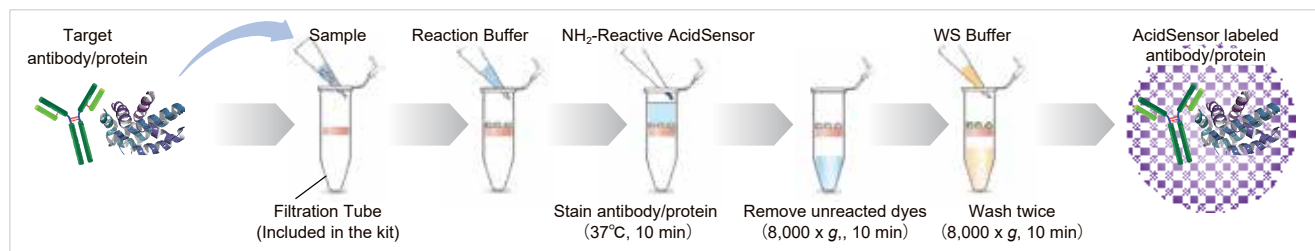


This kit is an all-in-one kit that allows visualization of the endocytosis uptake of a target substance. The NH<sub>2</sub>-Reactive AcidSensor (fluorescent probe) included in the kit has an intramolecular active ester group that forms a stable covalent bond when mixed with an amino group-containing target substance (protein). The AcidSensor label can be excited at 633 nm, allowing for multiple staining with green or red fluorescence (Figure 1). The AcidSensor label shows little fluorescence in neutral conditions and fluoresces when acidified in the cells where it is taken up by endocytosis (Figure 2). \*Notice:

- Unlike the endocytosis detection dye: ECGreen (code: E296), this kit stains target substances that enter the cell.
- This kit can label samples with molecular weights of more than 50,000 and with reactive amino groups.



This kit includes a filtration tube necessary to remove the unreacted dye, and allows you to perform everything from labeling to purification operations.\* In addition, even first-time users can easily label AcidSensor by conducting experiments according to the instruction manual. \* Protein/Antibody is not included.



Description	Unit	Code
AcidSensor Labeling Kit – Endocytic Internalization Assay	3 samples	A558-10

