Reagent for Cellular Function Analysis

Autophagy

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

Senescence

Code	Product Name	Page
SG03	Cellular Senescence Detection Kit - SPiDER-βGal	8
SG05	Cellular Senescence Plate Assay Kit - SPiDER-βGal	9
MT09	JC-1 MitoMP Detection Kit	27
MT10, MT11, MT12	MitoBright LT Series	31
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MD01	Mitophagy Detection Kit	28
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E297	Extracellular OCR Plate Assay Kit	26
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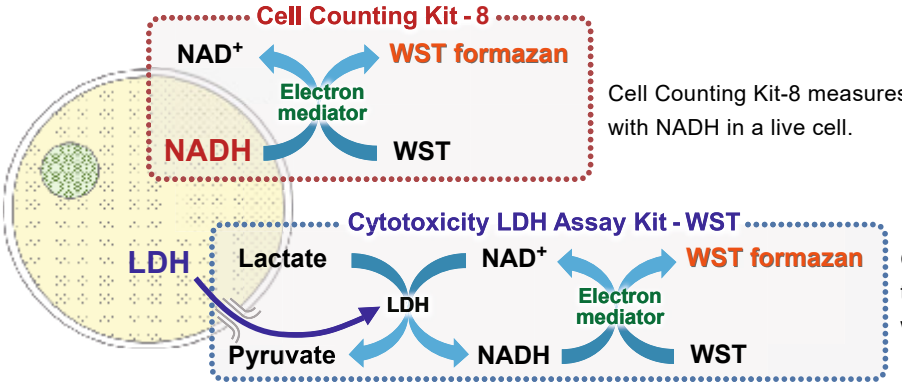
LD05	Lipid Droplet Assay Kit - Blue	41
LD06	Lipid Droplet Assay Kit - Deep Red	41

Cell Proliferation / Cytotoxicity Assay

Cell Counting Kit-8
Cytotoxicity LDH Assay Kit-WST

Cell Counting Kit-8 Cytotoxicity LDH Assay Kit-WST

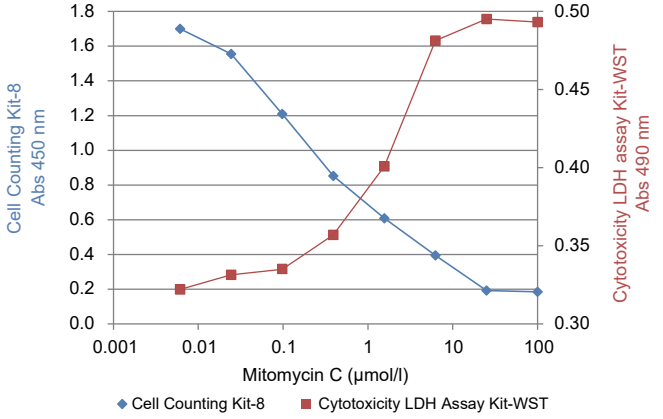
Detection Principle



Cell Counting Kit-8 measures the dehydrogenase activity with NADH in a live cell.

Cytotoxicity LDH Assay Kit-WST measures the LDH released by dead cells. when the plasma membrane is destructed.

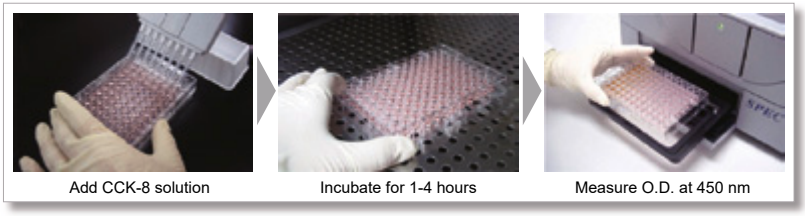
Simultaneous Usage of CCK-8 and Cytotoxicity LDH Assay Kit-WST



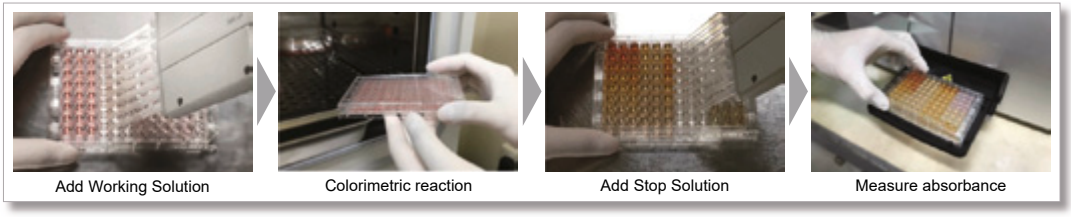
Drug: Mitomycin C
Cell Line: HeLa
Media: MEM, 10% FBS
Incubation: 37°C, 5% CO_2 for 48 hours
Measuring Condition: Cell Counting Kit-8 (450 nm)
Cytotoxicity LDH Assay Kit-WST (490 nm)

Simple Procedure

・Cell Counting Kit-8

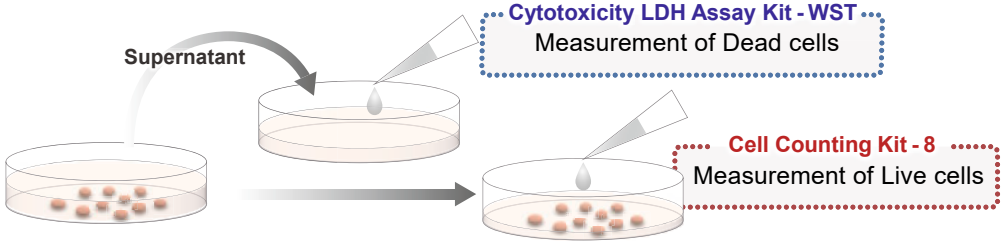


・Cytotoxicity LDH Assay Kit-WST



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

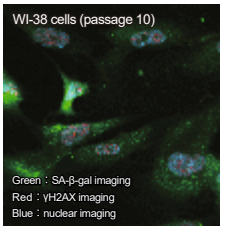
Senescence Detection

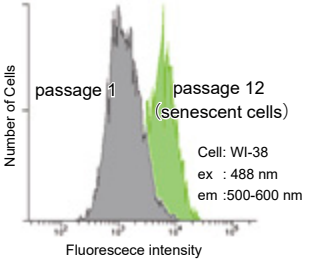
Cellular Senescence Detection Kit - SPiDER-βGal
Cellular Senescence Detection Kit - SPiDER Blue NEW

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

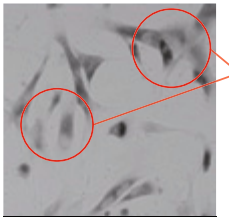




Compatible Instruments:
✓ Microscope ✓ Flow Cytometer ✓ Plate Reader

X-Gal

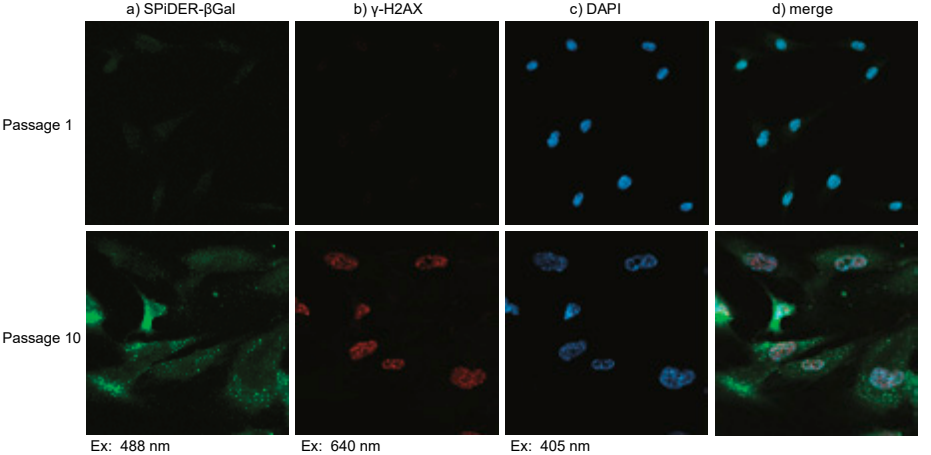
Difficult to quantify



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



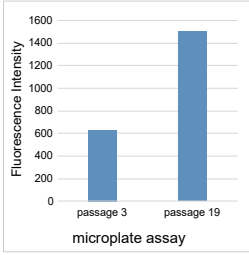
WI-38 cells were treated with anti- γ-H2AX antibody and observed under a confocal microscope. The procedure involved several steps, including fixing the cells, permeabilizing, blocking, adding primary and secondary antibodies, staining with DAPI, and washing the cells. The experiment aimed to detect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

