Reagent for Cellular Function Analysis

DOJINDO

7th Edition

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
- Cellular Senescence Detection Kit -SPiDER Blue

Neurodegenerative Diseases

Cancer

Senescence

Mitochondria

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

Cellular Metabolism

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

Ferroptosis

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

Cancer

Code	Product Name	Page
MT09	JC-1 MitoMP Detection Kit	31
MT10, MT11, MT12	MitoBright LT Series	35
MT13	MT-1 MitoMP Detection Kit	31
MT16	MtoBright ROS Deep Red - Mitochondrial Superoxide Detection	33
MD01	Mitophagy Detection Kit	32
MT02	Mtphagy Dye	32
E297	Extracellular OCR Plate Assay Kit	30
A562	Autophagic Flux Assay Kit	11
D675	DALGreen - Autophagy Detection	10
D676	DAPGreen - Autophagy Detection	10
D677	DAPRed - Autophagy Detection	10
F374	FerroOrange	16
L248	Liperfluo	14
M489	Mito-FerroGreen	34
UP05	Cystine Uptake Assay Kit	27
L267	Lipid Peroxidation Probe -BDP 581/591 C11-	15
G270	Glycolysis/OXPHOS Assay Kit	22
A550	ATP Assay Kit-Luminescence	24
L256	Lactate Assay Kit-WST	23
R252	ROS Assay Kit -Highly Sensitive DCFH-DA-	12
R253	ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	13
G257	GSSG/GSH Quantification Kit	17
G264	Glucose Assay Kit-WST	23
G268	Glutamate Assay Kit-WST	21

Neurodegenerative Diseases

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MT09	JC-1 MitoMP Detection Kit	31
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G270	Glycolysis/OXPHOS Assay Kit	22
A550	ATP Assay Kit-Luminescence	24
L256	Lactate Assay Kit-WST	23
L261	LysoPrime Green - High Specificity and pH Resistance	36
L264	LysoPrime Deep Red - High Specificity and pH Resistance	36
L265	pHLys Red - Lysosomal Acidic pH Detection	36
L266	Lysosomal Acidic pH Detection Kit	37
L268	Lysosomal Acidic pH Detection Kit-Green/Deep Red	37
LD01, LD02, LD03, LD04	Lipi-Blue, Lipi-Green, Lipi-Red, Lipi-Deep Red	40
LD05	Lipid Droplet Assay Kit - Blue	41
LD06	Lipid Droplet Assay Kit - Deep Red	41

Senescence

Code	Product Name	Page
SG03	Cellular Senescence Detection Kit - SPiDER-βGal	8
SG07	Cellular Senescence Detection Kit - SPiDER Blue	8
SG05	Cellular Senescence Plate Assay Kit - SPiDER-βGal	9
MT09	JC-1 MitoMP Detection Kit	31
MT10, MT11, MT12	MitoBright LT Series	35
MT13	MT-1 MitoMP Detection Kit	31
MT16	MtoBright ROS Deep Red - Mitochondrial Superoxide Detection	33
MD01	Mitophagy Detection Kit	32
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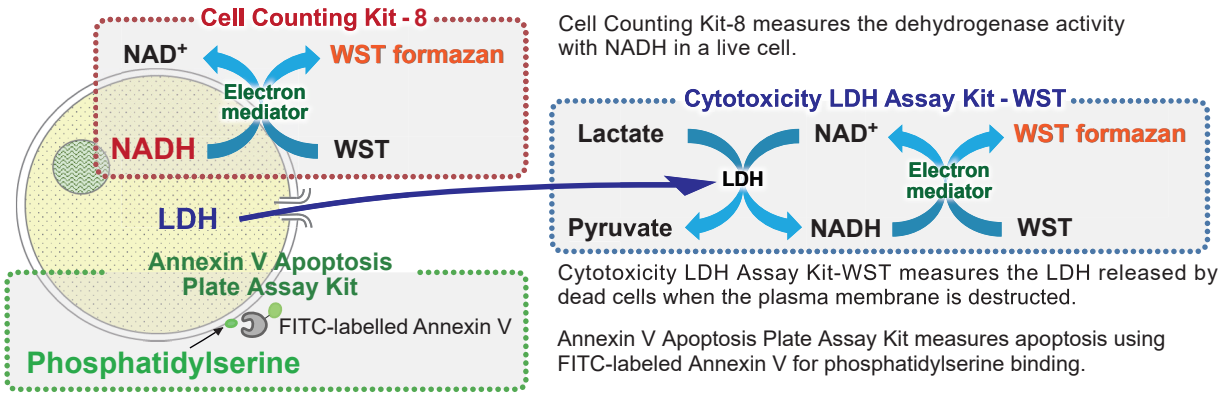
L267	Lipid Peroxidation Probe -BDP 581/591 C11-	15
G270	Glycolysis/OXPHOS Assay Kit	22
A550	ATP Assay Kit-Luminescence	24
L256	Lactate Assay Kit-WST	23
R252	ROS Assay Kit -Highly Sensitive DCFH-DA-	12
R253	ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	13
G257	GSSG/GSH Quantification Kit	17
LD01, LD02, LD03, LD04	Lipi-Blue, Lipi-Green, Lipi-Red, Lipi-Deep Red	40
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
Annexin V Apoptosis Plate Assay Kit

CK04 CK12 AD12

Detection Principle



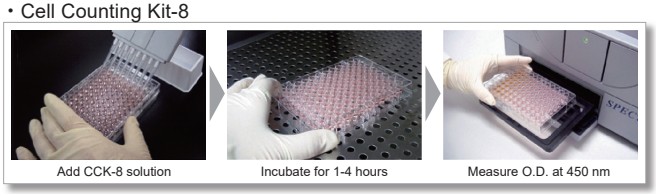
Cell Counting Kit-8 measures the dehydrogenase activity with NADH in a live cell.

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

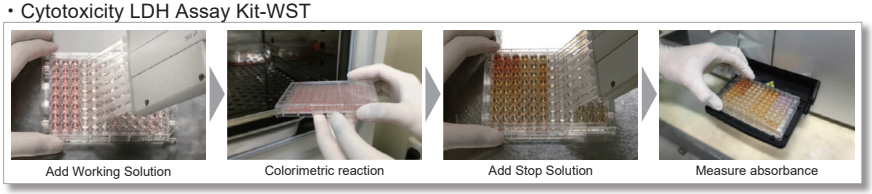
Annexin V Apoptosis Plate Assay Kit measures apoptosis using FITC-labeled Annexin V for phosphatidylserine binding.

Procedure

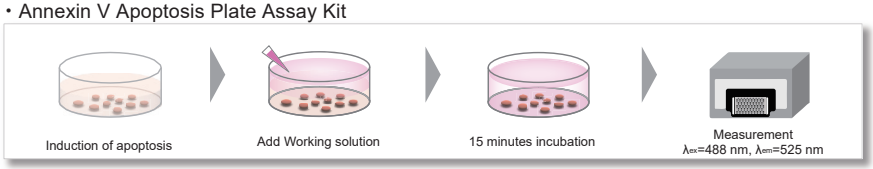
・Cell Counting Kit-8



・Cytotoxicity LDH Assay Kit-WST



・Annexin V Apoptosis Plate Assay Kit



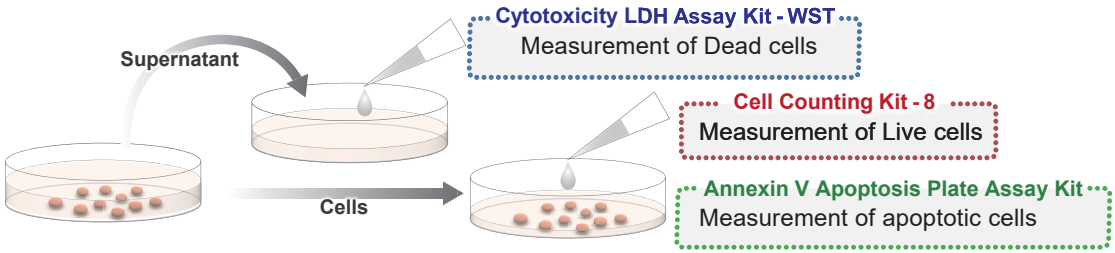
The Annexin V Apoptosis Plate Assay Kit contains a reagent that quenches the fluorescence of Annexin V not bound to phosphatidylserine, allowing rapid detection of multiple samples using a plate reader without washing procedures.

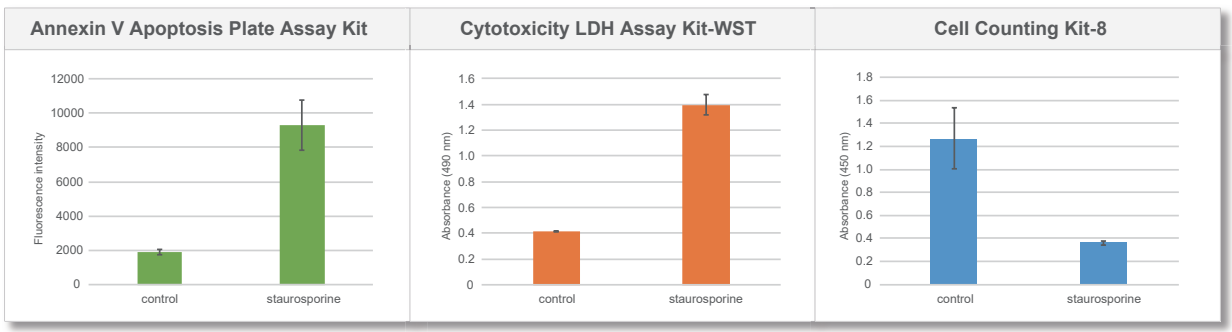
Experimental Example: Changes in various indicators due to Staurosporine

HepG2 cells were treated with staurosporine to induce apoptosis, and phosphatidylserine, extracellular LDH and cell proliferation were detected. Phosphatidylserine was measured as an apoptosis marker using the Annexin V Apoptosis Plate Assay Kit, extracellular LDH was measured as an indicator of dead cells using the Cytotoxicity LDH Assay Kit-WST , and cell proliferation was measured using the Cell Counting Kit-8. The results showed that staurosporine treatment increased phosphatidylserine and extracellular LDH, and decreased cell proliferation.

Same Samples can be used

In addition, cell samples are separated into cells and culture media and measured with different indicators (using a combination of our kits), allowing more detailed analysis of cell death.





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
Annexin V Apoptosis Plate Assay Kit	100 tests	AD12-10

Senescence Detection

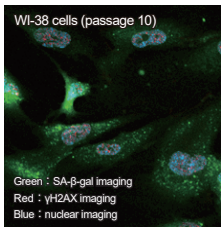
SG03 SG07

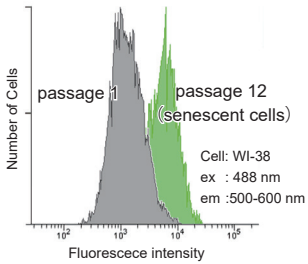
Cellular Senescence Detection Kit - SPiDER-βGal
Cellular Senescence Detection Kit - SPiDER Blue

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

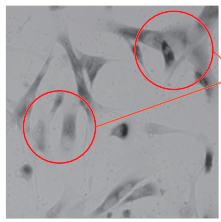




Compatible Instruments:
✓ Microscope ✓ Flow Cytometer ✓ Plate Reader

X-Gal

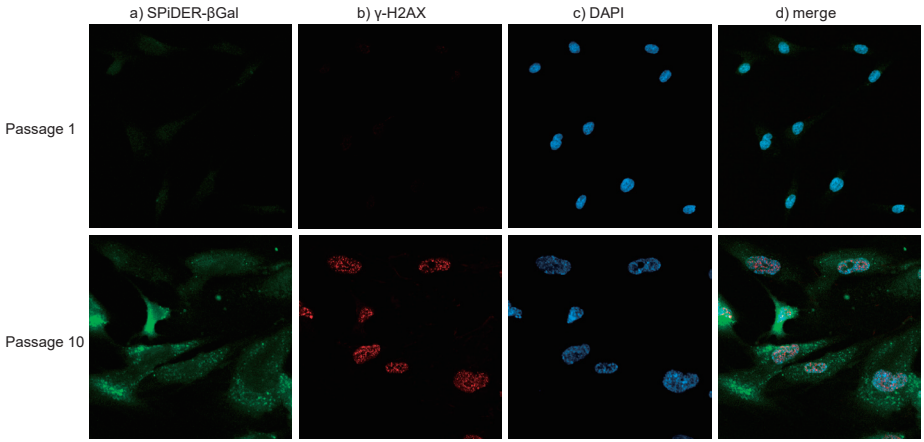
Difficult to quantify



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

Compatible Instruments:
✓ Microscope

Experimental Example: Co-staining of SA- β-gal and DNA Damage marker



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.

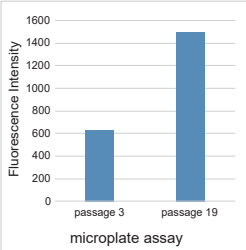
Description	Unit*	Code
Cellular Senescence Detection Kit - SPiDER-βGal	10 assays	SG03-10
Cellular Senescence Detection Kit - SPiDER Blue	1 Plate	SG07-10

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

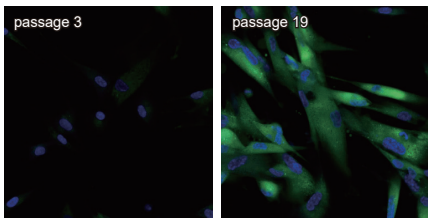
Cellular Senescence Plate Assay Kit - SPiDER-βGal

This kit allows you to quantify SA-β-gal activity and evaluate multiple samples in a 96-well plate by simply adding SPiDER-βGal, a reagent that can detect β-galactosidase.