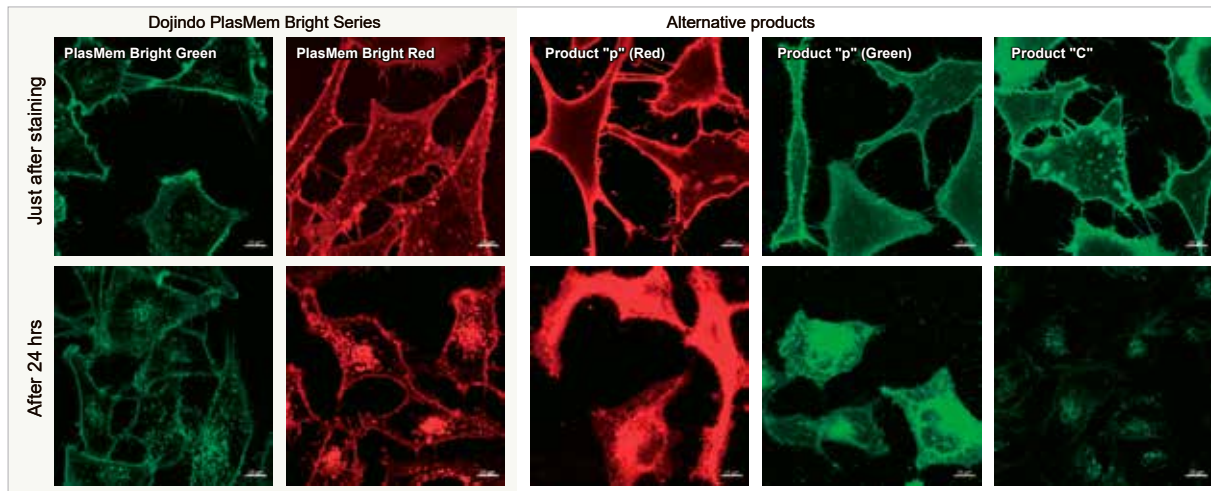
## Cell Membrane Staining

# PlasMem Bright Green / Red

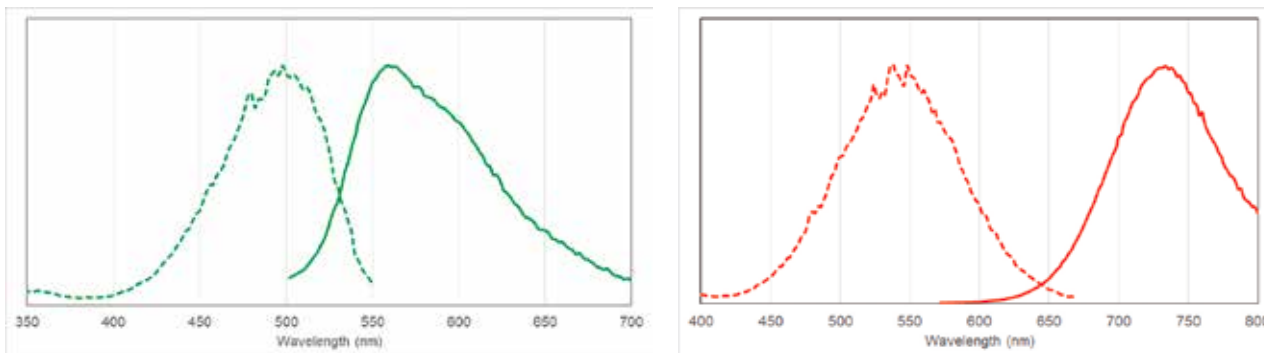


PlasMem Bright dyes overcome these limitations. PlasMem Bright dyes are designed to stain PMs for over a day. Furthermore, the PlasMem Bright dyes are more water-soluble compared with other commercially available dyes and can be diluted with culture medium. The PlasMem Bright dyes offer two different color options (green and red) and are provided as ready-to-use DMSO solutions. A working solution can be prepared easily via a single dilution step using growth medium or HBSS.

## High retentivity on plasma membrane



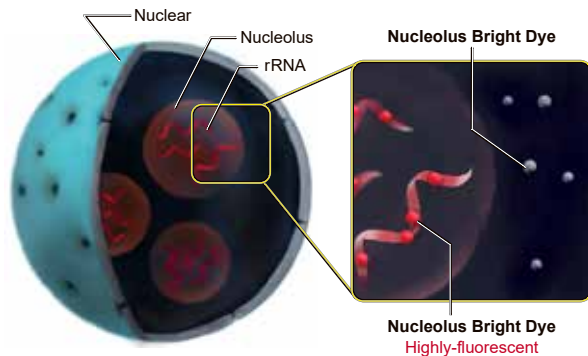
## Excitation and emission spectra of PlasMem Bright dyes



Description	Unit	Code
PlasMem Bright Green	100 µl	P504-10
PlasMem Bright Red	100 µl	P505-10