Description	Unit	Code
Cellular Senescence Detection Kit - SPiDER-βGal	10 assays	SG03-10
Cellular Senescence Detection Kit - SPiDER Blue	1 Plate	SG07-10

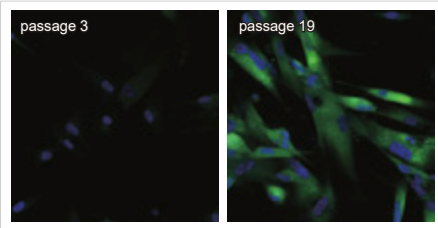
Cellular Senescence Plate Assay Kit - SPiDER-βGal

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



microplate assay



Imaging analysis

Plate Assay
Ex. 535nm / Em. 580nm

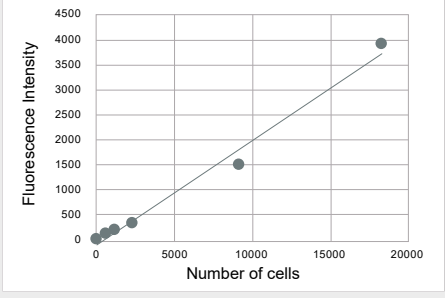
Imaging data
Green: Ex. 488nm / Em. 500-600nm (SA-β-Gal staining with Cellular Senescence Detection Kit – SPiDER-βGal(Code SG04))
Blue: Ex. 405nm / Em. 450-495nm (Nuclear staining with -Cellstain- DAPI solution(Code D523))

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



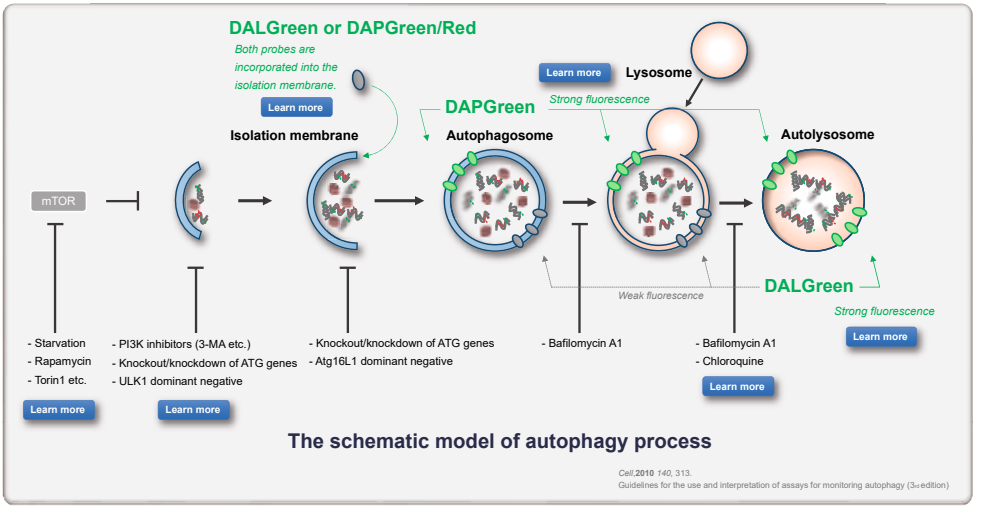
Description	Unit	Code
Cellular Senescence Plate Assay Kit - SPiDER-βGal	20 tests	SG05-01
	100 tests	SG05-05
Cell Count Normalization Kit	200 tests	C544-02
	1000 tests	C544-10

D675 D676 D677

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.

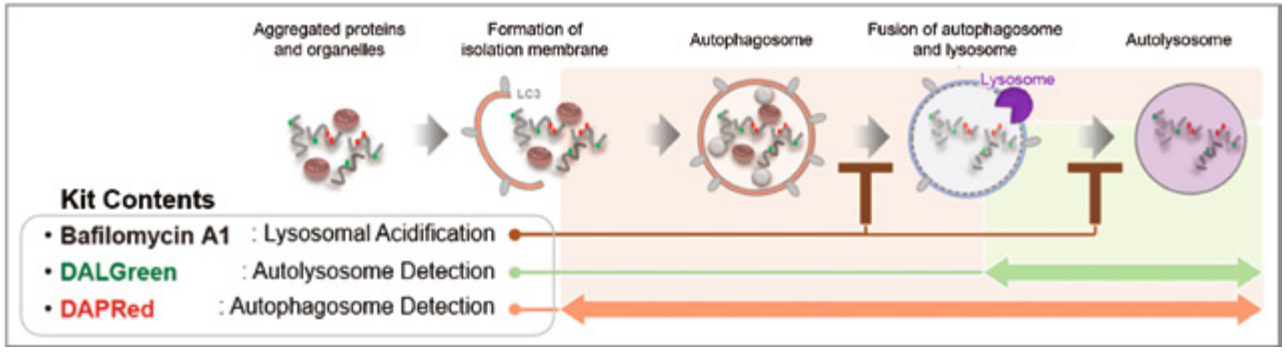


Feature of Each Dye

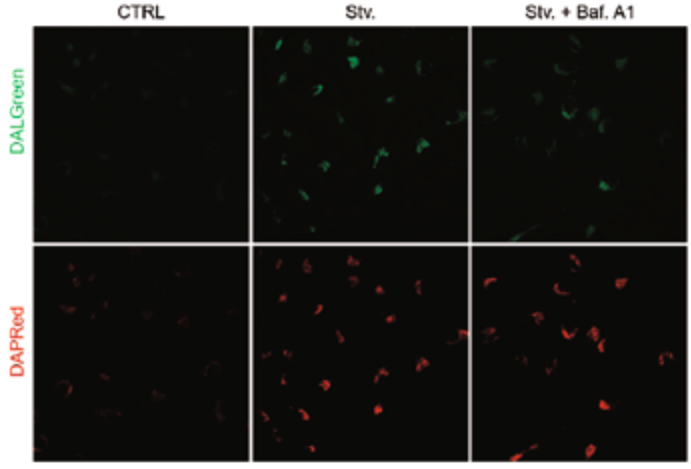
	Applicable instruments			Fluorescent properties	Volume / the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	✓	✓	✓	Ex = 425-475 nm Em = 500-560 nm * For confocal microscope,the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish: 25 (when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