Correlation with Imaging Data



microplate assay

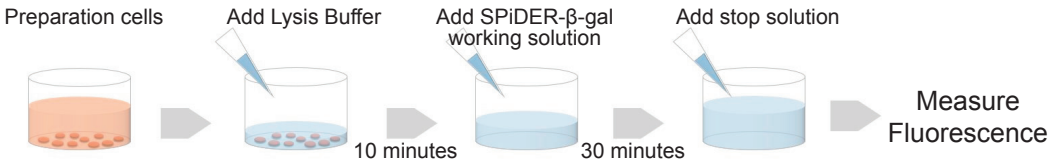


Imaging analysis

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))

Experimental Procedure

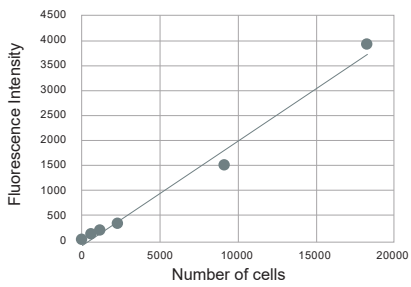


Cell Count Normalization Kit

Combined Cellular Senescence Plate Assay protocol available online

When normalized to the results obtained by quantifying nucleic acids using the Cell Count Normalization Kit, the measured values of SA-β-gal activity become available for evaluation of SA-β-gal activity according to cell number.

Highly correlated to cell number



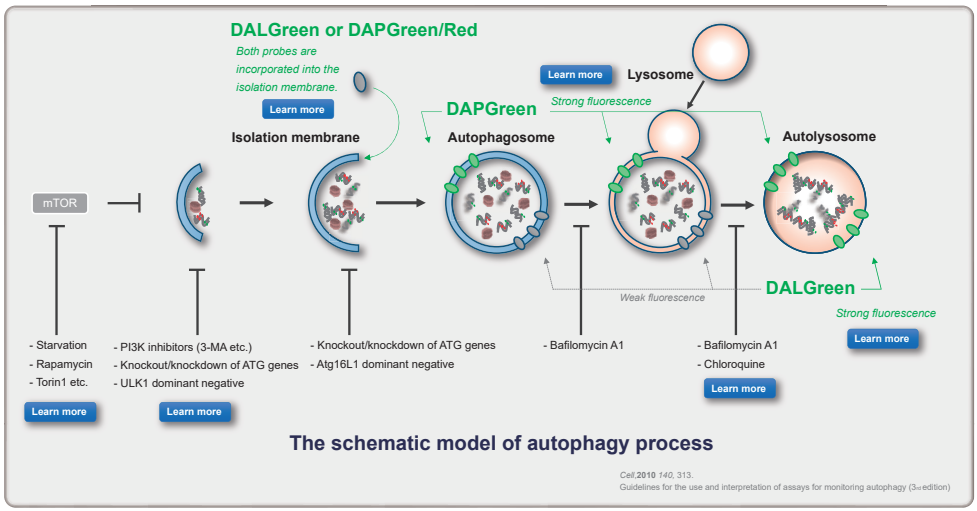
Description	Unit	Code
Cellular Senescence Plate Assay Kit - SPiDER-βGal	20 tests 100 tests	SG05-01 SG05-05
Cell Count Normalization Kit	200 tests 1000 tests	C544-02 C544-10

Autophagy

DAPGreen / Red - Autophagy Detection
DALGreen - Autophagy Detection

D675 D676 D677

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.

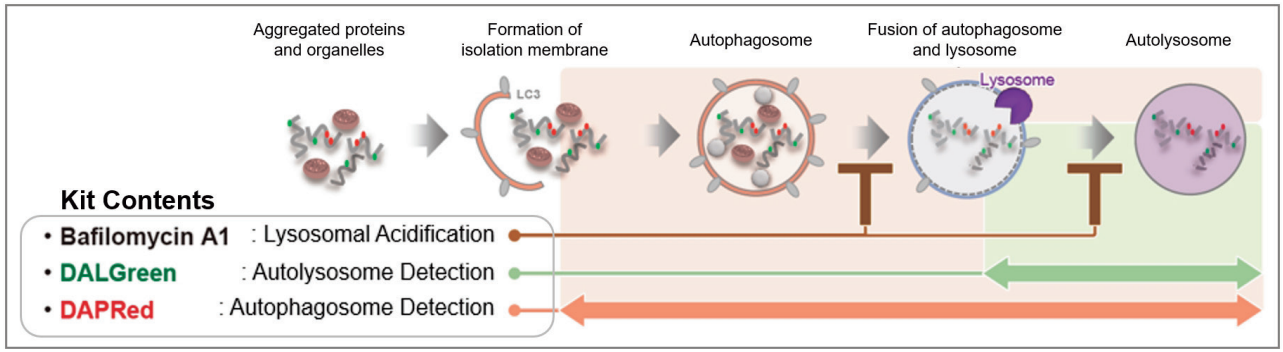


Feature of Each Dye

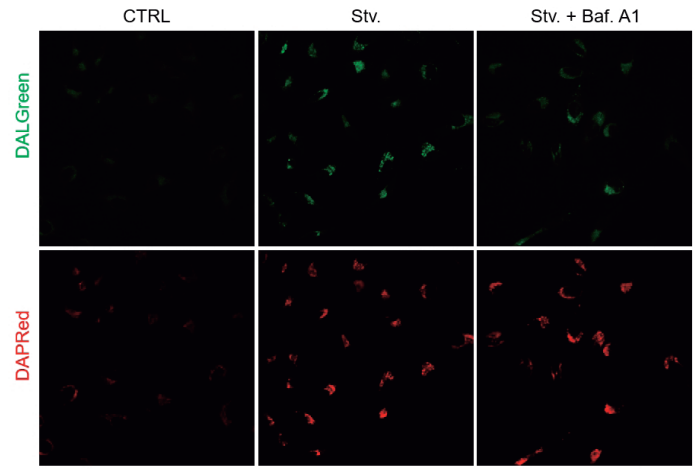
	Applicable instruments			Fluorescent properties	Volume / the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	○	○	○	Ex = 425-475 nm Em = 500-560 nm * For confocal microscope,the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish: 25 (when 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.

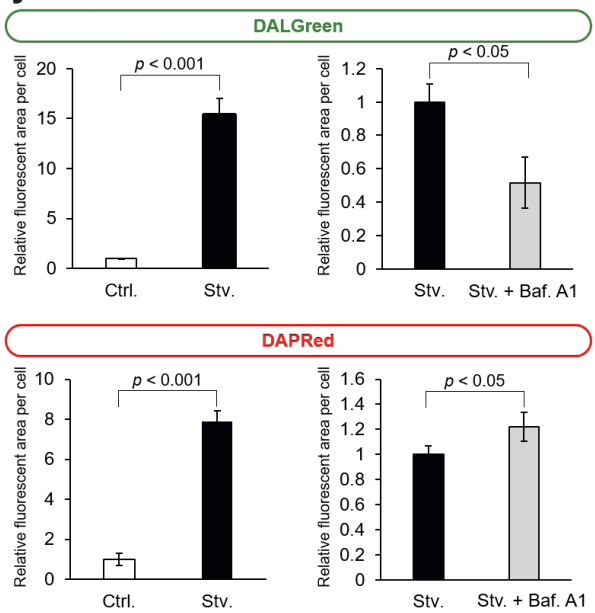
*Double staining imaging by DAPGreen and DALGreen is not possible

Autophagic Flux 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

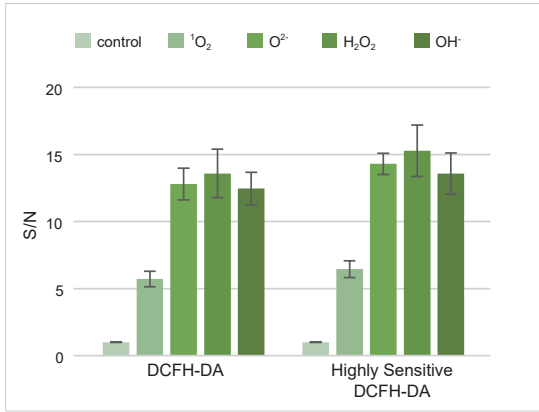
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Oxidative Stress

ROS Assay Kit -Highly Sensitive DCFH-DA-

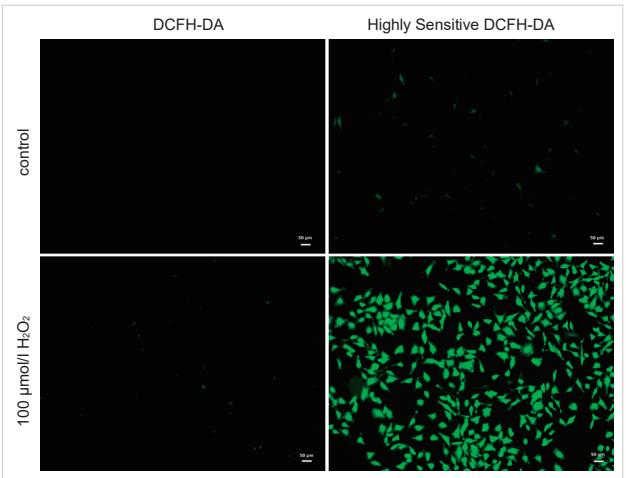
The selectivity for ROS

DCFH-DA is widely used for ROS detection, but it has some limitations like weak fluorescence signals and high background. Dojindo's ROS Assay Kit -Highly Sensitive DCFH-DA- allows ROS detection with higher sensitivity than DCFH-DA with the similar ROS selectivity.

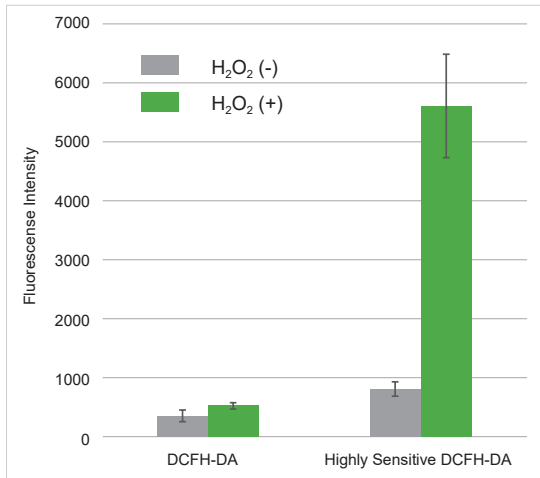


Experimental Example: High Sensitive Detection Compared with DCFH-DA

Detection using fluorescent microscope



Detection using microplate reader



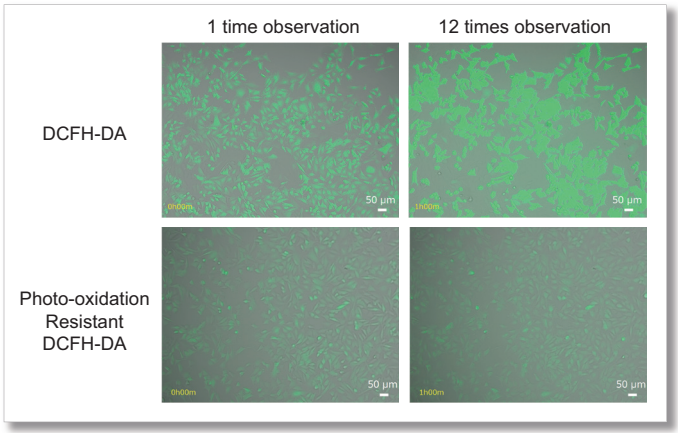
Hydrogen peroxide (H_2O_2)-treated HeLa cells (1×10^4 cells/ml) were stained with DCFH-DA or the ROS Assay Kit-Highly Sensitive DCFH-DA, and the fluorescence intensity of intracellular ROS was compared between two detection kits. As a result, the ROS Assay Kit-Highly Sensitive DCFH-DA in high-sensitivity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

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

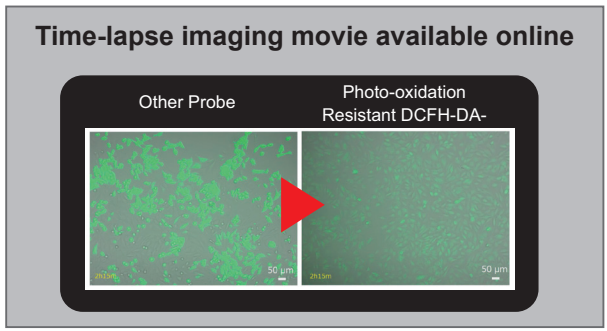
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-

The dye used in this kit allows ROS detection with higher sensitivity than DCFH-DA and long-term observation of live cells due to its resistance to photooxidation.

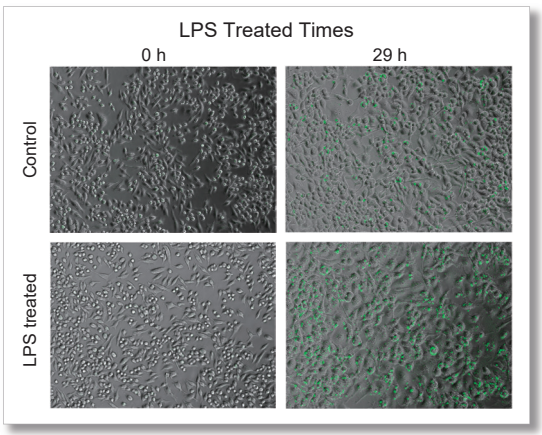
Resistant to Photo-oxidation

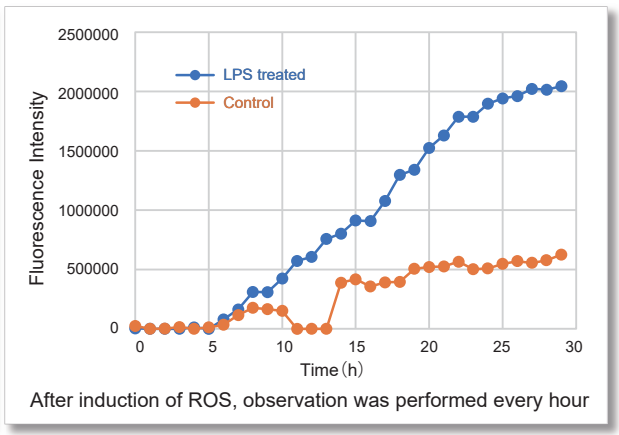


Comparison of photo-oxidation resistant ability in HeLa cells



Experimental Example: Simultaneous Detection of ROS in LPS-treated macrophages





After induction of ROS, observation was performed every hour

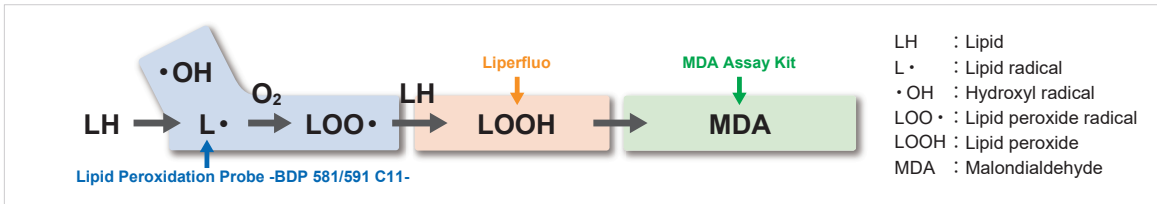
In Lipopolysaccharide (LPS) treated RAW 264.7 cells, after being stained with regular DCFH-DA, Highly Sensitive DCFH-DA, or Photo-oxidation Resistant DCFH-DA, the intracellular ROS level was compared.

