# Reagent for Cellular Function Analysis



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# **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-

Cancer			
Code	Product Name	Page	
MT09	JC-1 MitoMP Detection Kit	27	
MT10, MT11, MT12	MitoBright LT Series	31	
MT13	MT-1 MitoMP Detection Kit	27	
MT16	MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	29	
MD01	Mitophagy Detection Kit	28	
MT02	Mtphagy Dye	28	
E297	Extracellular OCR Plate Assay Kit	26	
A562	Autophagic Flux Assay Kit	11	
D675	DALGreen - Autophagy Detection	10	
D676	DAPGreen - Autophagy Detection	10	
D677	DAPRed - Autophagy Detection	10	
F374	FerroOrange	16	
L248	Liperfluo	14	
M489	Mito-FerroGreen	30	
UP05	Cystine Uptake Assay Kit	23	
L267	Lipid Peroxidation Probe -BDP 581/591 C11-	15	
G270	Glycolysis/OXPHOS Assay Kit	21	
A550	ATP Assay Kit-Luminescence	19	
L256	Lactate Assay Kit-WST	19	
R252	ROS Assay Kit -Highly Sensitive DCFH-DA-	12	
R253	ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	13	
G257	GSSG/GSH Quantification Kit	17	
G268	Glutamine Assay Kit-WST	19	
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	Neurodegenerative Diseases	
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MT10, MT11, MT12	MitoBright LT Series	31
MT13	MT-1 MitoMP Detection Kit	27
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L267	Lipid Peroxidation Probe -BDP 581/591 C11-	15
G270	Glycolysis/OXPHOS Assay Kit	21
A550	ATP Assay Kit-Luminescence	19
L256	Lactate Assay Kit-WST	19
L261	LysoPrime Green - High Specificity and pH Resistance	32
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	Senescence	
Code	Product Name	Page
SG03	Cellular Senescence Detection Kit - SPiDER-βGal	8
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MT10, MT11, MT12	MitoBright LT Series	31
MT13	MT-1 MitoMP Detection Kit	27
MT16	MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	29
MD01	Mitophagy Detection Kit	28
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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

Autophagy

# **Cell Proliferation / Cytotoxicity Assay**

# Cell Counting Kit-8 Cytotoxicity LDH Assay Kit-WST

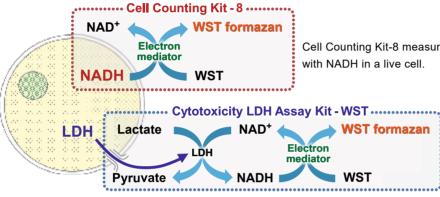


Cell Counting Kit-8



Cytotoxicity LDH Assay Kit-WST

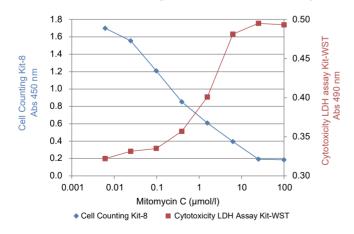
# **Detection Principle**



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<sub>2</sub> for 48 hours

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

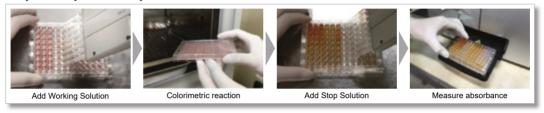
Cytotoxicity LDH Assay Kit-WST (490 nm)

# **Simple Procedure**

### · Cell Counting Kit-8



### · Cytotoxicity LDH Assay Kit-WST



### Same Samples can be used

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



Description	Unit	Code
	500 tests	CK04-05
Call Counting Vit 0	1000 tests	CK04-11
Cell Counting Kit-8	3000 tests	CK04-13
	10000 tests	CK04-20
	100 tests	CK12-01
Cytotoxicity LDH Assay Kit-WST	500 tests	CK12-05
	2000 tests	CK12-20

Oxidative Stress

### **Senescence Detection**

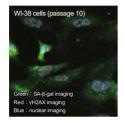
# Cellular Senescence Detection Kit - SPiDER-βGal Cellular Senescence Detection Kit - SPiDER Blue NEW

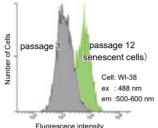


Cellular Senescence Detection Kit – SPiDER- $\beta$ Gal allows to detect SA- $\beta$ -gal with high sensitivity and ease of use. SPiDER- $\beta$ Gal is a new reagent to detect  $\beta$ -galactosidase which possesses a high cell-permeability and a high retentivity inside cells. SA- $\beta$ -gal are detected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

# SPiDER-βgal

### Compatible with quantitative analysis



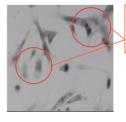


Compatible Instruments:

✓ Microscope ✓ Flow Cytometer ✓ Plate Reader

# X-Gal

### Difficult to quantify



Difficult to...