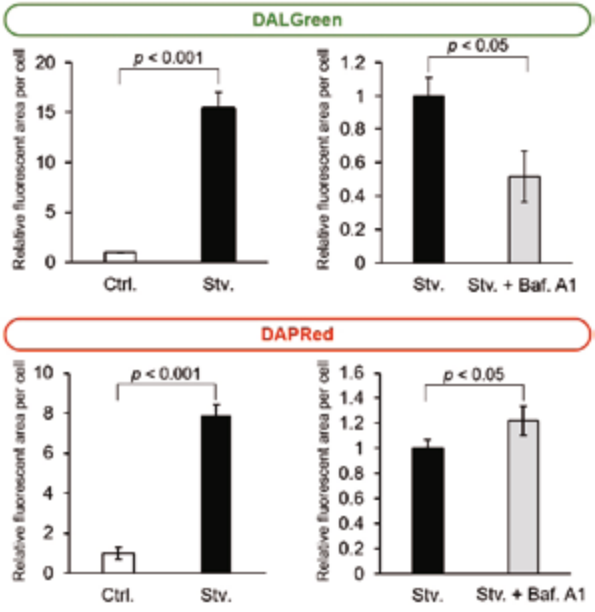
*Double staining imaging by DAPGreen and DALGreen is not possible

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)

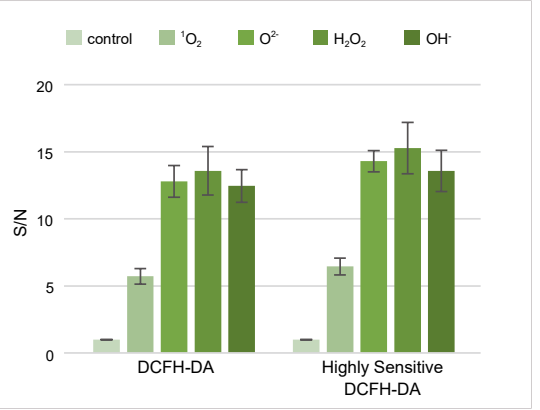
Oxidative Stress

ROS Assay Kit -Highly Sensitive DCFH-DA-

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

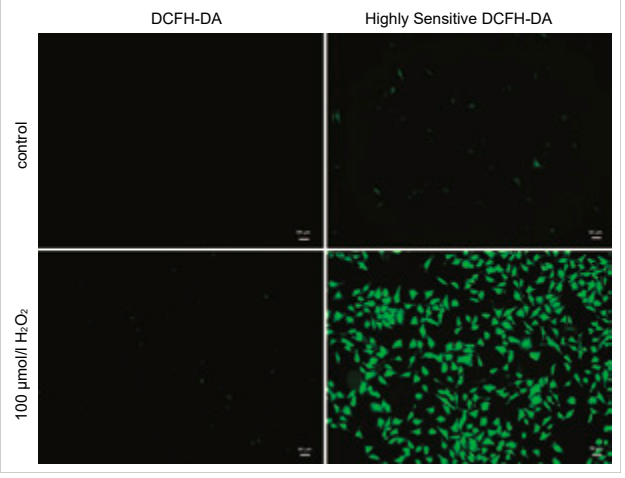
The reactivity of the Highly Sensitive DCFH-DA for ROS is similar to the reactivity of 2'-7' dichlorofluorescein diacetate (DCFH-DA). The Highly Sensitive DCFH-DA also has similar fluorescence characteristics (λ_{ex}: 505 nm, λ_{em}: 525 nm) to DCFH-DA. Therefore, ROS is detectable at the same excitation/fluorescence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

The selectivity for ROS

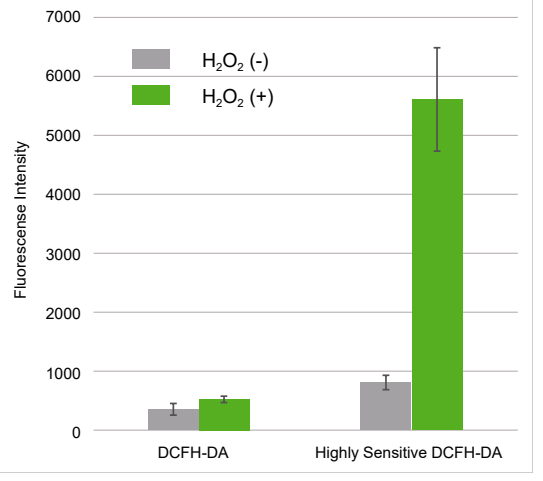


High Sensitive Detection Compared with DCFH-DA

Detection using fluorescent microscope



Detection using microplate reader



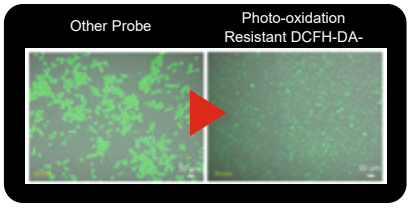
Hydrogen peroxide (H_2O_2)-treated HeLa cells (1×10^4 cells/ml) were stained with DCFH-DA or the ROS Assay Kit-Highly Sensitive DCFH-DA, and the fluorescence intensity of intracellular ROS was compared between two detection kits. As a result, the ROS Assay Kit-Highly Sensitive DCFH-DA in high-sensitivity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

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

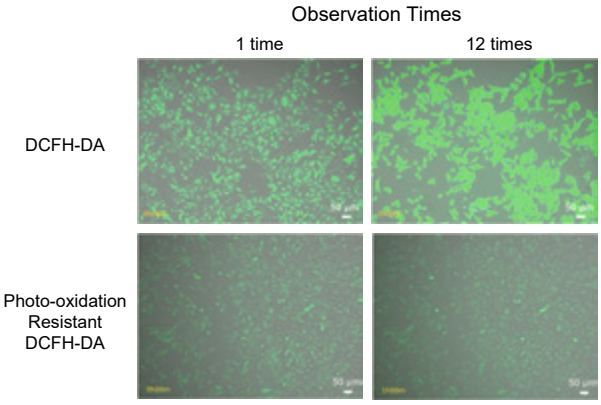
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-

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

Time-lapse imaging movie Available

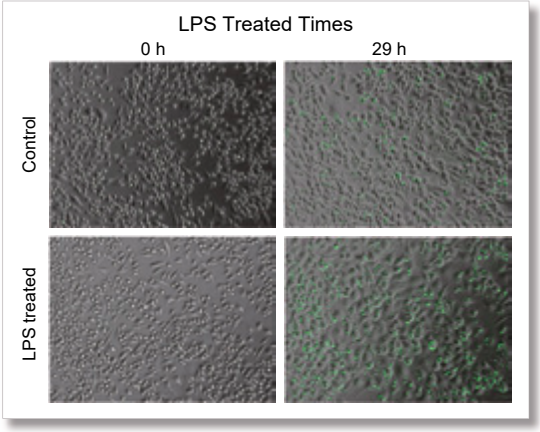


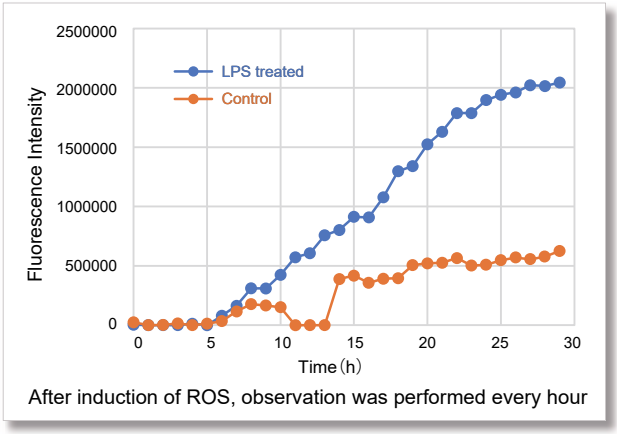
Resistant to Photo-oxidation



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

Simultaneous Detection of ROS in LPS-treated macrophages





After induction of ROS, observation was performed every hour

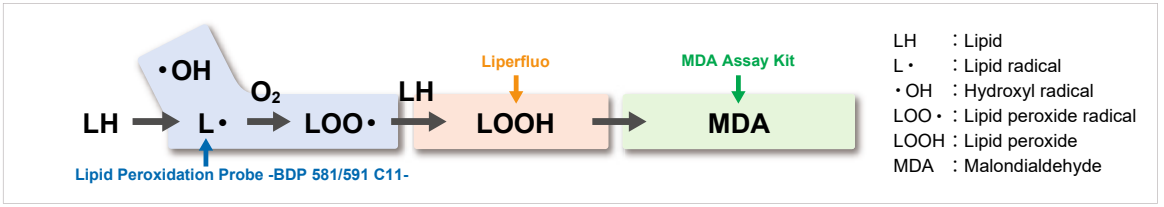
In Lipopolysaccharide (LPS) treated RAW 264.7 cells, after being stained with regular DCFH-DA, Highly Sensitive DCFH-DA, or Photo-oxidation Resistant DCFH-DA, the intracellular ROS level was compared. The results showed that the Dojindo Laboratories' probes could detect intracellular ROS with higher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