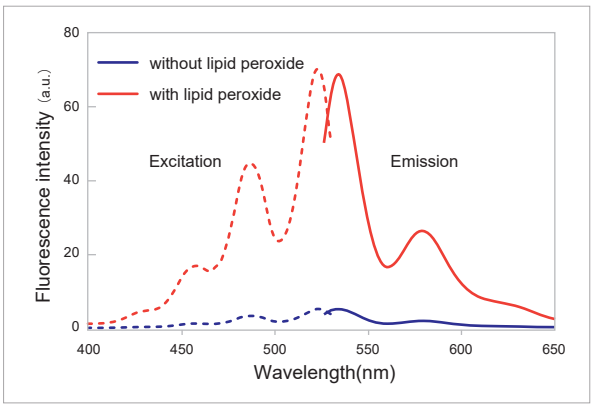
Description	Unit	Code
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	100 tests	R253-10

Lipid Peroxide Detection

Liperfluo

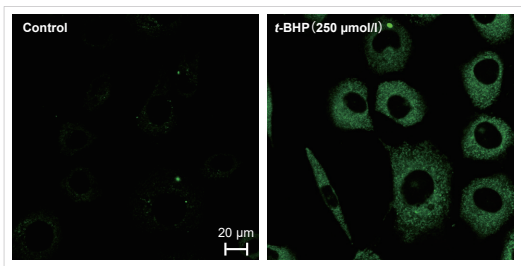
Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and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

Experimental Example: 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.

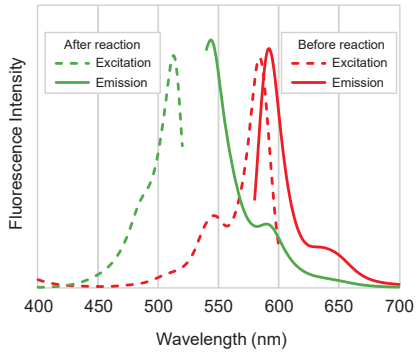
Cell line: L929
Microscope: Zeiss LSM510META
Filter type: FITC (GFP, Alexa488) wide filter
HFT UV/488
NFT490
BP505-550

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

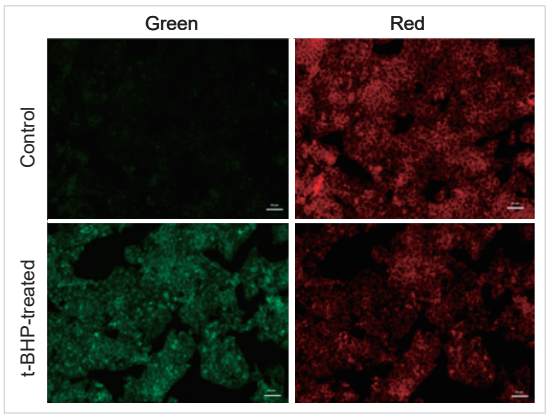
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

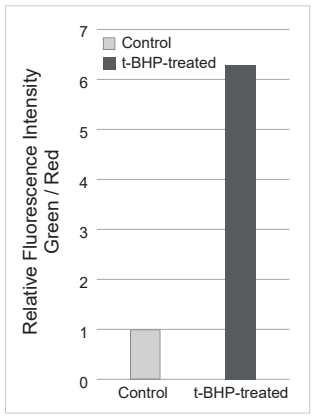
Lipid Peroxidation Probe -BDP 581/591 C11-

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.



Experimental Example: 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 µm

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

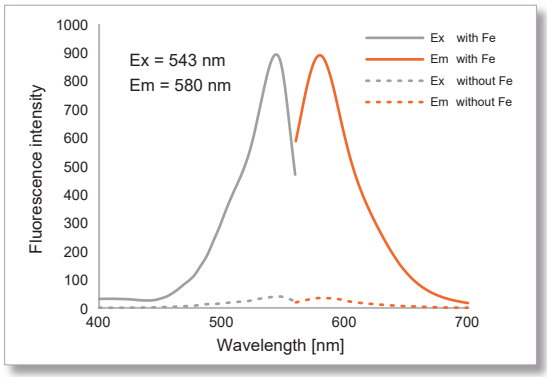
HepG2 cells stained with this probe were stimulated with HBSS solution containing 200 µmol/l *t*-BHP for 2 hours, and the fluorescence intensity was compared with control cells. As a result, a decrease in red fluorescence and an increase in green fluorescence were observed with high sensitivity in *t*-BHP-treated 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.

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

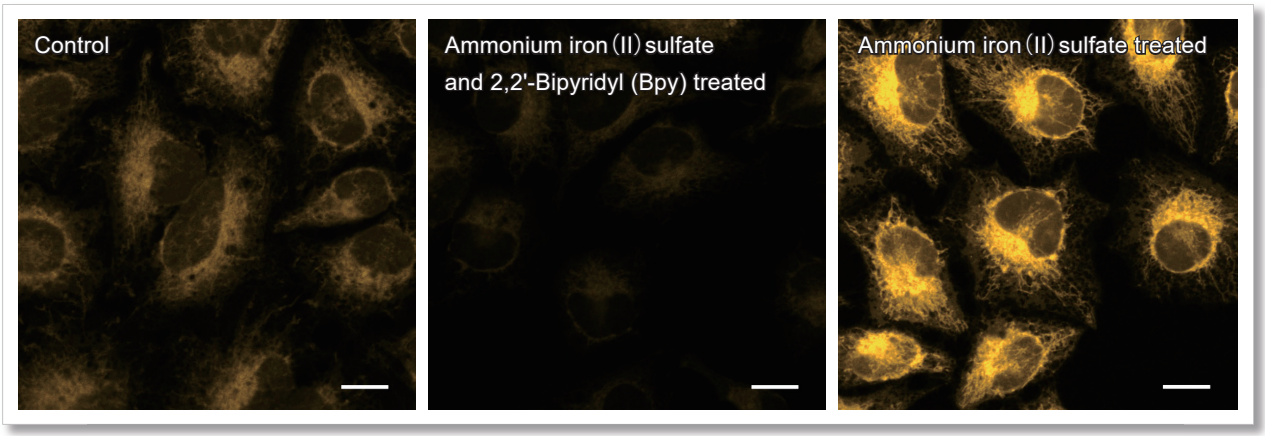
Intracellular Iron Ion Measurement

FerroOrange

FerroOrange is a specific probe for ferrous ions (Fe^{2+}) and reacts irreversibly, which differs from the detection principle of chelating ability. Fluorescence imaging or quantification of intracellular Fe^{2+} is often used to evaluate iron-related non-apoptotic cell death, ferroptosis.



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

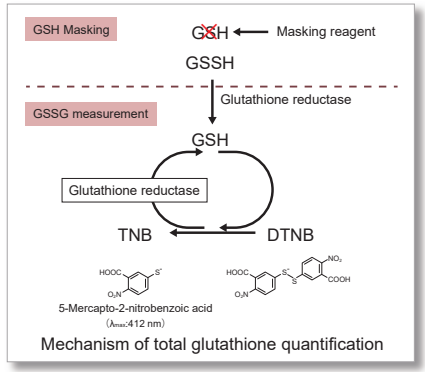
Description	Unit*	Code
FerroOrange	1 tube	F374-10
	3 tube	F374-12

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

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

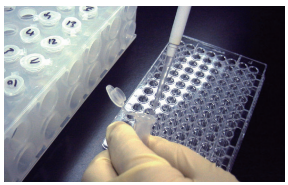
GSSG/GSH Quantification Kit

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.

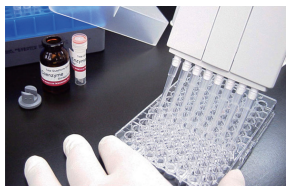


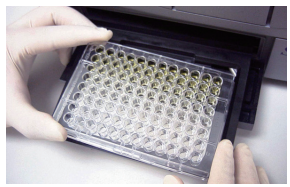
Mechanism of total glutathione quantification

Experimental Procedure: Experimental time is only 1-2 hours

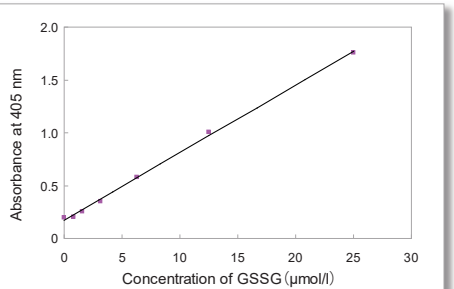

1) GSSG/GSH Standard Solution and add Sample A or Sample B to each well.
2) Add Buffer solution to each well


3) Incubate at 37℃ for 1 h.

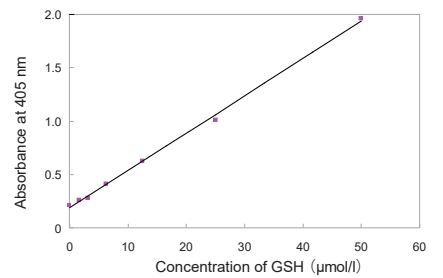

4)-5) Add substrate working solution and Enzyme/ Coenzyme working solution to each well.


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)

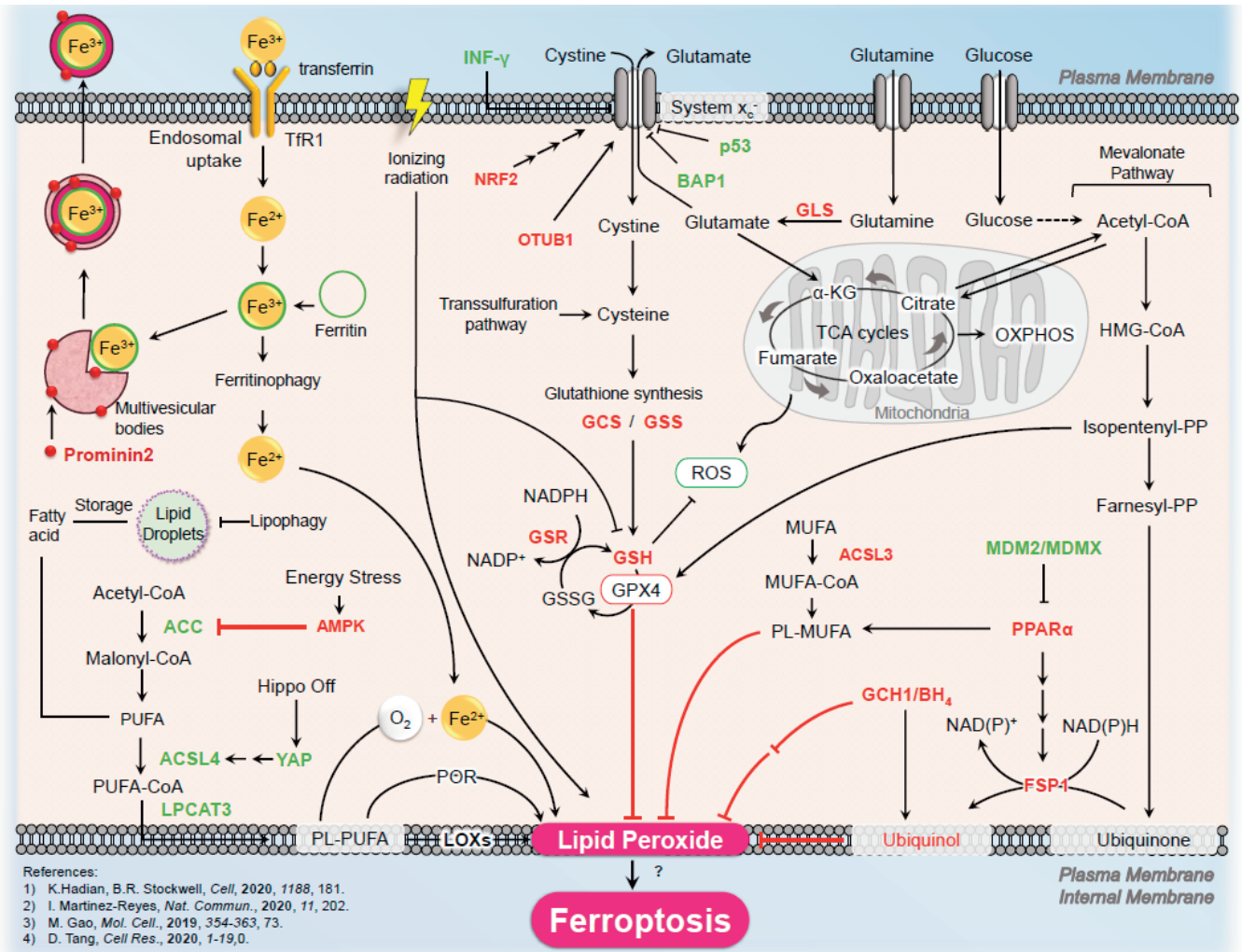
Description	Unit	Code
GSSG/GSH Quantification Kit	200 tests	G257-10

Related Products

Ferroptosis research

"Ferroptosis" was coined by Stockwell et al. at Columbia University in 2012 and described as a form of iron-dependent cell death. * It was reported to be a form of programmed cell death by the Nomenclature Committee on Cell Death (NCCD) in 2018.
Ferroptosis is a form of programmed cell death caused by iron ion-dependent accumulation of lipid peroxides. Ferroptosis has been shown to follow a different cell death pathway from apoptosis and thus is attracting attention as a new target for cancer therapy. It has also been found to be associated with various diseases, such as neurodegenerative diseases, cerebral apoplexy, and hepatitis (NASH).