# Nucleolus Staining

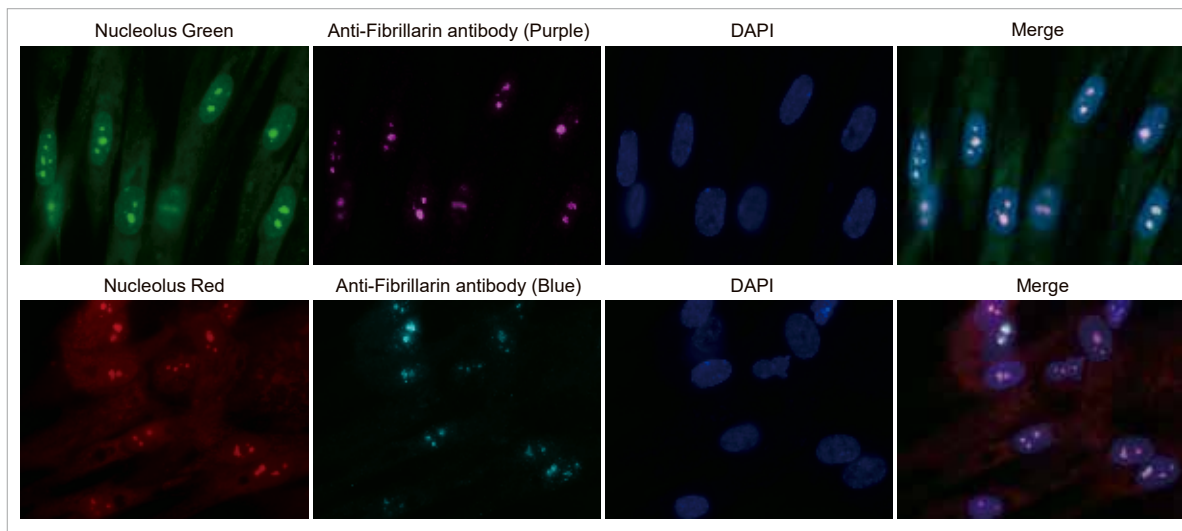
## Nucleolus Bright Green / Red



Nucleolus Bright reacts to RNAs present besides nucleolus, but it shows strong fluorescence in nucleolus, which is the site of rRNA production. We recommend to co-stain with DAPI in order to image nucleolus clearly. For co-staining protocol, please refer to the Q&A tab.

	Maximum Excitation Wavelength	Maximum Emission Wavelength	Fluorescence of MeOH fixed cells	Fluorescence of PFA fixed cells
<b>Nucleolus Bright Green</b>	513 nm	538 nm	○	○
<b>Nucleolus Bright Red</b>	537 nm	605 nm	○	○

## Nucleolus Localization



Description	Unit	Code
Nucleolus Bright Green	60 nmol	N511-10
Nucleolus Bright Red	60 nmol	N512-10

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

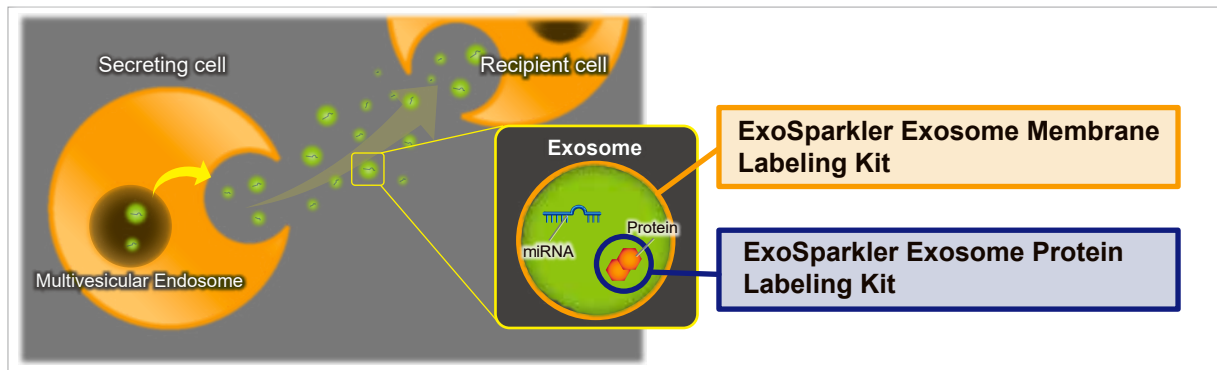
Other Organelles  
Exosome, Lipid Droplet, etc.