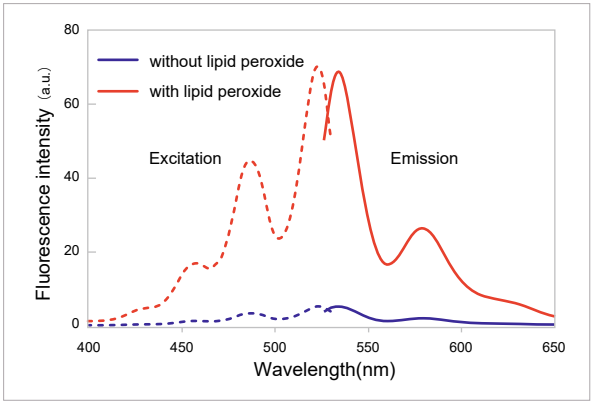
Description	Unit	Code
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	100 tests	R253-10

Lipid Peroxide Detection

Liperfluo

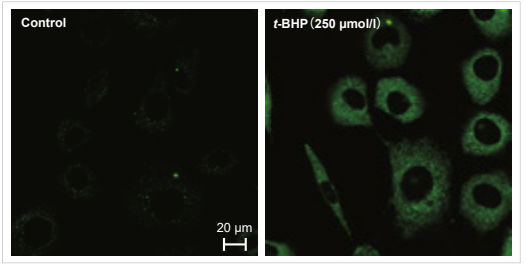
Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and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





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

Lipid Peroxide Detection in Living Cells



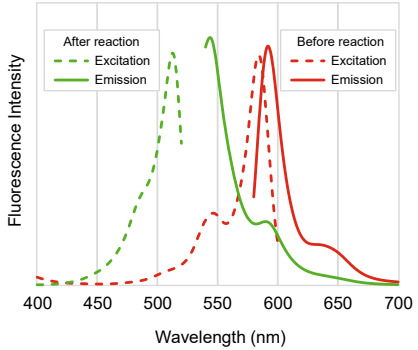
Liperfluo added to cells, t-BHP induced lipid peroxidation and cells were observed under confocal microscope to study ferroptosis.

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

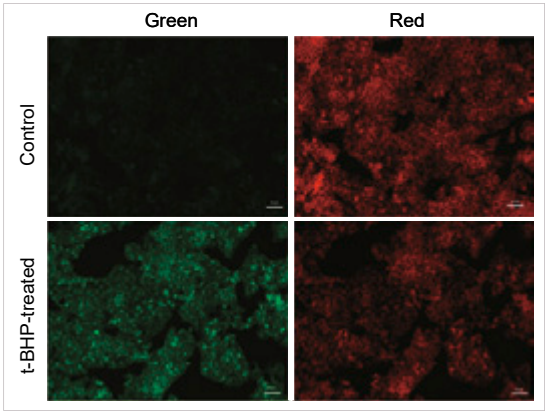
Description	Unit	Code
Liperfluo	1 set (50 μg × 5)	L248-10

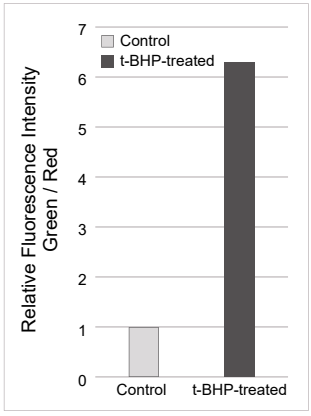
Lipid Peroxidation Probe -BDP 581/591 C11-

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



Lipid Peroxidation Assay





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

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

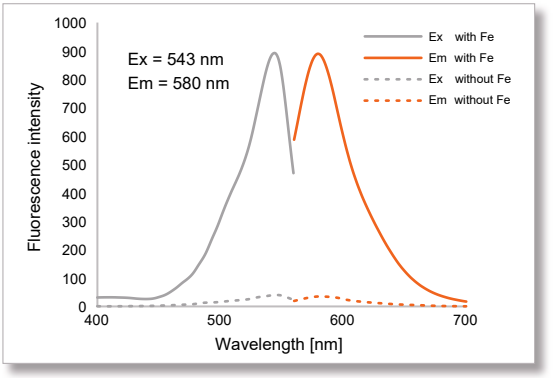
Fluorescent Plate Reader
Green: Ex = 490 nm, Em = 520-540 nm
Red: Ex = 570 nm, Em = 600-620 nm

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

Intracellular Iron Ion Measurement

FerroOrange

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



Experimental Example

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

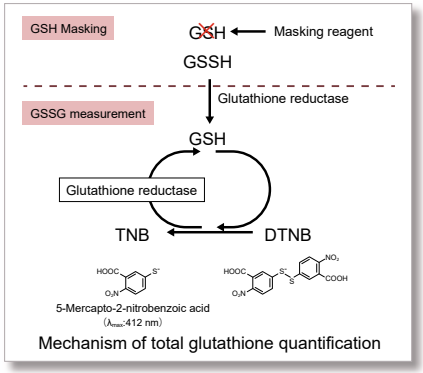
Ex = 561 nm, Em = 570-620 nm, Scale bars 20 μm

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

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

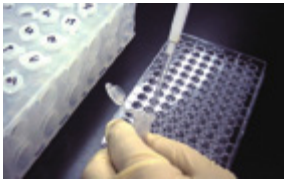
GSSG/GSH Quantification Kit

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

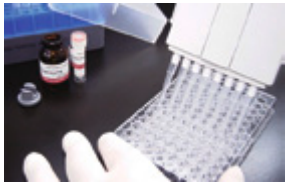


Mechanism of total glutathione quantification

Assay Procedure

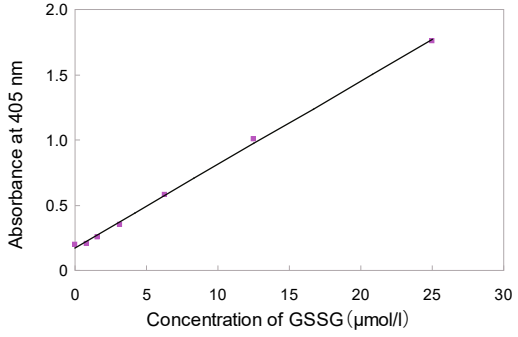

1) GSSG/GSH Standard Solution and add Sample A or Sample B to each well.
2) Add Buffer solution to each well


3) Incubate at 37℃ for 1 h.

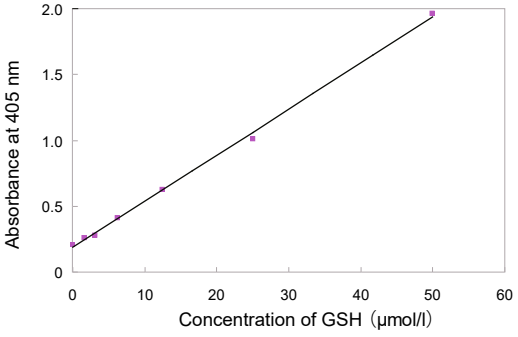

4)-5) Add substrate working solution and Enzyme/ Coenzyme working solution to each well.