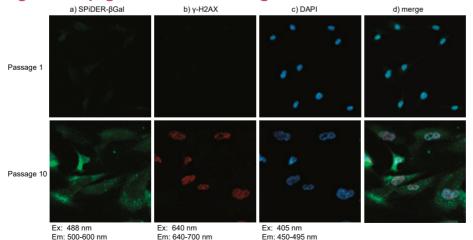
• count the cells

• difficult to distinguish / negative cells

Compatible Instruments: 

Microscope

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



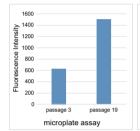
WI-38 cells were treated with anti- y-H2AX antibody and observed under a confocal microscope. The procedure involved several steps, including fixing the cells, permeabilizing, blocking, adding primary and secondary antibodies, staining with DAPI, and washing the cells. The experiment aimed to detect 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-\(\beta\)Gal

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

# **Correlation with Imaging Data**



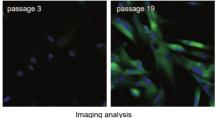


Plate Assav Ex. 535nm / Em. 580nm

### Imaging data

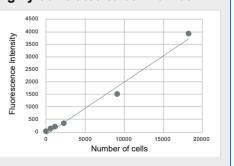
Green: Ex. 488nm / Em. 500-600nm (SA-β-Gal staining with Cellular Senescence Detection Kit – SPiDER-\(\beta\)Gal(Code SG04)) Blue: Ex. 405nm / Em. 450-495nm (Nuclear staining with -Cellstain- DAPI solution(Code D523))

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
Collular Sanasanaa Plata Assay Vit. SPiDER (Col.	20 tests	SG05-01
Cellular Senescence Plate Assay Kit - SPiDER-βGal	100 tests	SG05-05
Call Count Name alimation Vit	200 tests	C544-02
Cell Count Normalization Kit	1000 tests	C544-10

# **Autophagy**

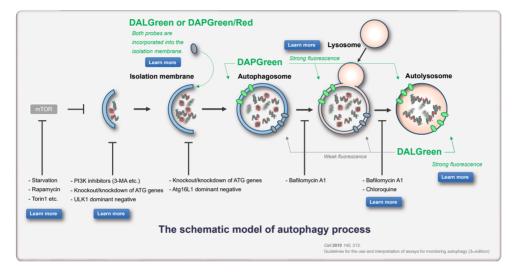
# **DAPGreen / Red - Autophagy Detection DALGreen - Autophagy Detection**







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**

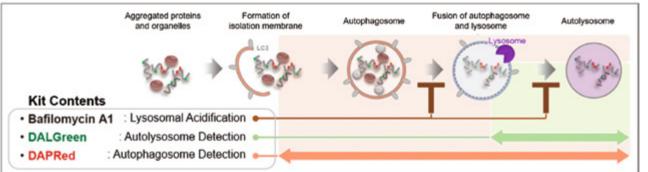
	Appl Fluorescent	licable instrum	ents Microplate	Fluorescent properties	Volume / the number of usable assays	Existing methods
	Microscope	cytometer	reader	p.opol.uod	asazis assays	
DAPGreen	✓	✓	✓	Ex = 425-475 nm Em = 500-560 nm * For confocal microscope,the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish: 25 (when used in 1.0 μmol/l)	LC3-GFP MDC
DAPRed	✓			Ex = 500-560 nm Em = 690-750 nm	5 nmol x 1 / 35 mm dish: 25 (when used in 1.0 μmol/l)	Cyto-ID etc.
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.

<sup>\*</sup>Double staining imaging by DAPGreen and DALGreen is not possible

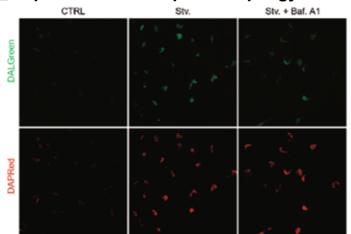


Stv. + Baf. A1

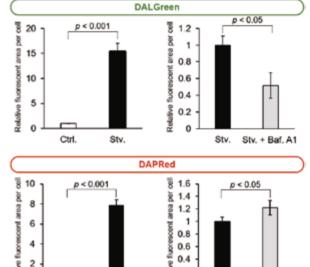
**Autophagic Flux Assay Kit** 



# 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.



0.2

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

00

### Oxidative Stress

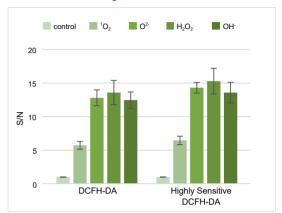
# **ROS Assay Kit -Highly Sensitive DCFH-DA-**