# Exosome Staining

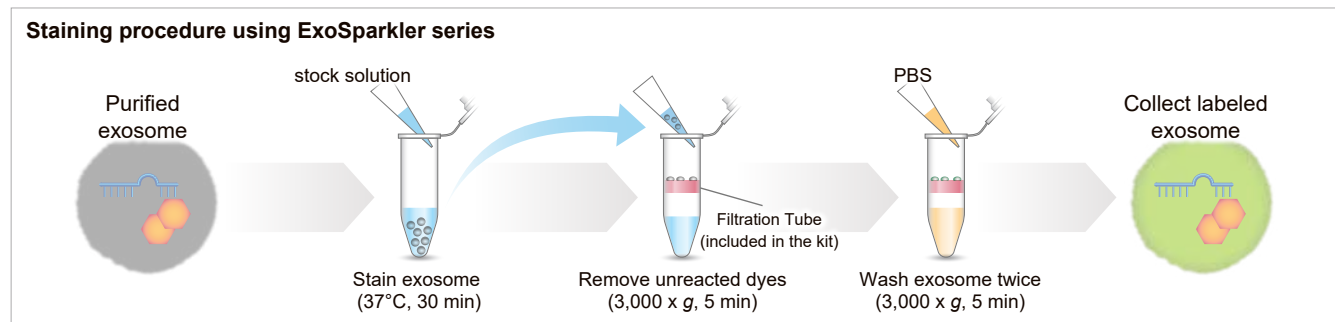
## Exosome Labeling Kits



The ExoSparkler series can be used to stain purified exosomal membrane or protein and allows imaging of labeled exosomes taken up by cells.



## Labelling Procedure



ExoSparkler series contains filtration tubes available for the removal of dyes unreacted after fluorescence labeling, as well as an optimized protocol for labeling exosomes. Our ExoSparkler series makes it possible to prepare fluorescence labeling of exosomes using the simple procedure.

Description	Unit	Code
ExoSparkler Exosome Membrane Labeling Kit-Green	5 samples	EX01-10
ExoSparkler Exosome Membrane Labeling Kit-Red	5 samples	EX02-10
ExoSparkler Exosome Membrane Labeling Kit-Deep Red	5 samples	EX03-10
ExoSparkler Exosome Protein Labeling Dye-Green	5 samples	EX04-10
ExoSparkler Exosome Protein Labeling Dye-Red	5 samples	EX05-10
ExoSparkler Exosome Protein Labeling Dye-Deep Red	5 samples	EX06-10

Proliferation  
Cytotoxicity

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Autophagy

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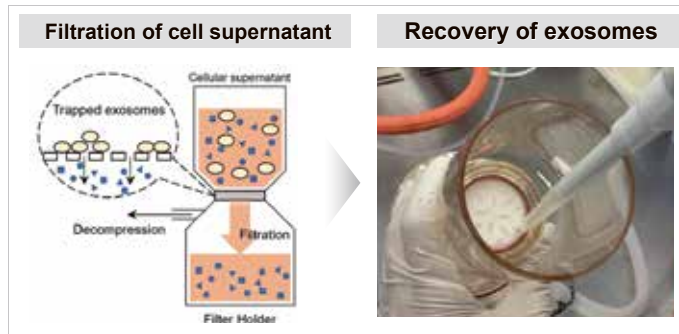
Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.



Exo/solator Exosome Isolation Kit can collect exosomes from cell supernatants with a recovery rate equivalent to the ultracentrifugation(UC) method. Science Exo/solator Exosome Isolation Kit requires only the filtration procedure, unlike the UC, exosomes are obtained quickly without any complicated operations.

## Easy to Use no Technique Required



Exo/solator Exosome Isolation Kit includes Filter Holder and Isolation Filter can collect exosomes from cell supernatant by adding PBS to the filter surface after filtration. The exosomes recovery rate is high and easy to use, no technique is required during the whole process. [Patent applied]

## Recovery Rate Equivalent to Ultracentrifugation

Fig.1

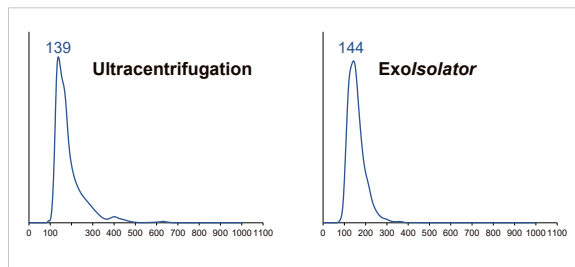


Fig.2a

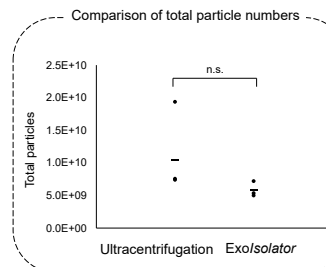
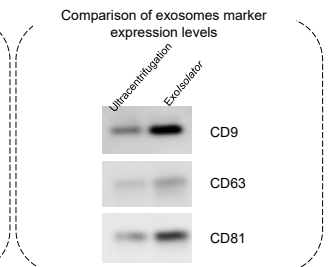