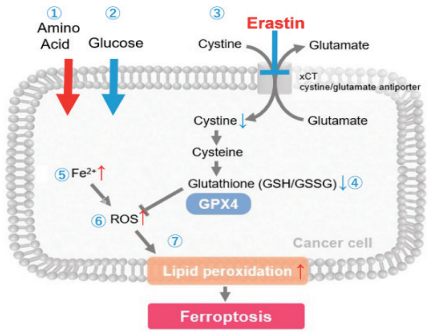
Ferroptosis Pathway



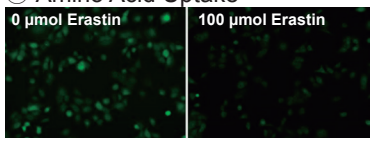
References:
1) K.Hadian, B.R. Stockwell, *Cell*, **2020**, *1188*, 181.
2) I. Martinez-Reyes, *Nat. Commun.*, **2020**, *11*, 202.
3) M. Gao, *Mol. Cell.*, **2019**, *354-363*, 73.
4) D. Tang, *Cell Res.*, **2020**, *1-19*,0.

Experimental Example: Evaluating Intracellular Uptake and Redox Balance

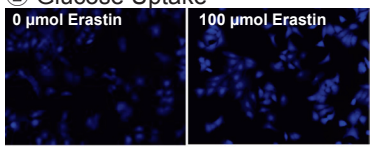
We investigated the transition of cellular metabolisms in A549 cells treated with Erastin, a known ferroptosis inducer. Our results revealed the following.



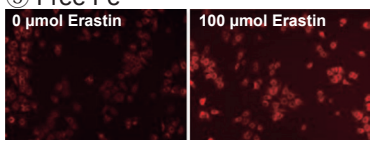
① Amino Acid Uptake



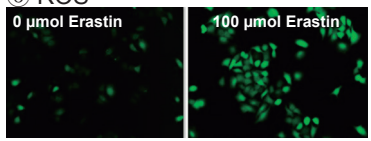
② Glucose Uptake



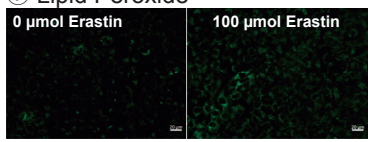
⑤ Free Fe^{2+}



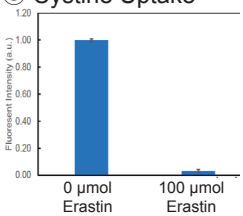
⑥ ROS



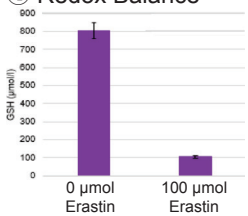
⑦ Lipid Peroxide



③ Cystine Uptake



④ Redox Balance



<Experimenta Condition>
A549 cells were treated with 100 μmol/l Erastin/MEM for 3 hours.

- The inhibition of cystine uptake by Erastin led to a depletion of cysteine, which in turn increased the compensatory uptake of other amino acids.
- Glucose uptake, which typically promotes ferroptosis, was found to decrease upon Erastin treatment, suggesting a potential cellular self-defense mechanism.
- The depletion of cysteine resulted in a decrease in glutathione levels and an increase in Fe^{2+}, ROS, and lipid peroxides, all of which are recognized markers of ferroptosis.

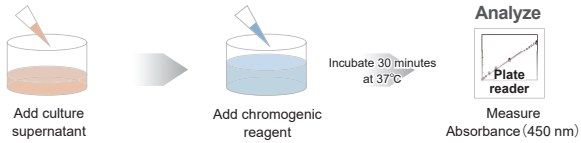
Description	Unit	Code
Lipidperoxide / Ferros Ion Detection		
Liperfluo (Used in Experiment ⑦)	50 μg x 5	L248-10
Lipid Peroxidation Probe -BDP 581/591 C11-	200 tests	L267-10
FerroOrange (Used in Experiment ⑤)	1 tube 3 tubes	F374-10 F374-12
Mito-FerroGreen	1 set (50 μg x 2)	M489-10
Oxidative Stress Detection		
ROS Assay Kit -Highly Sensitive DCFH-DA- (Used in Experiment ⑥)	100 tests	R252-10
GSSG/GSH Quantification Kit (Used in Experiment ④)	200 tests	G257-10
MDA Assay Kit	100 tests	M496-10
Metabolism Assay Kit		
Amino Acid Uptake Assay Kit (Used in Experiment ①)	20 tests 100 tests	UP04-10 UP04-12
Glucose Uptake Assay Kit (Used in Experiment ②)	1 set (100 tests)	UP01-10 (Blue) UP02-10 (Green) UP03-10 (Red)
Cystine Uptake Assay Kit (Used in Experiment ③)	20 tests 100 tests	UP05-10 UP05-12
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10

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.

<table>
<tr><th>Determination index</th><th>Detection</th><th>Operation</th></tr>
<tr><td rowspan="2">Glucose
Lactate
Glutamine
Glutamate
NAD/NADH
NADP/NADPH</td><td rowspan="2">Colorimetric</td><td>Simply transfer the culture supernatant to a plate and mix it with the chromogenic reagent
 
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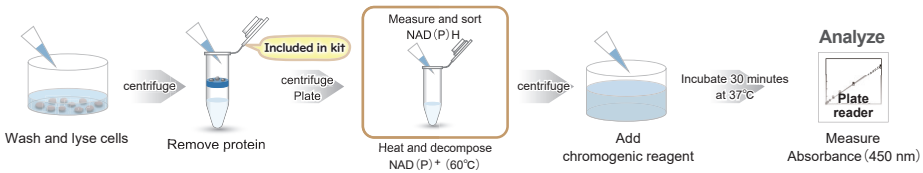
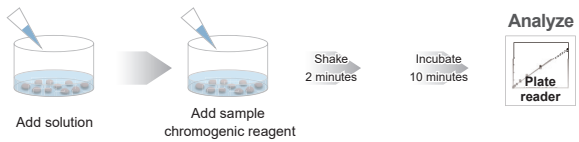
<tr><td> 
</td></tr>
<tr><td>ATP
ADP/ATP</td><td>Luminescent</td><td>Kit includes ATP standard - very easy to use
 
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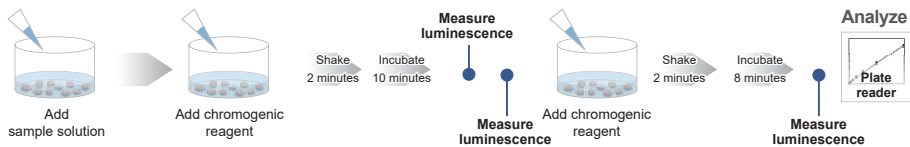
<tr><td colspan="2"> 
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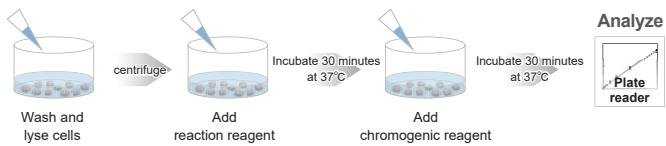
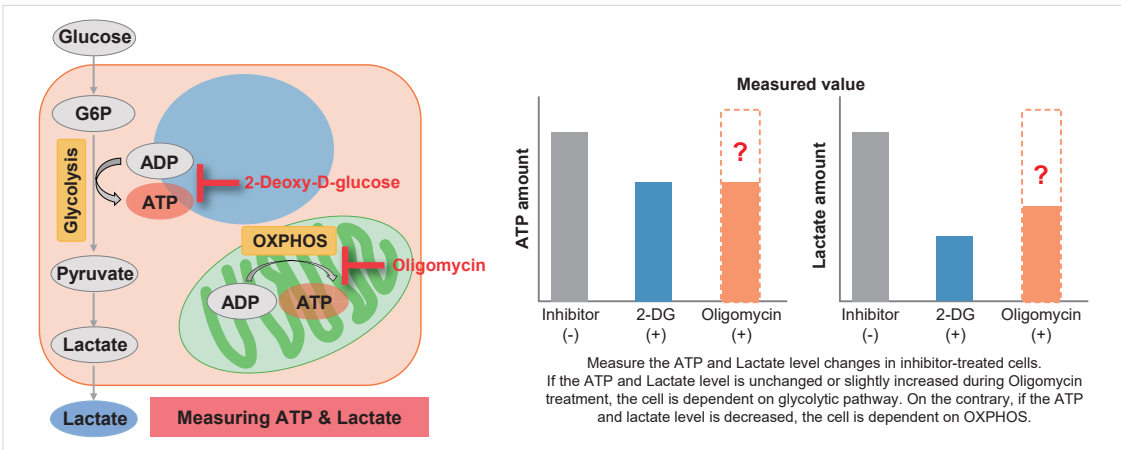
<tr><td>α-ketoglutaric acid</td><td>Fluorescent</td><td>Less variable results than existing assays
 
</td></tr>
</table>

Intracellular Metabolism

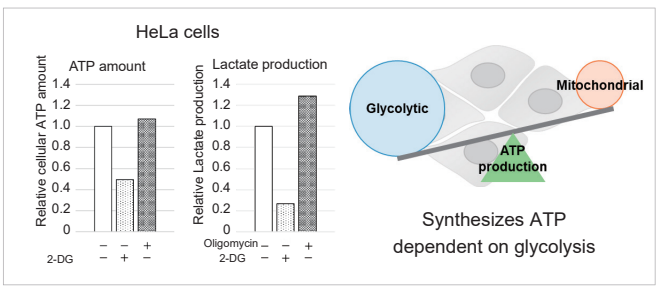
Glycolysis/OXPHOS Assay Kit

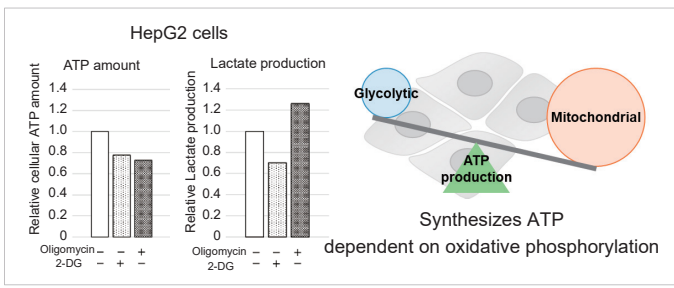
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

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



Experimental Example:
Comparison of metabolic pathway dependence in different cell line



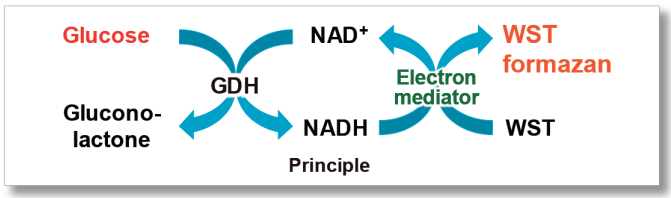


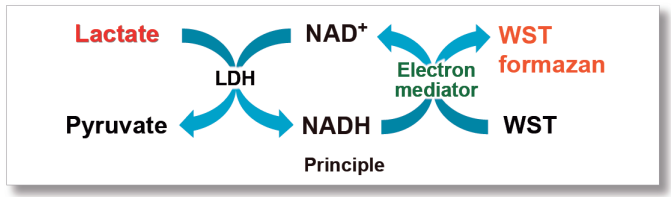
Description	Unit	Code
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10

Glucose Assay Kit-WST
Lactate Assay Kit-WST

G264 L256

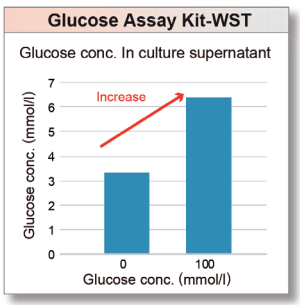
The Glucose Assay Kit-WST and Lactate Assay Kit-WST are colourimetric kits for the quantification of glucose and lactate, respectively, both with a lower detection limit of 0.02 mmol/L. These two indicators are crucial for understanding glycolytic metabolism and are among the most commonly measured parameters in metabolic studies. In addition, the use of Dojindo's proprietary WST dye in the assay systems allows for easy and highly accurate detection.

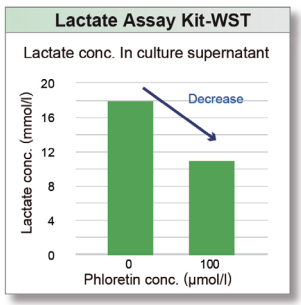




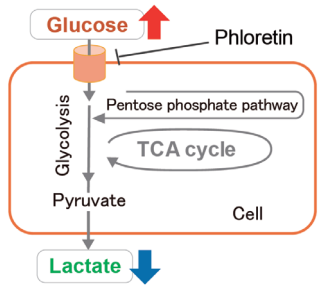
Experimental Example: Evaluation of culture supernatant using two indicators

Phloretin, the glucose transporter inhibitor, was added to Jurkat cells and the intracellular metabolism change was evaluated using Glucose Assay Kit-WST and Lactate Assay Kit-WST.





<Experimental condition>
Cell Line: Jurkat cells ($5x10^5$ cells)
Stimulation condition:
Phloretin (final conc.: 100 μmol/l), overnight incuvation
Sample: Culture supernatant"



<Description>
Glucose consumtion has decreased due to inhibition of glucose uptake by Phloretin, resulting in increase of glucose and decrease in lactate in culture supernatant.