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

Calibration Curve



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



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

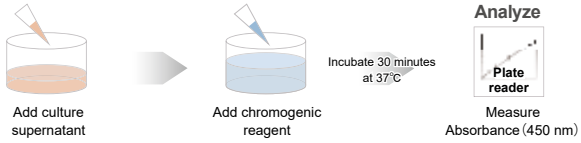
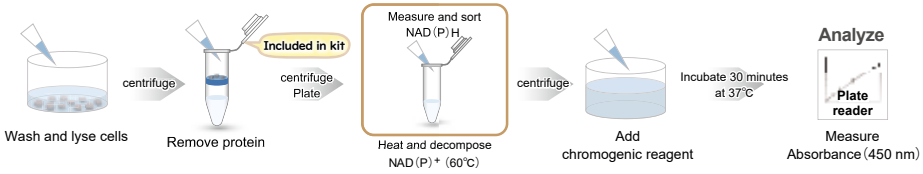
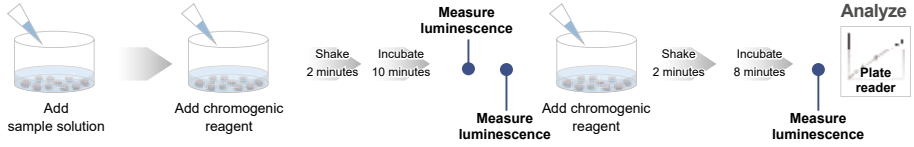
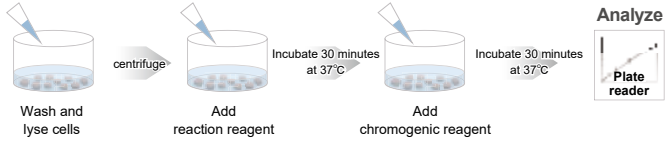
Description	Unit	Code
GSSG/GSH Quantification Kit	200 tests	G257-10

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
Lactate Assay Kit-WST	50 tests	L256-10
	200 tests	L256-20
NAD/NADH Assay Kit-WST	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

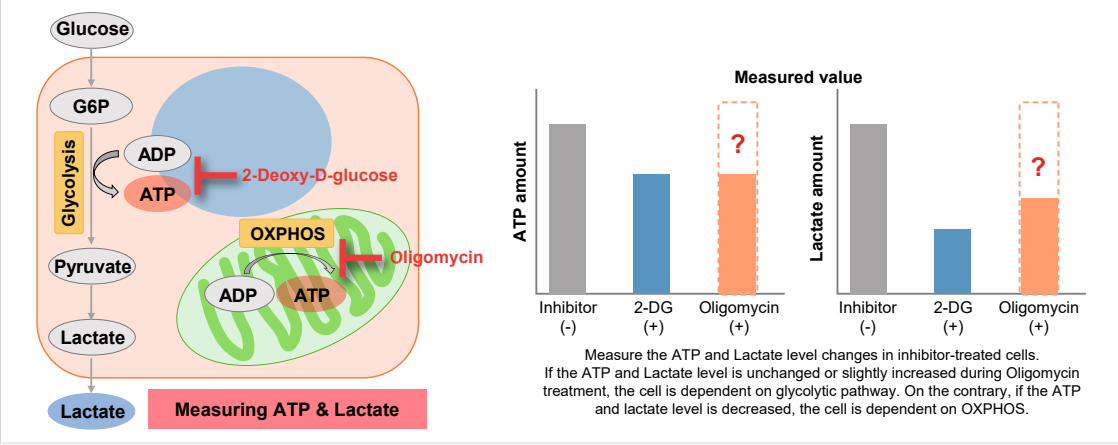
For a first-time user, the kit includes the reagents and components necessary for measuring samples. You'll soon realize how easy it is to use.

Determination index	Detection	Operation
Glucose Lactate Glutamine Glutamate NAD/NADH NADP/NADPH	Colorimetric	**Simply transfer the culture supernatant to a plate and mix it with the chromogenic reagent**  
ATP ADP/ATP	Luminescent	**Kit includes ATP standard - very easy to use**  
α-ketoglutaric acid	Fluorescent	**Less variable results than existing assays** 

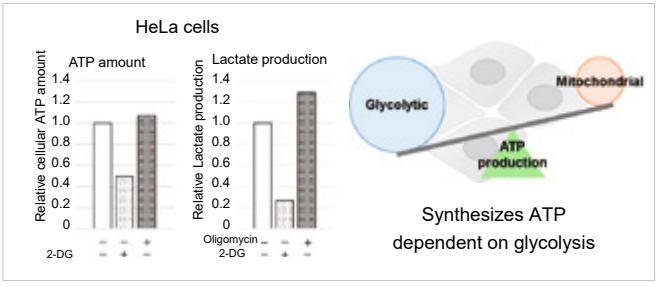
Glycolysis/OXPHOS Assay Kit

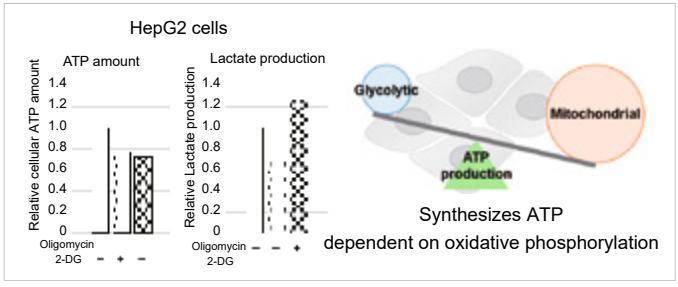
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

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



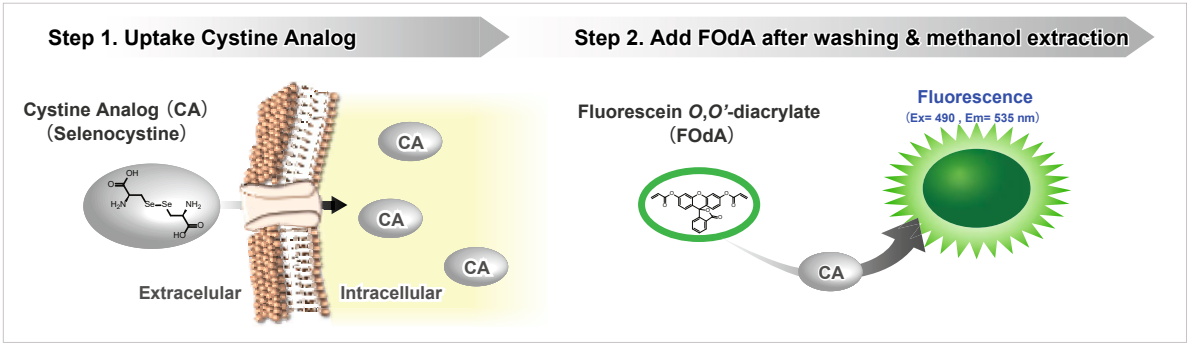


Description	Unit	Code
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10

Cystine Uptake Assay Kit

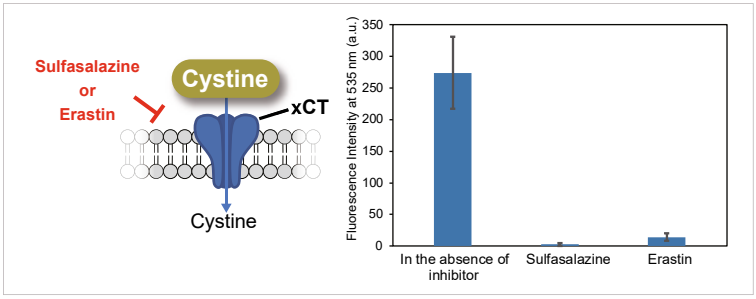
- Easier way to cystine uptake assay
- Applied for plate assay