Fig.2b



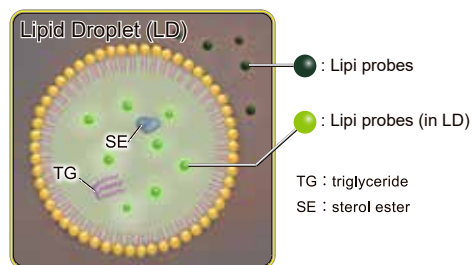
Ultracentrifugation is the most commonly used method to isolate exosomes. We isolated the exosomes from the supernatant of HEK293S using both of ultracentrifugation method and the Exo/solator method. The particle size distribution (Fig. 1), the number of particles (Fig. 2(a)) and the expression level of exosome markers (Fig. 2(b)) of the isolated exosomes were tested and compared. The results showed that the Exo/solator recovered exosomes with the same particle size distribution and the number of particles as the ultracentrifugation method, and the amount of exosome marker expression per protein was higher, indicating that Exo/solator recovered exosomes with higher purity than the ultracentrifugation method.

	Description	Unit	Code
	Exo/solator Exosome Isolation Kit	3 tests	EX10-10
	Exo/solator Isolation Filter	10 pieces	EX11-10



## Lipid Droplet Staining

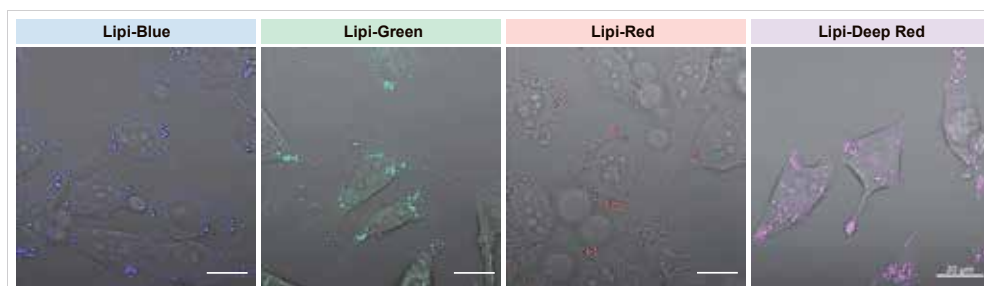
# Lipi-Blue / Green / Red / Deep Red



Lipi probes are small molecules that emit strong fluorescence in a hydrophobic environment such as LDs, which can be observed without any washing steps after staining with Lipi probes.

A medium that contained oleic acid (200  $\mu\text{mol/l}$ ) was added and incubated overnight. Then, the supernatant was removed and the cells were washed with PBS. Each Lipi product series (1  $\mu\text{mol/l}$ ) was added and the cells were incubated for 15 minutes.

Lipi-Blue: Ex. 405 nm / Em. 450 – 500 nm, Lipi-Green: Ex. 488 nm / Em. 500 – 550 nm,  
Lipi-Red: Ex. 561 nm / Em. 565 – 650 nm, Lipi-Deep Red: Ex. 640 nm / Em. 650-700 nm



## Comparison of Reagents

	Dojindo			Other Products		
	Lipi-Blue	Lipi-Green	Lipi-Red	Oil Red O	Nile Red	Reagent B
Live Cells	✓	✓	✓		✓	✓
Fixed Cells	✓	✓	✓	✓	✓	✓
Selectivity towards Lipid Droplet (Level of Background)	✓	✓	✓			
General Filter Accommodation <sup>*1</sup>	✓	✓	✓ <sup>*2</sup>	n.d.	<sup>*3</sup>	✓
Retention in Live Cells	✓	✓		n.d.		