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

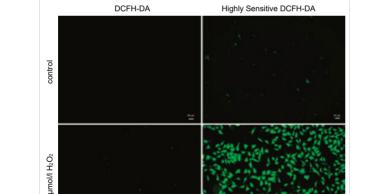
The reactivity of the Highly Sensitive DCFH-DA for ROS is similar to the reactivity of 2'-7' dichlorofluorescein diacetate (DCFH-DA). The Highly Sensitive DCFH-DA also has similar fluorescence characteristics ( $\lambda_{ex}$ : 505 nm,  $\lambda_{em}$ : 525 nm) to DCFH-DA. Therefore. ROS is detectable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

# The selectivity for ROS

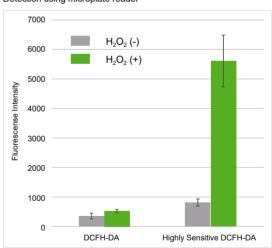


# **High Sensitive Detection Compared with DCFH-DA**

Detection using fluorescent microscope



Detection using microplate reader



Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-treated HeLa cells (1×10<sup>4</sup> cells/ml) were stained with DCFH-DA or the ROS Assav Kit-Highly Sensitive DCFH-DA, and the fluorescence intensity of intracellular ROS was compared between two detection kits. As a result, the ROS Assav 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-**

Proliferation Cytotoxicity Senescence

Autophagy

Oxidative Stress

Metabolism

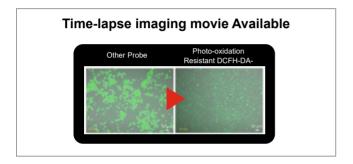
Mitochondria

Lysosome

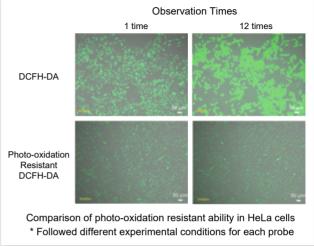
Endocytosis

Other Organelles Exosome, Lipid Droplet, etc.

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

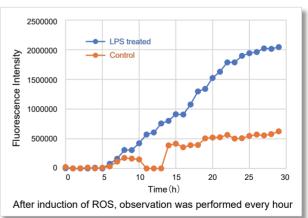


# Resistant to Photo-oxidation



# Simultaneous Detection of ROS in LPS-treated macrophages





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

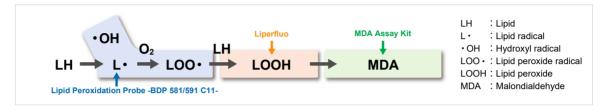
Description	Unit	Code
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	100 tests	R253-10

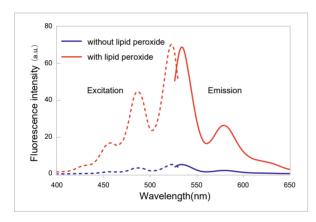
### **Lipid Peroxide Detection**

# Liperfluo



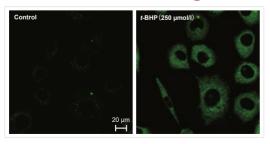
Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and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

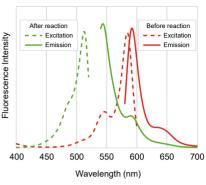
Description	Unit	Code
Liperfluo	1 set (50 $\mu$ g $ imes$ 5)	L248-10

Proliferation Cytotoxicity Senescence

Autophagy

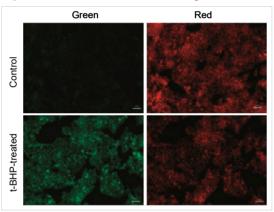
**Lipid Peroxidation Probe -BDP 581/591 C11-**

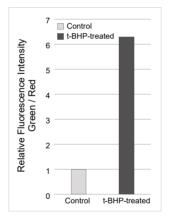
Lipid Peroxidation Probe -BDP 581/591 C11- is a fluorescent probe for detecting lipid peroxidation. This fluorescent probe does not react with lipid peroxides but reacts with lipid radicals generated when lipids are peroxidized, resulting in the detection of lipid peroxidation. The unreacted probe emits 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.