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

Mitochondrial Research

Mitochondrial Superoxide Detection

MitoBright ROS 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^{2+}

Mitophagy Detection

Mitophagy Detection Kit

Live-cell fluorescent imaging of mitophagy without transfection

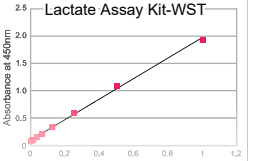
Measurement of Glucose

Glucose Assay Kit-WST

Measurement of intracellular glucose concentrations via fluorescence

Measurement of Lactate

Lactate Assay Kit-WST

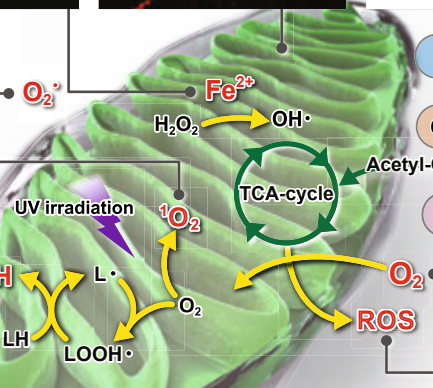


Measuring lactate to infer glycolytic activity

Singlet Oxygen Detection

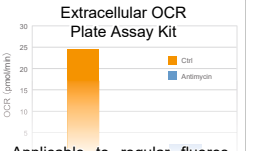
Si-DMA for Mitochondrial Singlet Oxygen Imaging

Real-time visualization of $^1O_2$ generation



Oxygen consumption rate (OCR) Detection

Extracellular OCR Plate Assay Kit



Applicable to regular fluorescent plate reader with temperature-controlled incubation

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

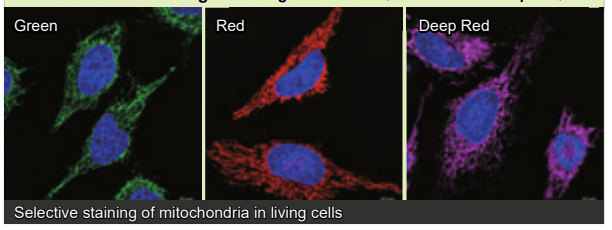
Monitoring and observation even after fixation, with more sensitive detection than JC-1

Total ROS Detection

ROS Assay Kit -Highly Sensitive DCFH-DA-

Detection with higher sensitivity than the original DCFH-DA

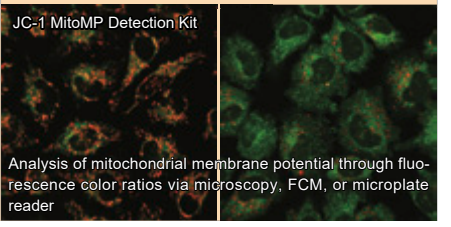
Mitochondrial Staining MitoBright LT Series (Green / Red / DeepRed)



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 µg × 3	MT02-10
Mitochondrial Staining		
MitoBright LT Green	400 µl	MT10-12
MitoBright LT Red	400 µl	MT11-12
MitoBright LT Deep Red	400 µl	MT12-12
MitoBright IM Red for Immunostaining	20 µl×1	MT15-10
	20 µl×3	MT15-12
Oxidative Stress		
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	100 nmol × 1	MT16-10
	100 nmol × 3	MT16-12
Mito-FerroGreen	1 set (50 µg × 2)	M489-10
Si-DMA for Mitochondrial Singlet Oxygen Imaging	2 µg	MT05-10
MitoPeDPP	5 µg × 3	M466-10

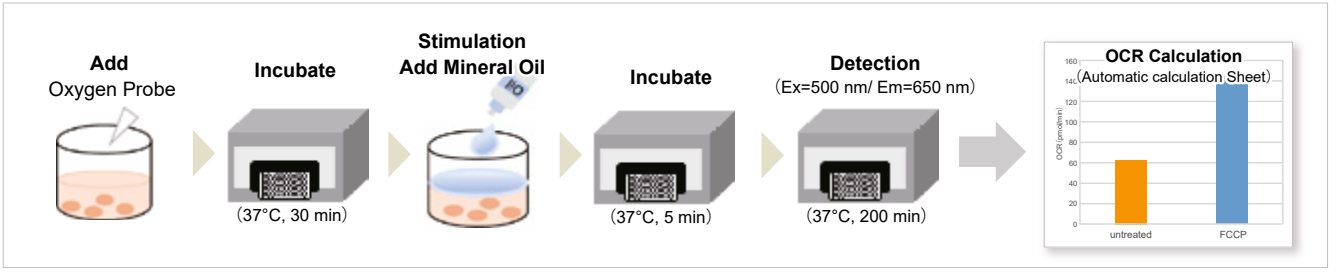
Mitochondrial Research

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

Kit Contents

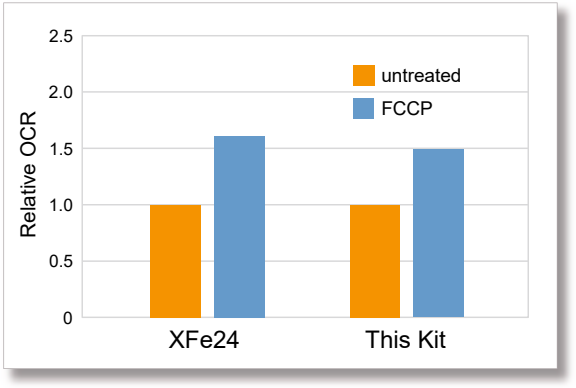
Procedure



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: 5×10^4 cells/well
Stimulation: FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone)
FCCP Concentration: 2 µmol/l



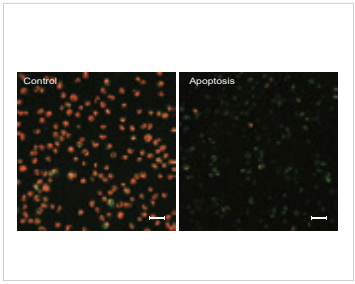
Description	Unit	Code
Extracellular OCR Plate Assay Kit	100 tests	E297-10

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 a indicator of mitochondrial condition.

Fluorescence Microscope



Flow Cytometry

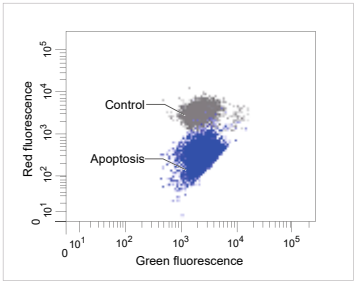
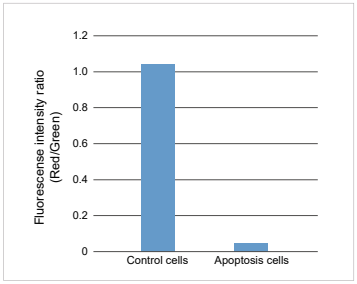


Plate Reader



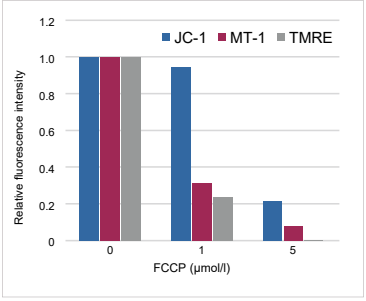
Description	Unit	Code
JC-1 MitoMP Detection Kit	1 set	MT09-10

Mitochondrial Membrane Potential Detection

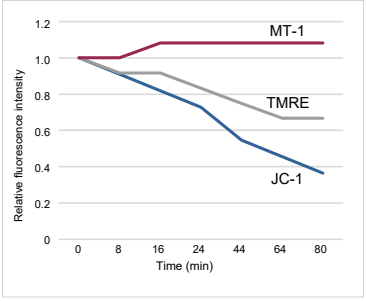
MT-1 MitoMP Detection Kit

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.