Description	Unit	Code
Glucose Assay Kit-WST	50 tests 200 tests	G264-05 G264-20
Lactate Assay Kit-WST	50 tests 200 tests	L256-10 L256-20

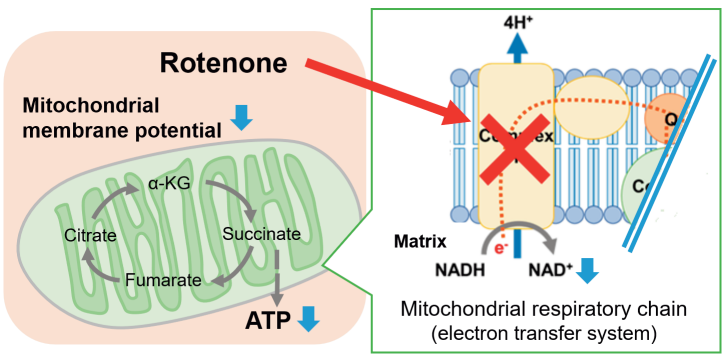
Intracellular Metabolism

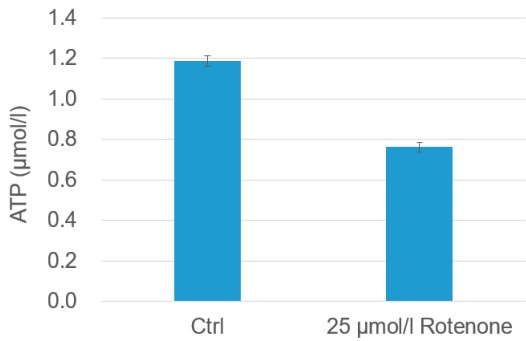
ATP Assay Kit-Luminescence

ATP Assay Kit-Luminescence is a luciferase luminescence assay kit for quantification of intracellular ATP. ATP is an important energy source of living cells that is synthesized in both glycolysis and mitochondrial oxidative phosphorylation. Mitochondrial dysfunction reduces ATP levels in cells and the decreased ATP levels are known to be associated with cancer, aging, and neurodegenerative diseases. Therefore, ATP level is used as an indicator for mitochondrial activity. ATP level is a lso focused in Cancer research since the recent studies have revealed that although cancer cells were known to rely on glycolysis for ATP synthesis, a shift from glycolysis to oxidative phosphorylation occurs when glycolysis is suppressed.

Experimental Example: Change in intracellular metabolism of rotenone-treated cells

Rotenone, which is known to inhibit the mitochondrial electron transport chain, was added to Jurkat cells, followed by measurement of intracellular ATP using the ATP Assay Kit-Luminescence. As a result, ATP production in the mitochondrial respiratory chain (the electron transport chain) was inhibited, and ATP concentrations were lower than those in the control cells.



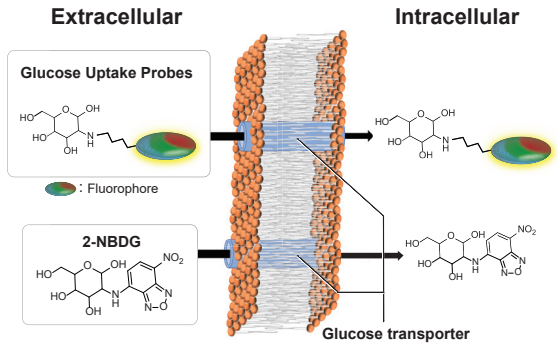


Description	Unit	Code
ATP Assay Kit-Luminescence	50 tests 200 tests	A550-10 A550-12

Glucose Uptake Assay Kit

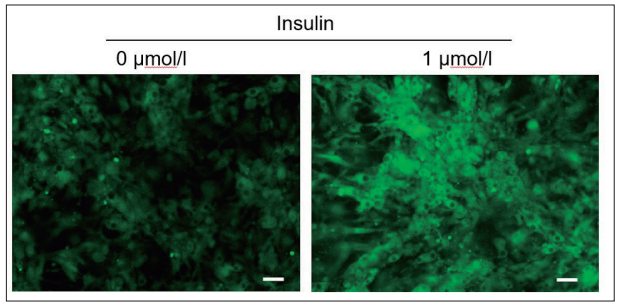
- Highly sensitive and simple measurement of glucose uptake capacity
- Applicable for microscopy & FCM
- Reduces dye leakage after staining

Principle



Experimental Example

Glucose uptake enhancement by insulin



Detailed results and other data are available online.

Comparison with Existing Method

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

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

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

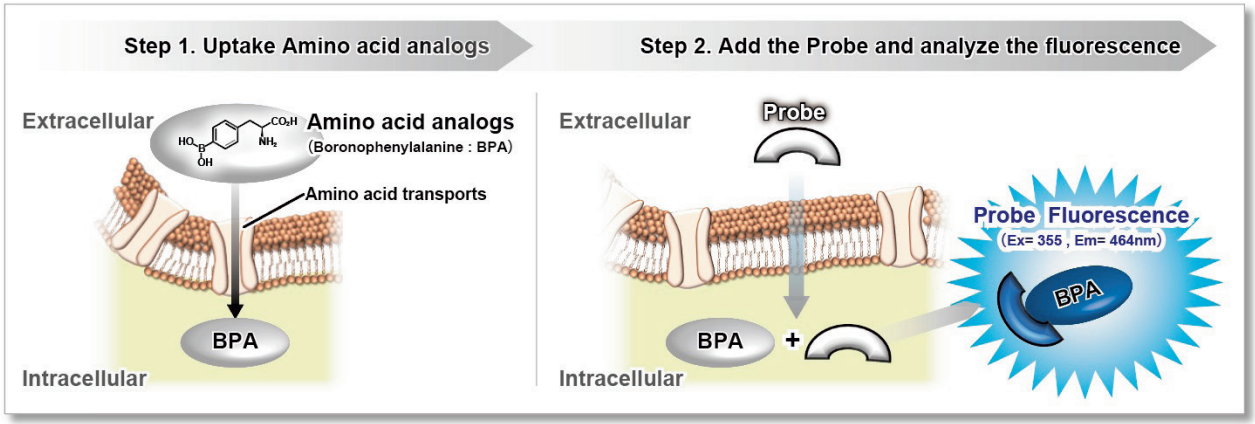
Description	Unit*	Code
Glucose Uptake Assay Kit-Blue	1 set	UP01-10
Glucose Uptake Assay Kit-Green	1 set	UP02-10
Glucose Uptake Assay Kit-Red	1 set	UP03-10

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Intracellular Metabolism

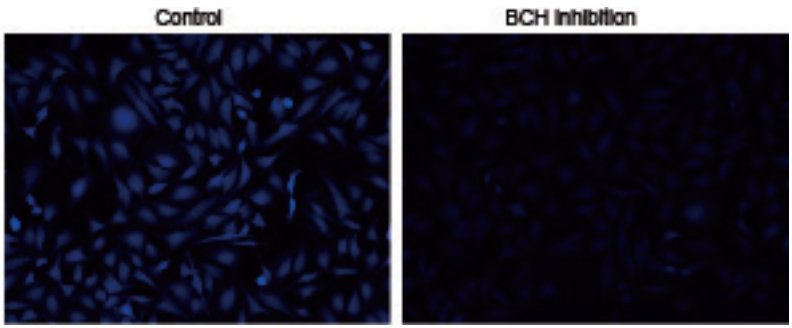
Amino Acid Uptake Assay Kit

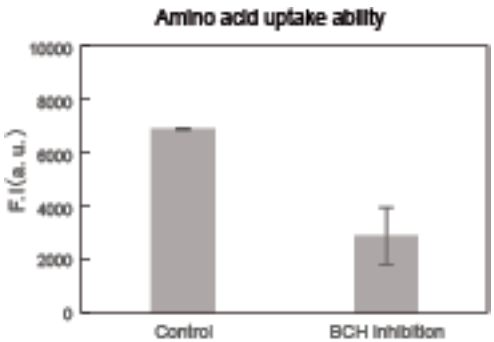
Principle



After amino acid analogs (BPA) are taken up into cells via amino acid transporters, the fluorescent probe permeates the cell membrane and binds to the amino acid analogs, emitting fluorescence

Experimental Example: Evaluation of BCH (Inhibitor of amino acids transporter)





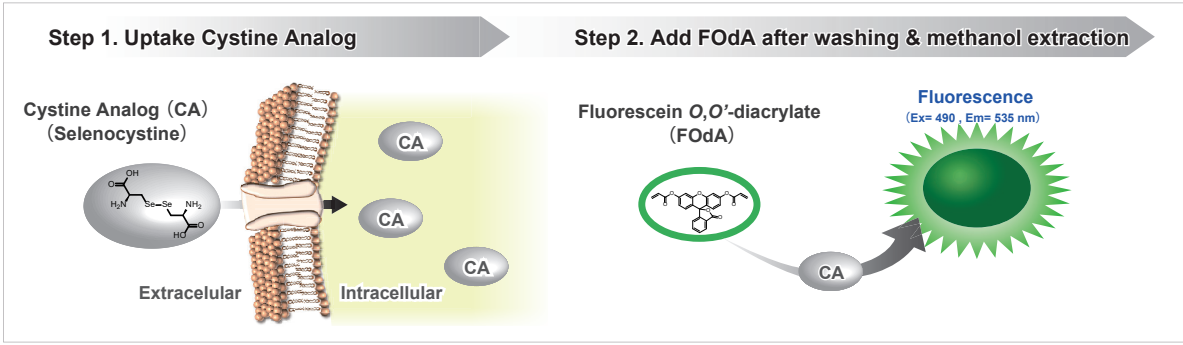
Experiment Condiitons
Cell Line: HeLa cells
Medium: MEM (5.5 mmol/l Glucose)
Incubation: 1 mmol/l BCH/HBSS (Hanks' Balanced Salt Solution), 37°C, 30 min
Instrument: Fluorescent Microscopy (Ex=340-380 nm, Em: 435-485 nm)
Instrument: Plate Reader (Ex=360 nm, Em: 460 nm)

Description	Unit	Code
Amino Acid Uptake Assay Kit	20 tests 100 tests	UP04-10 UP04-12

Cystine Uptake Assay Kit

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

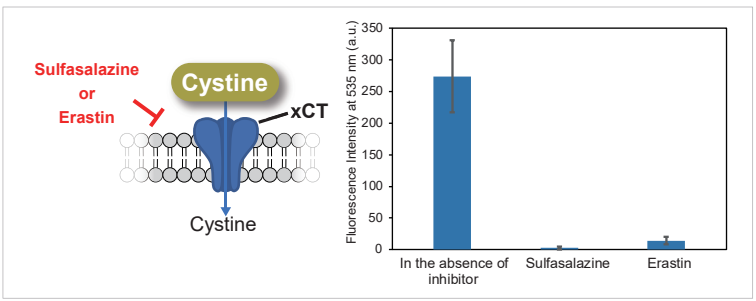
Principle



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]

Experimental Example: 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

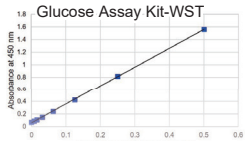
Live-cell fluorescent imaging of intracellular Fe^{2+}

Mitophagy Detection

Mitophagy Detection Kit

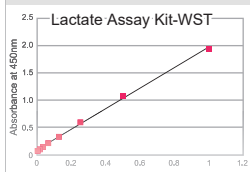
Live-cell fluorescent imaging of mitophagy without transfection

Measurement of Glucose



Measurement of intracellular glucose concentrations via fluorescence

Measurement of Lactate

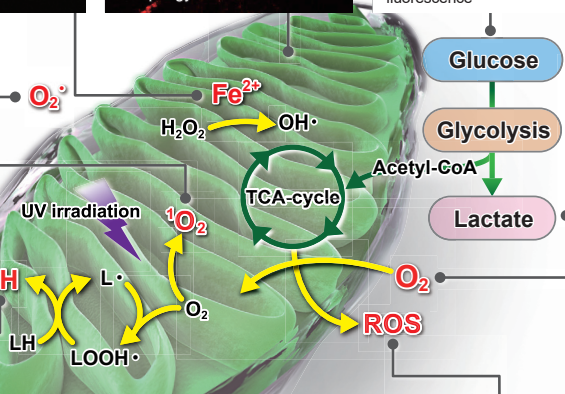


Measuring lactate to infer glycolytic activity

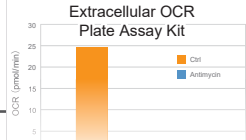
Singlet Oxygen Detection

Si-DMA for Mitochondrial Singlet Oxygen Imaging

Real-time visualization of $^1O_2$ generation



Oxygen consumption rate (OCR) Detection



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

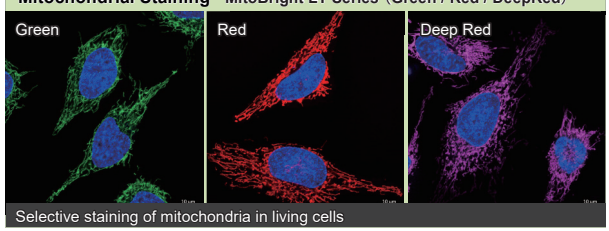
Monitoring and observation even after fixation, with more sensitive detection than JC-1

Total ROS Detection

ROS Assay Kit -Highly Sensitive DCFH-DA-

Detection with higher sensitivity than the original DCFH-DA

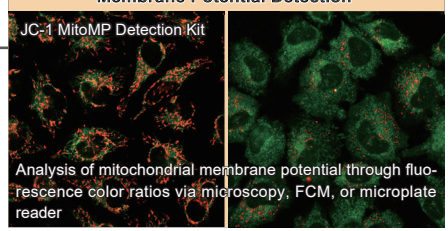
Mitochondrial Staining MitoBright LT Series (Green / Red / DeepRed)



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

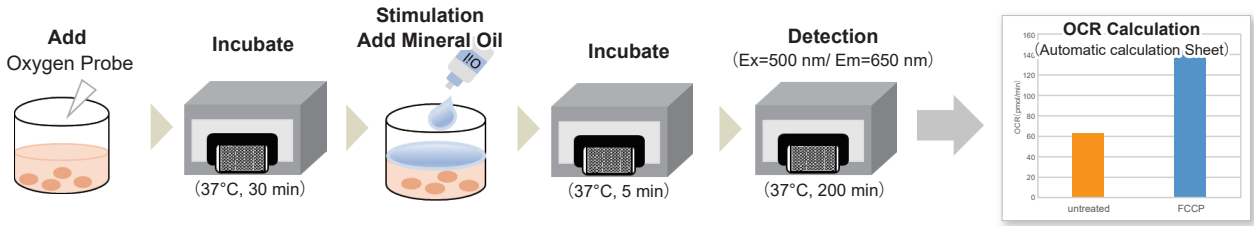
Extracellular OCR Plate Assay Kit

- 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

Oxygen Probe　Mineral Oil

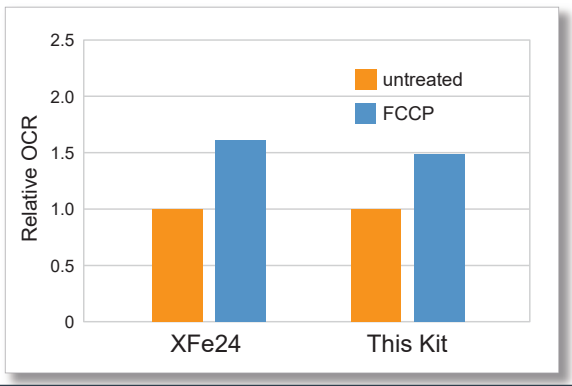
Procedure



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 300 tests	E297-10 E297-12

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

MT09 MT13

JC-1 indicates mitochondrial condition by changing from red to green fluorescence as mitochondrial membrane potential (MMP) decreases. While JC-1 and similar dyes such as TMRE and TMRM are popular for MMP detection, they suffer from low photostability and poor retention. Dojindo's MT-1 MitoMP Detection Kit overcomes these limitations and improves experimental reproducibility.