HepG2 cells stained with this probe

# **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 nmRed: Ex = 570 nm. Em = 600-620 nm

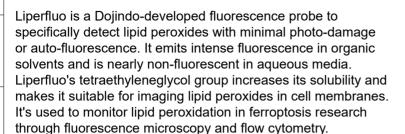
were stimulated with HBSS solution containing 200 µmol/l t-BHP for 2 hours, and the fluorescence intensity was compared with control cells. As a result, a decrease in red fluorescence and an increase in green fluorescence were observed with high sensitivity in t-BHP-treated cells compared to untreated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

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

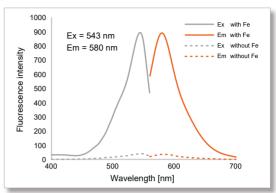
Oxidative Stress

### Intracellular Iron Ion Measurement

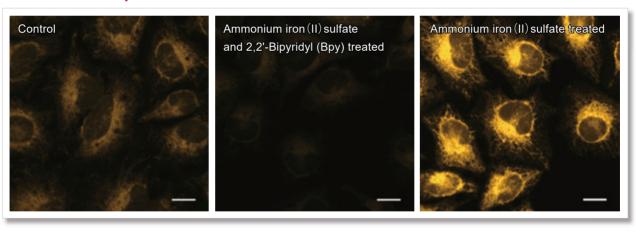
# **FerroOrange**







# **Experimental Example**



HeLa cells treated with chelator of iron 2,2'-bipyridyl (Bpy) (100  $\mu$ mol/l) or Ammonium iron (II) sulfate (100  $\mu$ mol/l) were prepared. The change of intracellular Fe<sup>2+</sup> in HeLa cells was detected by the FerroOrange. Ex = 561 nm, Em = 570-620 nm, Scale bars 20  $\mu$ m

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

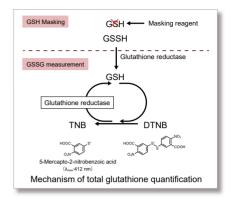
Autophagy

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

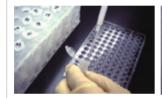
# **GSSG/GSH Quantification Kit**



The GSSG/GSH Quantification kit contains Masking Reagent of GSH. GSH will be deactivated in the sample by simply adding the Masking Reagent. Then, using the enzymatic recycling system, only the GSSG will be detected by measuring the absorbance ( $\lambda$ max = 412 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  $\mu$ mol/l to 50  $\mu$ mol/l and GSSG concentrations from 0.5  $\mu$ mol/l to 25  $\mu$ mol/l can be quantified.



# **Assay Procedure**

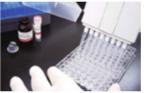


 GSSG/GSH Standard Solution and add Sample A or Sample B to each well.

2) Add Buffer solution to each well



3) Incubate at 37℃ for 1 h.

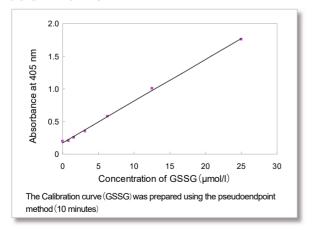


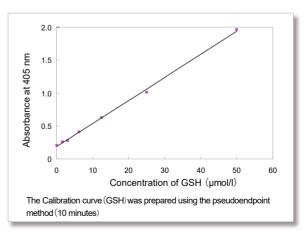
 4) -5) Add substrate working solution and Enzyme/ Coenzyme working solution to each well



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

### **Calibration Curve**





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

Oxidative Stress

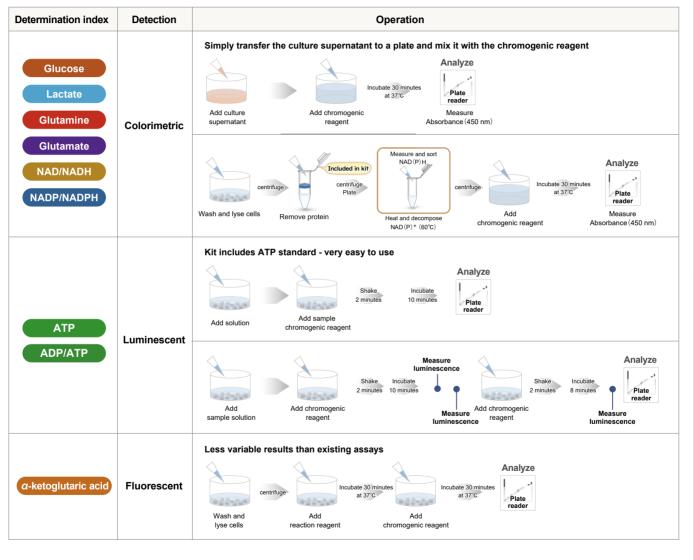
# **Measurements of Intracellular Metabolism**