High Sensitivity Detection



Allow to monitor mitochondrial membrane potential

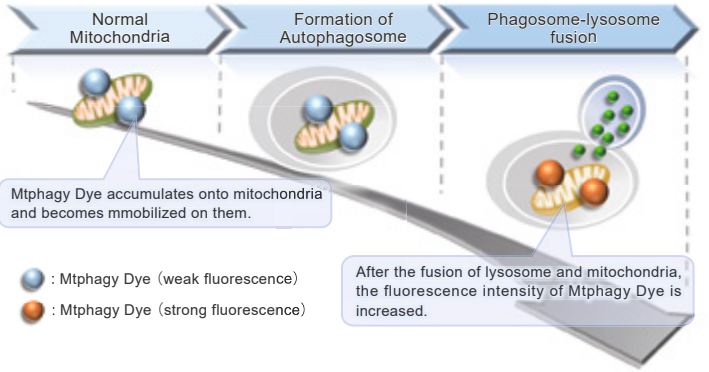


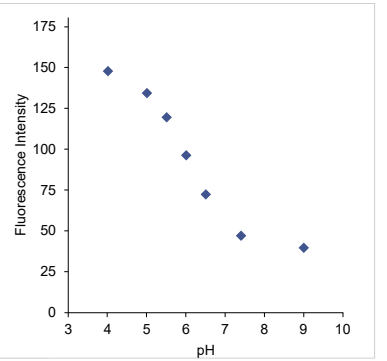
Description	Unit	Code
MT-1 MitoMP Detection Kit	1 set	MT13-10

Mitochondrial Research

Mitophagy Detection Kit

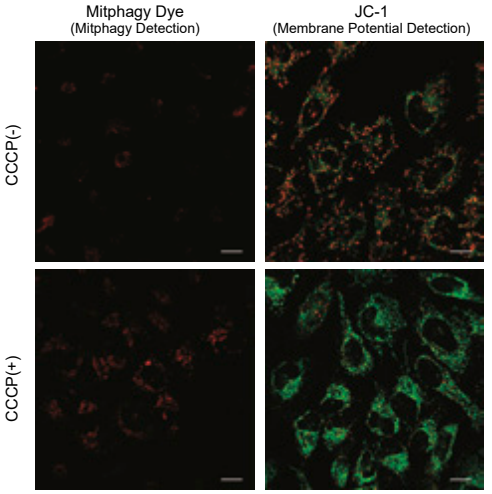
This kit is composed of Mtphagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtphagy Dye accumulates in intact mitochondria, is immobilized on it with chemical bond and exhibits a weak fluorescence from the influence of surrounding condition. When Mitophagy is induced, the damaged mitochondria 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 incresased 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).

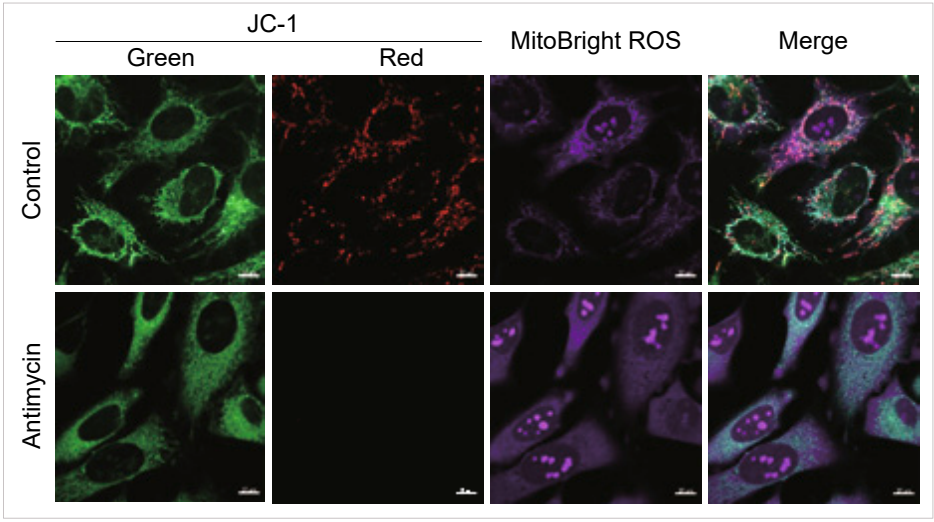
Description	Unit	Code
Mitophagy Detection Kit	1 set	MD01-10
Mtphagy Dye	5 µg × 3	MT02-10

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.

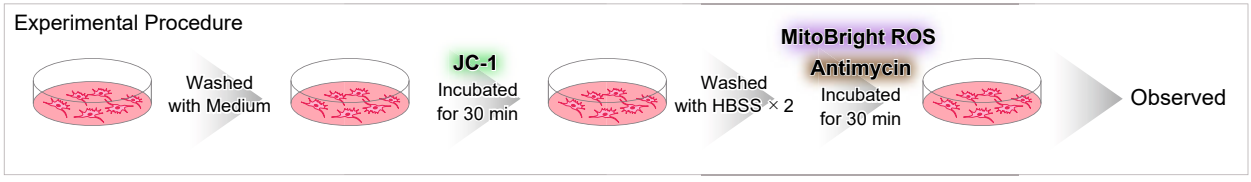
Experimental Example

Simultaneously Evaluation of Mitochondrial Superoxide and Membrane Potential



<Imaging Conditions>
(Confocal microscopy)
JC-1: Green Ex = 488, Em = 490-520 nm,
Red: Ex = 561, Em = 560-600 nm
MitoBright ROS Deep Red: Ex = 633 nm,
Em = 640-700 nm
Scale bar: 10 μm

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

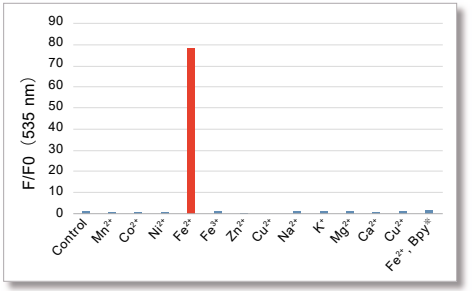
M489 F374

Mitochondrial Superoxide Detection

Mito-FerroGreen

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

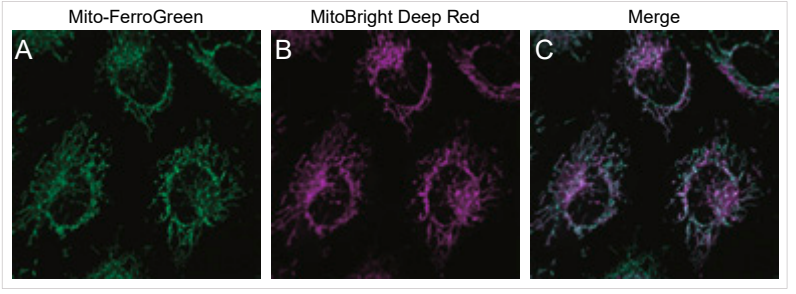
Metal ion Selectivity



※ Bpy : 2,2′ -Bipyridyl

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 mitocondrial staining probe

Mito-FerroGreen (5 μmol/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	λex 505 nm, λem 535 nm	λex 543 nm, λem 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 μg x 2) 10 assays at 35 mm dish (final concentration 5 μmol/l)	1 tube (24 μg) 17 assays at 35 mm dish (final concentration 1 μmol/l)