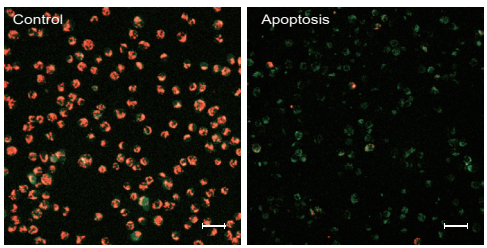
Comparison of Reagents

	Features	Sensitivity	Fixation	Monitoring	Fluorescence change (upon loss of mitochondrial membrane potential)	Detection (ex/em)
JC-1 (JC-1 MitoMP Detection Kit)	Recomended for starting-up	✓			Color change from red to green	Green: 450-490 nm / 500-550 nm Red: 530-560 nm / 570-640 nm
MT-1 (MT-1 MitoMP Detection Kit)	Recommended for more detailed analysis	✓ (High)	✓	✓	Decrease in fluorescence intensity	530-560 nm / 570-640 nm
TMRE	Widely used	✓ (High)			Decrease in fluorescence intensity	530-560 nm / 570-640 nm

Experimental Example

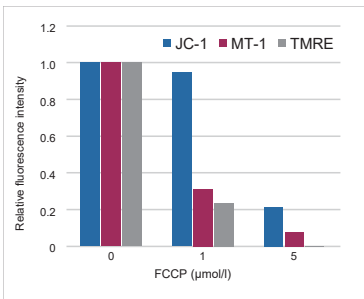
JC-1

Detection of MMP in Apoptotic Cells

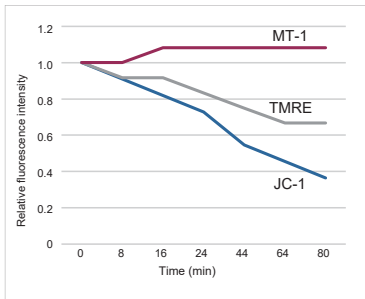


MT-1

High Sensitivity Detection



Allow to monitor MMP



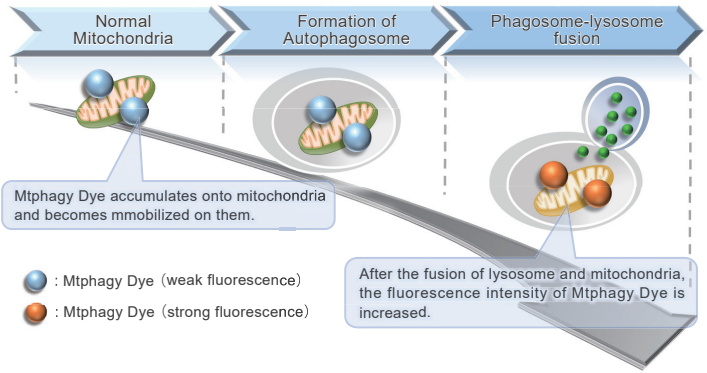
Description	Unit*	Code
JC-1 MitoMP Detection Kit	1 set	MT09-10
MT-1 MitoMP Detection Kit	1 set	MT13-10

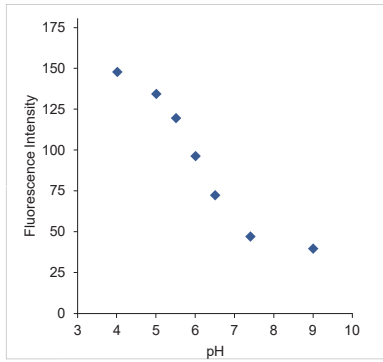
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

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.





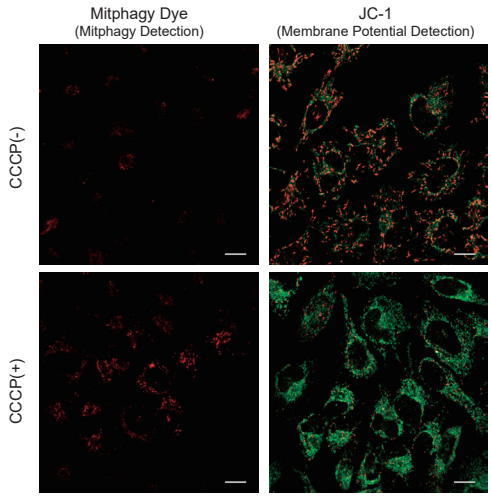
The fluorescent intensity of Mtphagy Dye is incresased at pH 4-5.

Experimental Example

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).

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

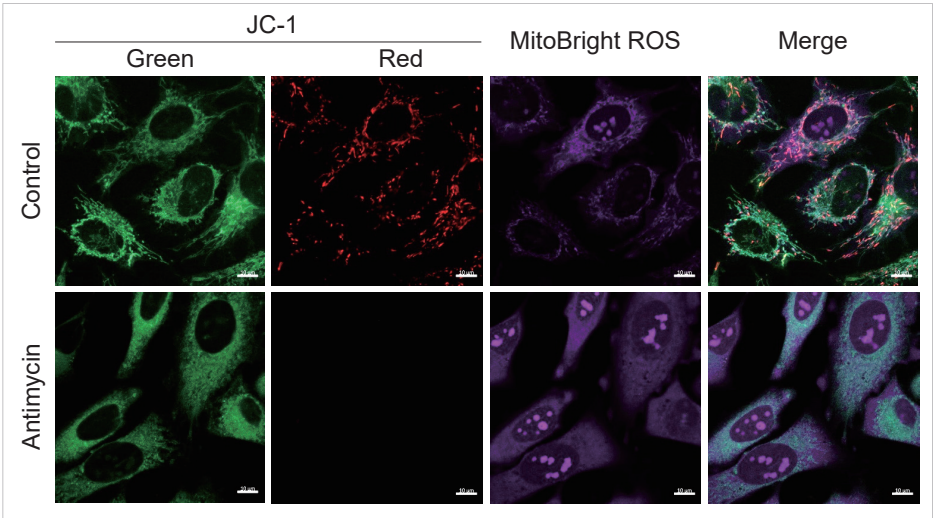
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

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 × 1 100 nmol × 3	MT16-10 MT16-12

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

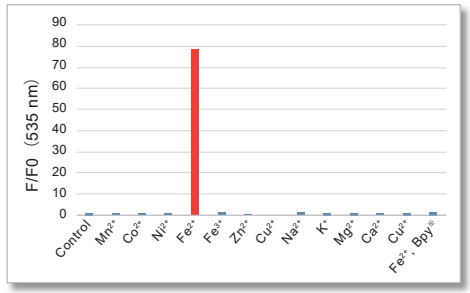
Mitochondrial Superoxide Detection

Mito-FerroGreen

M489 F374

Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion (Fe^{2+}) in mitochondria. Mito-FerroGreen has 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.

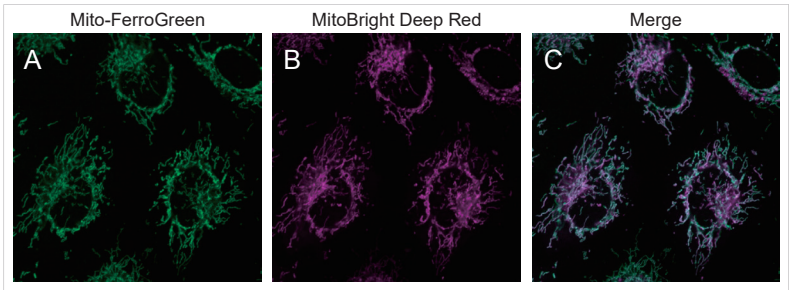
Metal ion Selectivity



※ Bpy : 2,2′ -Bipyridyl

Experimental Example

Double staining with mitochondrial staining probe



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

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

Selection Guide of 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

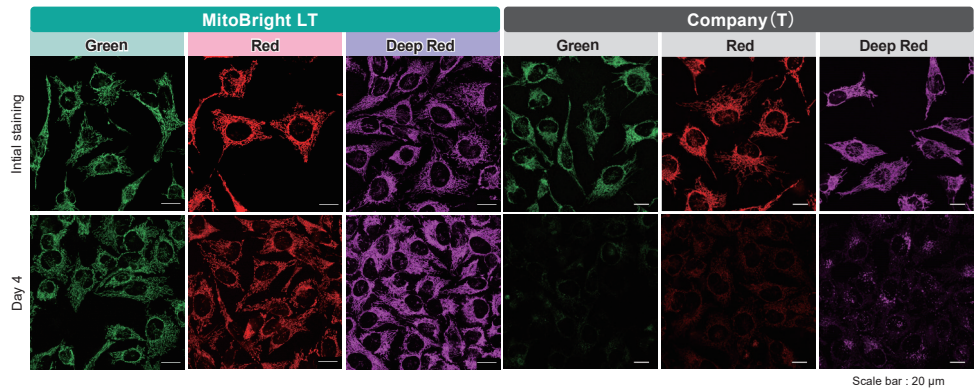
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

MitoBright LT Series

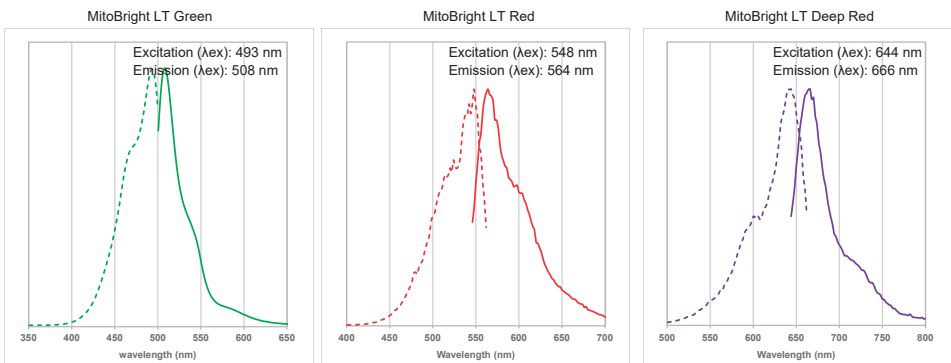
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color options (Green, Red and Deep Red), and are provided as a ready-to-use DMSO solution.

Experimental Example: 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 4 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

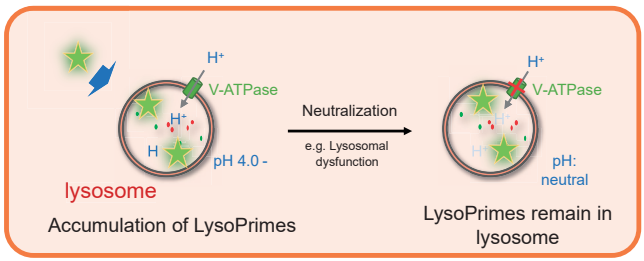
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Lysosomal Analysis

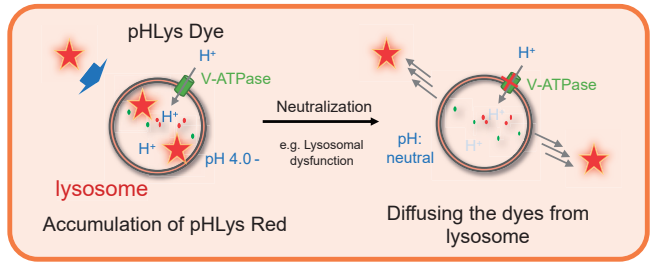
LysoPrime Green / Deep Red - High Specificity and pH Resistance
pHLys Red - Lysosomal Acidic pH Detection

L261 L264 L265

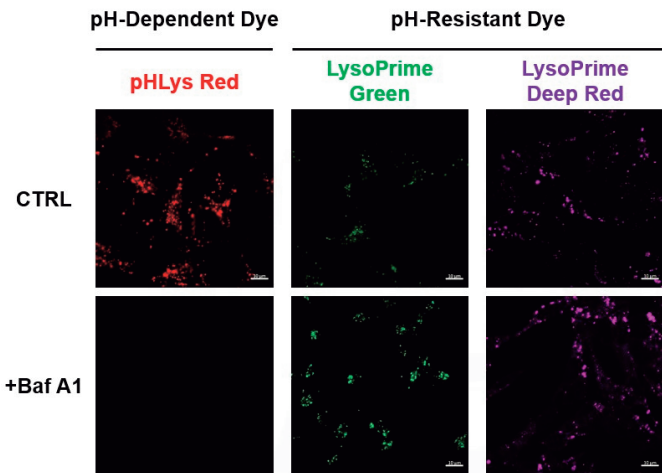
pH-resistant Probe
LysoPrime Green / Deep Red



pH-dependent Probe
pHLys Red

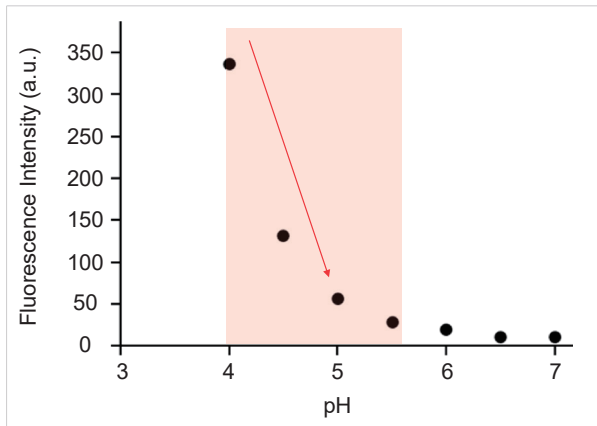


Resistance to pH changes



pHLys Red is highly specific to lysosomes and shows pH-dependent changes in fluorescence, and pH-resistant LysoPrime Green/Deep Red is retained in lysosomes even after adding Bafilomycin A1, a lysosomal acidity inhibitor. The lysosomal pH and mass of the same sample can be measured using these two dyes for a detailed analysis of lysosomal function.