<sup>\*1</sup> Please refer to our website for the co-staining filter.

<sup>\*2</sup> When co-staining with a green fluorescent dye, a green fluorescent emission filter less than 550 nm is recommended.

<sup>\*3</sup> Leaks in GFP filter (500 ~ 540 nm)

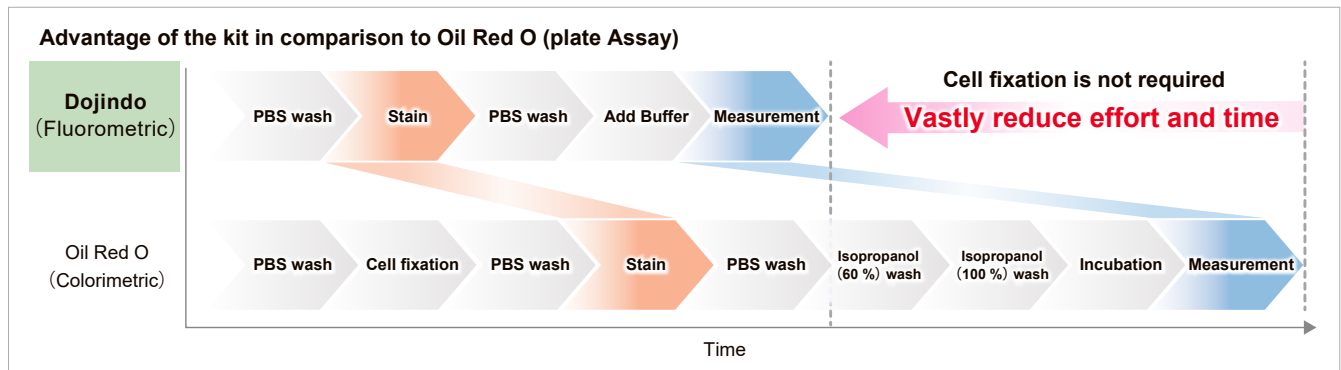
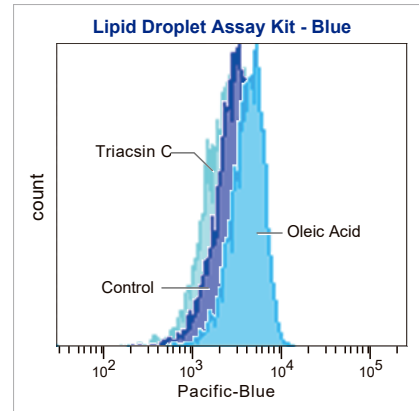
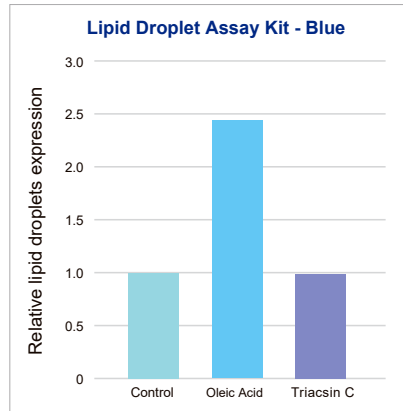
Description	Unit	Code
Lipi-Blue	10 nmol × 1	LD01-10
Lipi-Green	10 nmol × 1	LD02-10
Lipi-Red	100 nmol × 1	LD03-10
Lipi-Deep Red	10 nmol × 1	LD04-10

## Lipid Droplet Staining

# Lipid Droplet Assay Kit - Blue / Deep Red



The Lipid Droplet Assay Kit simplifies the quantification of fat droplets with provided protocols and buffers. It works for live cells, and its fluorescent dye is suitable for both live and fixed cells. Compared to colorimetric reagents, it reduces measuring time and increases experiment repeatability by avoiding dye deposition in the plate.



Description	Unit	Code
Lipid Droplet Assay Kit-Blue	1 set	LD05-10
Lipid Droplet Assay Kit-Deep Red	1 set	LD06-10

Proliferation  
Cytotoxicity

Senescence

Autophagy

Oxidative  
Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

Other Organelles  
Exosome, Lipid Droplet, etc.



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