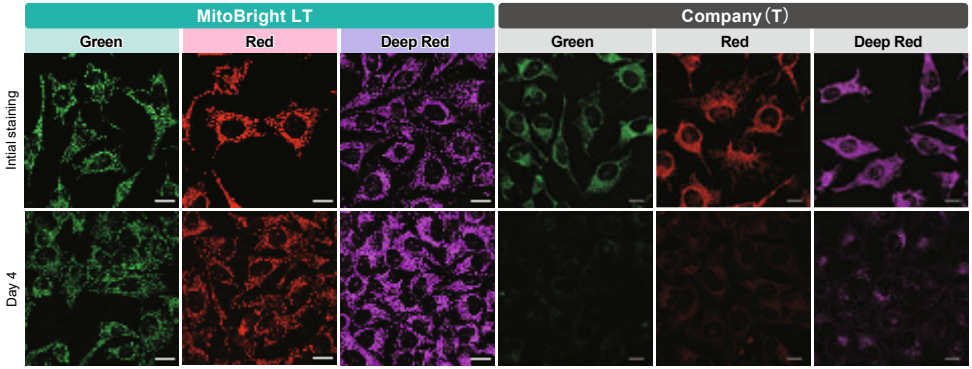
Description	Unit	Code
Mito-FerroGreen	1 set (50 μg × 2)	M489-10
FerroOrange	1 tube	F374-10
	3 tube	F374-12

MitoBright LT Series

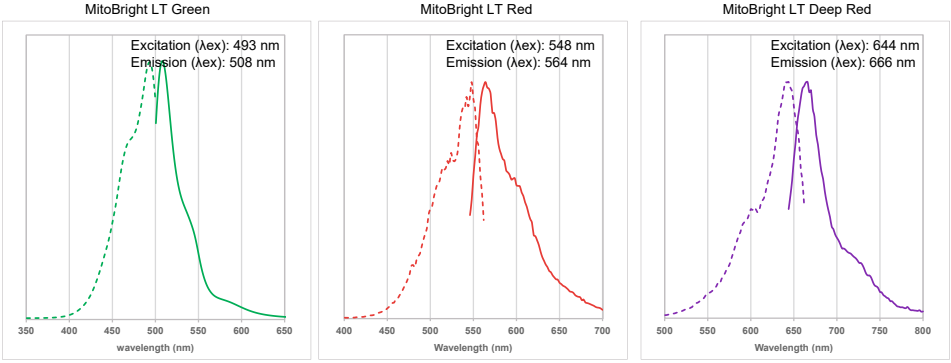
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color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



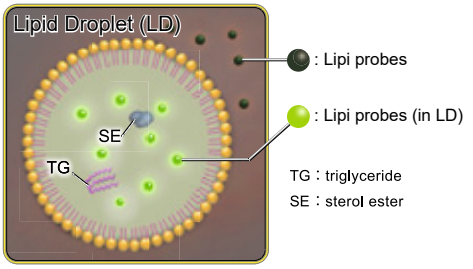
Fluorescence Properties



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

Lipid Droplet Staining

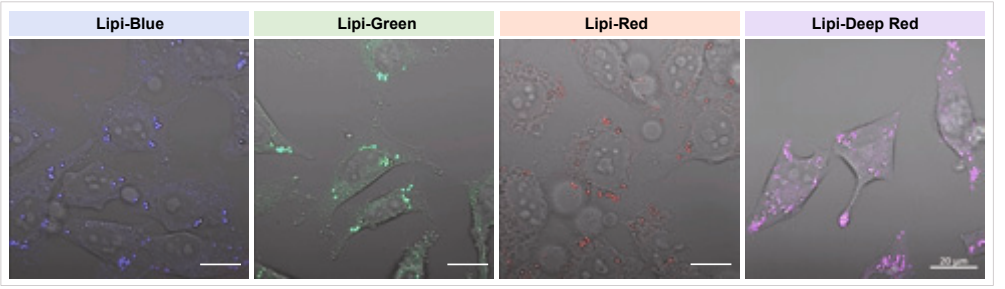
Lipi-Blue / Green / Red / Deep Red



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 µmol/l) was added and incubated overnight. Then, the supernatant was removed and the cells were washed with PBS. Each Lipi product series (1 µmol/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

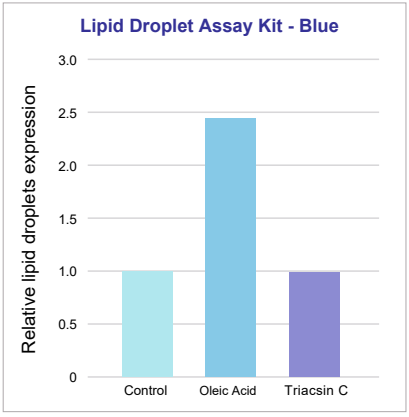
	Dojindo			Other Products		
	Lipi-Blue	Lipi-Green	Lipi-Red	Oil Red O	Nile Red	Reagent B
Live Cells	✓	✓	✓		✓	✓
Fixed Cells	✓	✓	✓	✓	✓	✓
Selectivity towards Lipid Droplet (Level of Background)	✓	✓	✓			
General Filter Accommondation*1	✓	✓	✓*2	n.d.	*3	✓
Retention in Live Cells	✓	✓		n.d.		

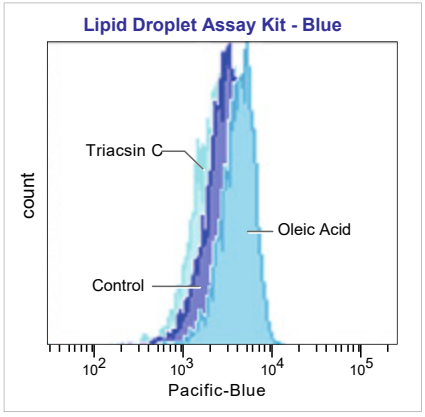
*1 Please refer to our website for the co-staining filter.
*2 When co-staining with a green fluorescent dye, a green fluorescent emission filter less than 550 nm is recommended.
*3 Leaks in GFP filter (500 ～ 540 nm)

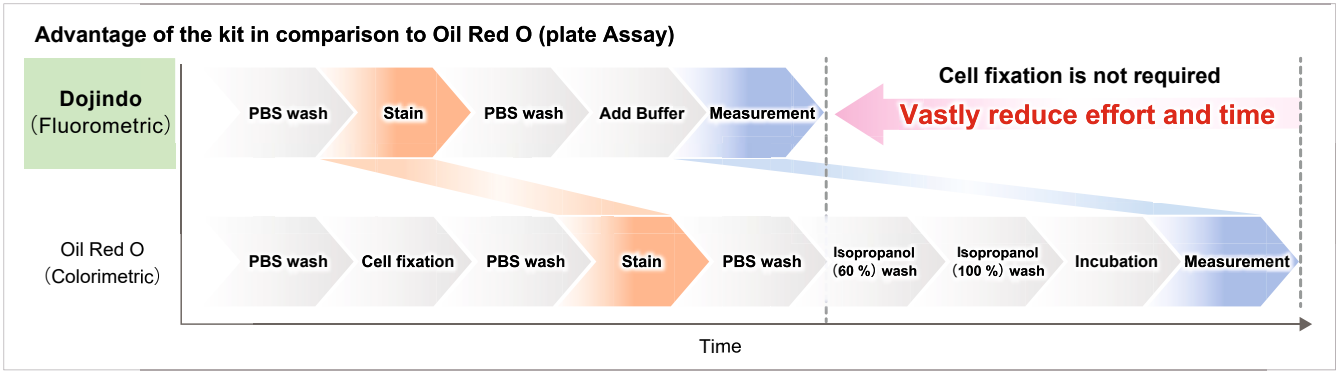
Description	Unit	Code
Lipi-Blue	10 nmol × 1	LD01-10
Lipi-Green	10 nmol × 1	LD02-10
Lipi-Red	100 nmol × 1	LD03-10
Lipi-Deep Red	10 nmol × 1	LD04-10

Lipid Droplet 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

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