pH dependence of pHLys Red

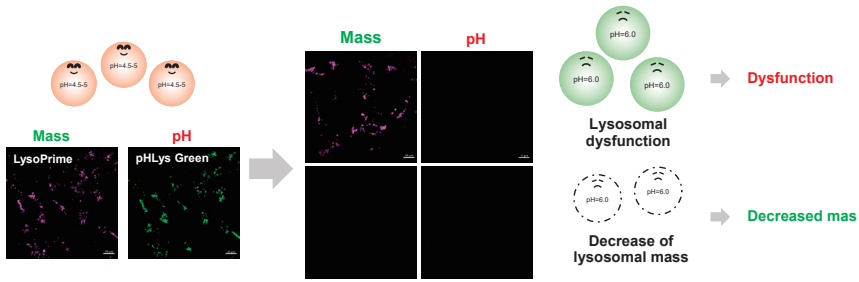


The fluorescence intensity of pHLys Red at each pH was confirmed in vitro, and it was confirmed that the fluorescence intensity changed sensitively within the range of lysosomal pH (pH 4.0-5.5).

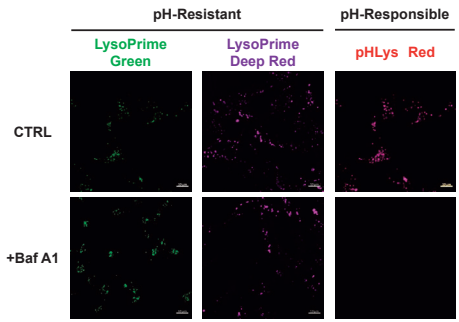
Lysosomal Acidic pH Detection Kit

L266 L268

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.

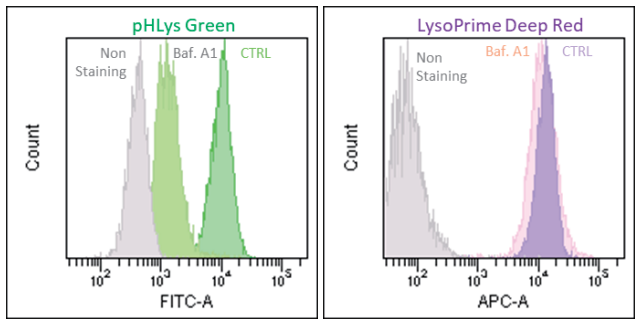


Imaging Analysis: Green/Red (#L266-10)



<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 10 µl × 3	L261-10 L261-12
LysoPrime Deep Red - High Specificity and pH Resistance	1 tube 3 tube	L264-10 L264-12
pHLys Red - Lysosomal Acidic pH Detection	1 tube 3 tube	L265-10 L265-12

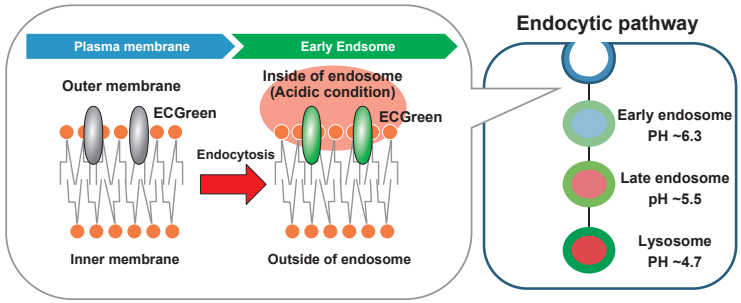
*1 Green/Red: combination of LysoPrime Green and pHLys Red, *2 Green/Deep Red: combination of pHLys Green and LysoPrime Deep Red
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Endocytosis

ECGreen-Endocytosis Detection

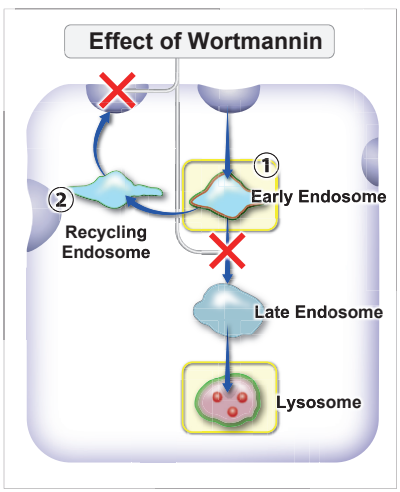
ECGreen-Endocytosis Detection is a pH dependent fluorescence dye that localizes to vesicle membrane. The visualization of endocytosis using the ECGreen is a more direct method than fluorescent analogs and allows visualization endocytosis from the stage of early endosomes.

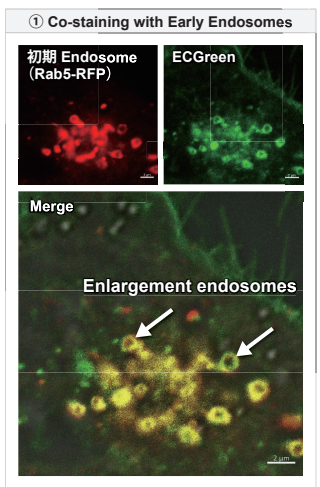
The detection mechanism of endocytosis

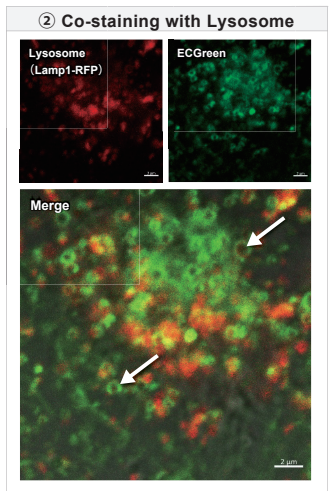


Clear visualization of intracellular vesicular trafficking

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 µl	E296-10

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

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_2-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).

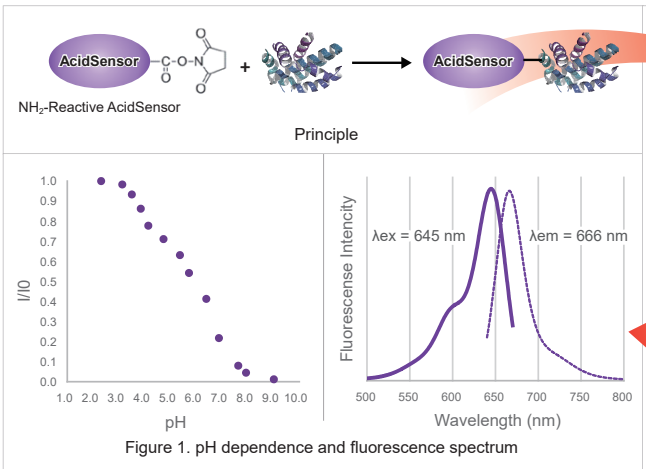


Figure 1. pH dependence and fluorescence spectrum

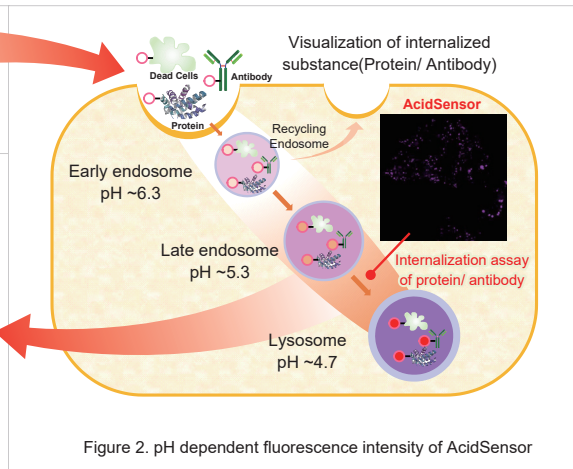
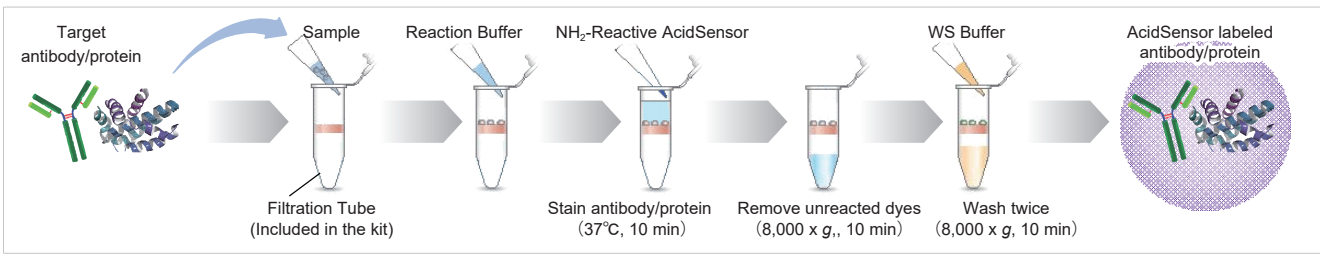


Figure 2. pH dependent fluorescence intensity of AcidSensor

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.

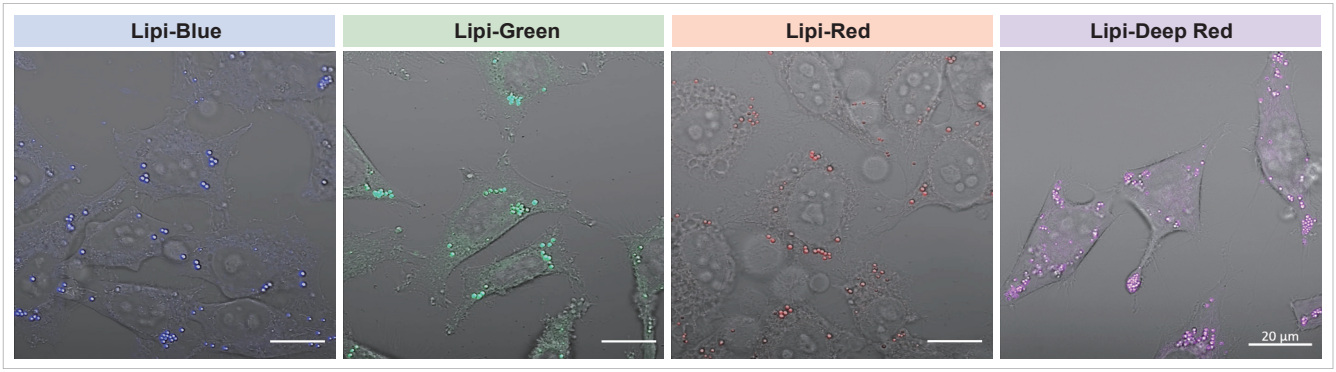


Description	Unit	Code
AcidSensor Labeling Kit – Endocytic Internalization Assay	3 samples	A558-10

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)

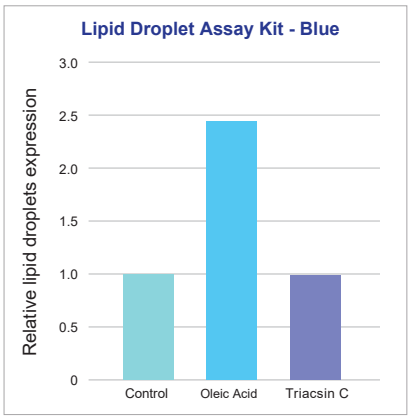
Description	Unit*	Code
Lipi-Blue	10 nmol × 1	LD01-10
Lipi-Green	10 nmol × 1	LD02-10
Lipi-Red	100 nmol × 1	LD03-10
Lipi-Deep Red	10 nmol × 1	LD04-10

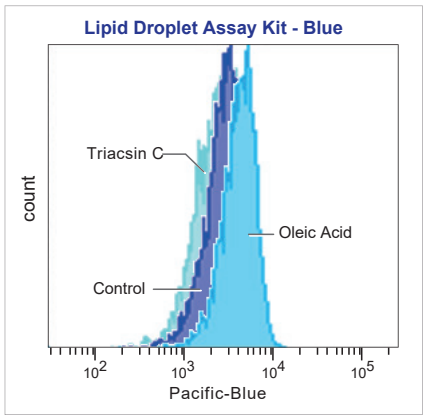
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

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 both live and fixed cells. Compared to colorimetric reagents, it reduces measuring time and increases experiment repeatability by avoiding dye deposition in the plate.

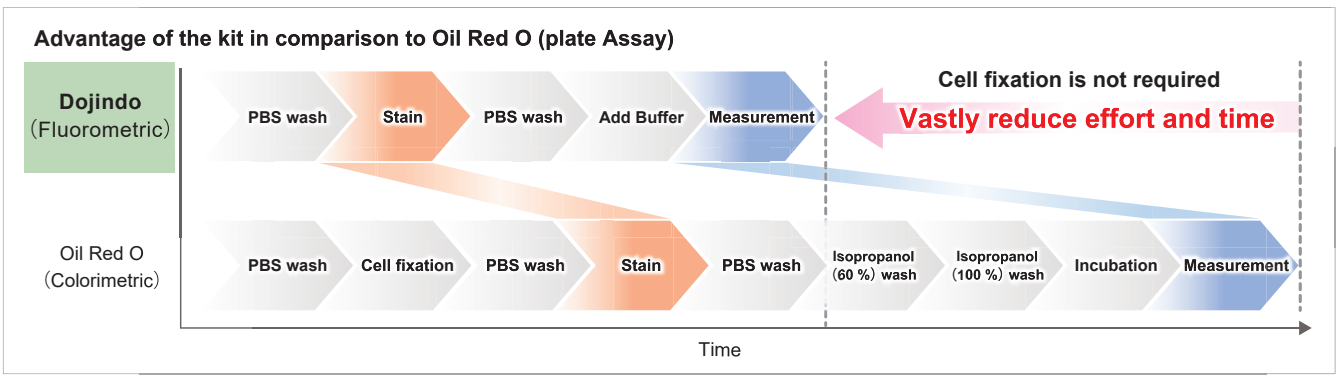
Experimental Example of plate assay and flow cytometry





Changes in lipid droplets were examined after the addition of oleic acid or Triacsin C (acyl-CoA synthetase inhibitor) to the A549 cell culture medium.

Advantage of the kit in comparison to Oil Red O (Plate Assay)



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

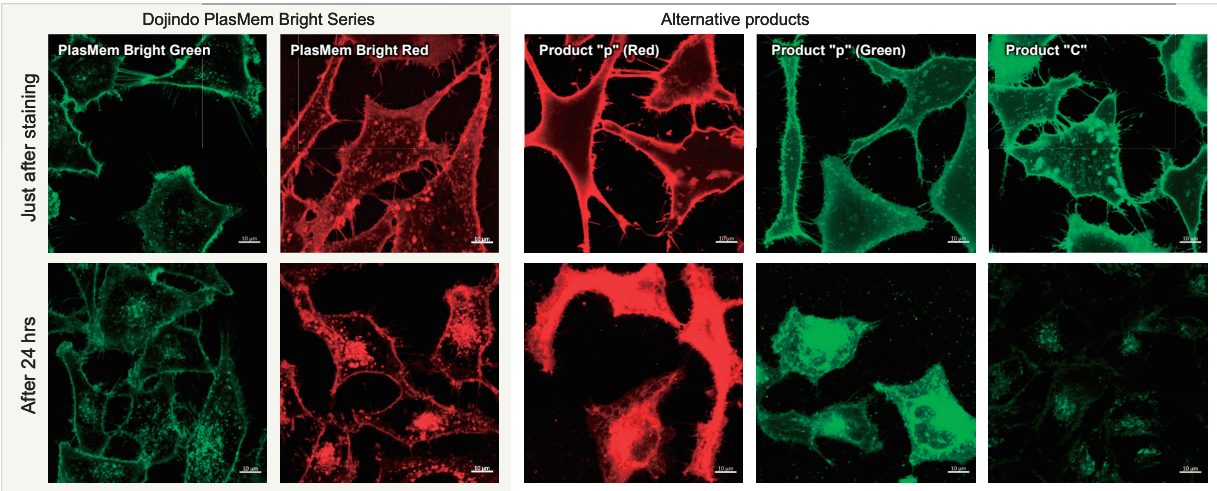
*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Cell Membrane Staining

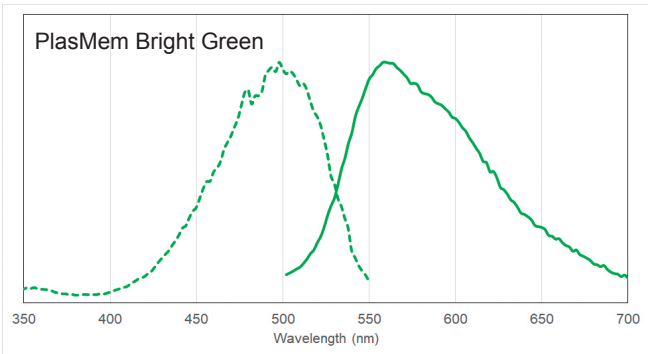
PlasMem Bright Green / Red

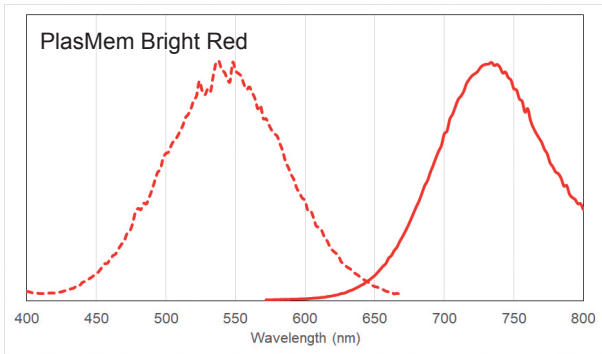
PlasMem Bright dyes are designed to stain plasma membrane for over a day. Furthermore, the PlasMem Bright dyes are more water-soluble compared with other commercially available dyes and can be diluted with culture medium. The PlasMem Bright dyes offer two different color options (green and red) and are provided as ready-to-use DMSO solutions.

Experimental Example: High retentivity on plasma membrane



Fluorescence Properties





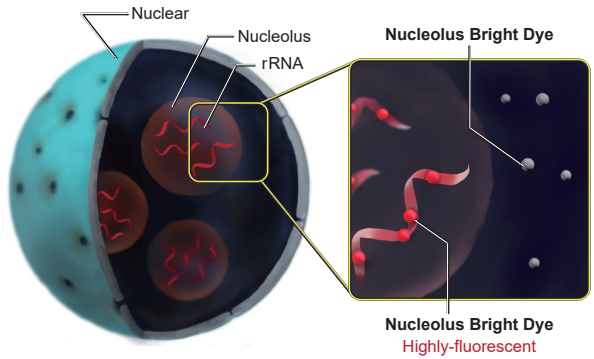
Description	Unit*	Code
PlasMem Bright Green	100 µl	P504-10
PlasMem Bright Red	100 µl	P505-10

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Nucleolus Bright Green / Red

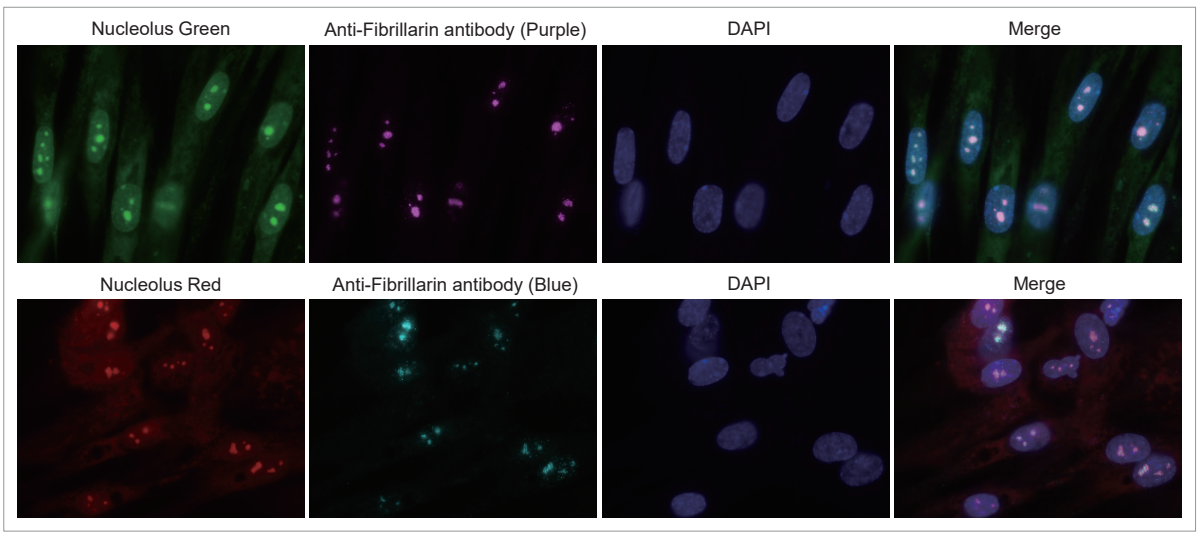
Detection Principle

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.



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

Nucleolus Localization



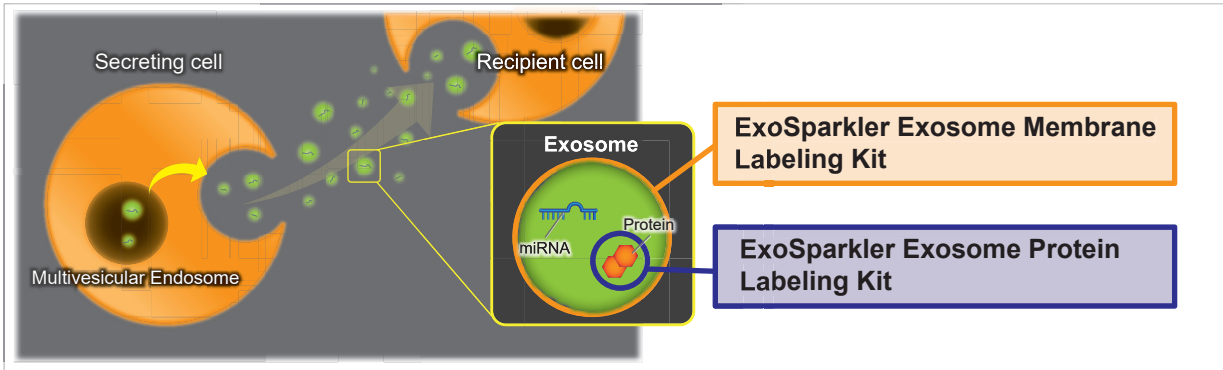
Description	Unit*	Code
Nucleolus Bright Green	60 nmol	N511-10
Nucleolus Bright Red	60 nmol	N512-10

*Approximate usage depends on the experiment. Please refer to the Q&A section on our product webpage.

Exosome Staining

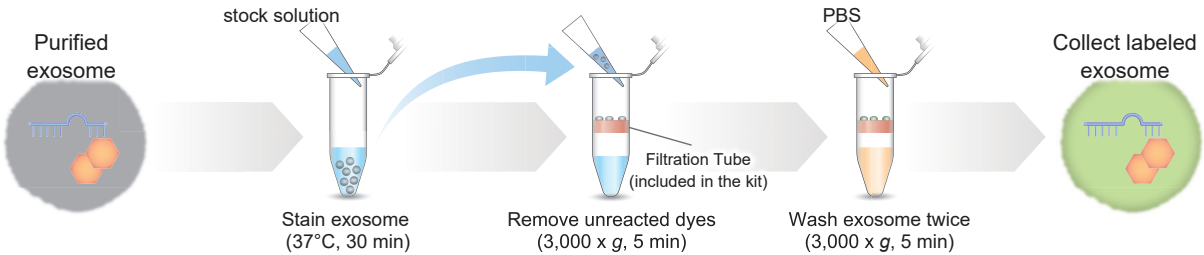
Exosome Labeling Kits

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

Staining procedure using ExoSparkler series

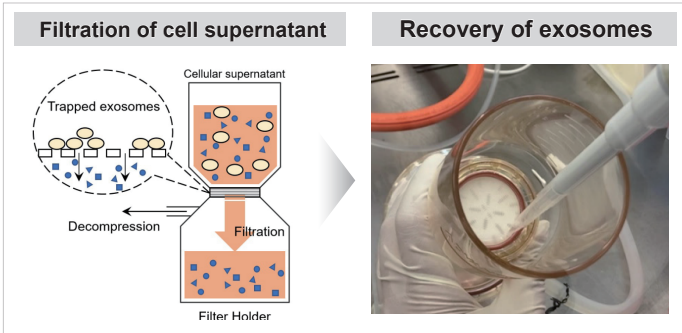


ExoSparkler series contains filtration tubes available for the removal of dyes unreacted after fluorescence labeling, as well as an optimized protocol for labeling exosomes. Our ExoSparkler series makes it possible to prepare fluorescence labeling of exosomes using the simple procedure.

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

Exo*Isolator* Exosome Isolation Kit

Easy to Use No Special Technique Required



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

Recovery Rate Equivalent to Ultracentrifugation

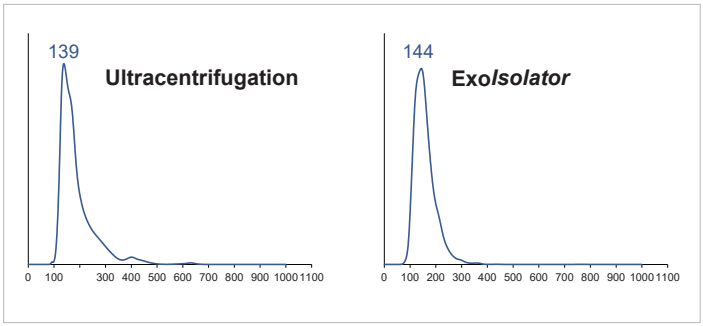


Fig. 1 Particle size distribution

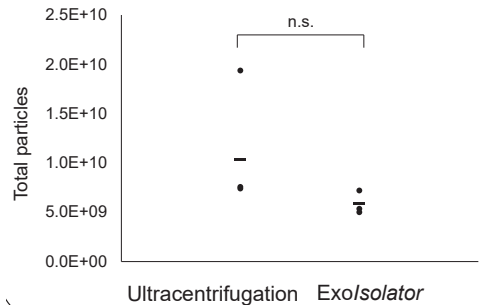


Fig. 2a The number of particles

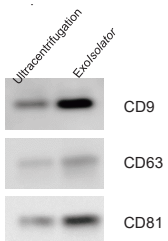


Fig. 2b Expression level of exosome markers

We isolated the exosomes from the supernatant of HEK293S using both the ultracentrifugation method and the Exo*Isolator* method.
The results showed that the Exo*Isolator* recovered exosomes with the same particle size distribution and the number of particles as the ultracentrifugation method, and the amount of exosome marker expressions per protein were higher, indicating that Exo*Isolator* recovered exosomes with higher purity than the ultracentrifugation method.

Description	Unit	Code
Exo*Isolator* Exosome Isolation Kit	3 tests	EX10-10
Exo*Isolator* Isolation Filter	10 pieces	EX11-10

For inquiries, please contact Dojindo Europe GmbH.

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