Description	Unit	Code
tarter Kit		
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10
Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272-10
uantification for Intracellular Metabolism		
ATD A I/A I	50 tests	A550-10
ATP Assay Kit-Luminescence	200 tests	A550-12
ADP/ATP Ratio Assay Kit-Luminescence	100 tests	A552-10
OL A LCUMOT	50 tests	G264-05
Glucose Assay Kit-WST	200 tests	G264-20
Glutamine Assay Kit-WST	100 tests	G268-10
Glutamate Assay Kit-WST	100 tests	G269-10
α-Ketoglutarate Assay Kit-Fluorometric	100 tests	K261-10
	50 tests	L256-10
Lactate Assay Kit-WST	200 tests	L256-20
NAD/NADH Assay Kit-WST	100 tests	N509-10
NADP/NADPH Assay Kit-WST	100 tests	N510-10
ptake 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
	20 tests	UP04-10
Amino Acid Uptake Assay	100 tests	UP04-12
	20 tests	UP05-10
Cystine Uptake Assay Kit	100 tests	UP05-12
Fatty Acid Uptake Assay Kit	100 tests	UP07-10

# **Simple Procedure for First Time User**

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



### Intracellular Metabolism

# Glycolysis/JC-1 MitoMP Assay Kit



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

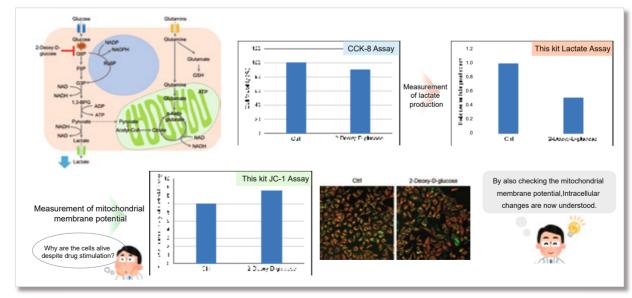
Intracellular metabolic changes caused by any stimulations can be detected by measuring lactate production and mitochondrial membrane potential. In certain instances, cells manage to survive despite sustaining damage to their glycolytic system or mitochondrial function, the principal pathways for energy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

# Experimental Example:

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

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

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



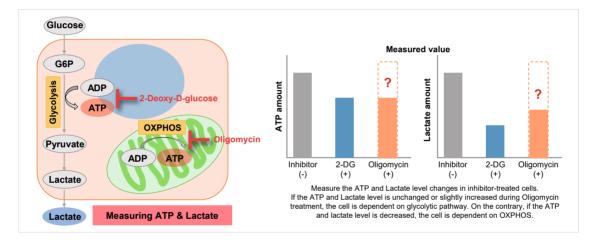
D	escription	Unit	Code
Glycolysis/JC-1 MitoMP Assay Kit		50 tests	G272-10

Proliferation Cytotoxicity

Glycolysis/OXPHOS Assay Kit

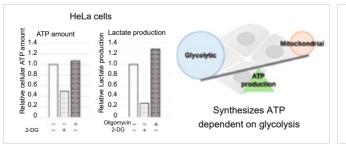
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

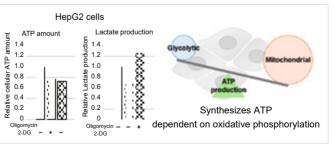
Combining methods (1) and (2) can be used to measure the metabolic pathway dependency of cells. Cells are treated with oligomycin or 2-DG to inhibit OXPHOS or ATP synthesis in the glycolytic pathway, and the amounts of ATP and lactate production are measured, respectively. Changes in the amount of ATP 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





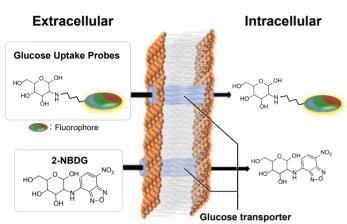
Description	Unit	Code
Glycolysis/OXPHOS Assay Kit	50 tests	G270-10

### **Intracellular Metabolism**

# **Glucose Uptake Assay Kit**



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



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

# **Comparison with Existing Method**

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

product name	Fluorescence microscope	Plate reader detection	FCM detection	Retention ability	Fluorescence characteristics
Glucose Uptake Assay Kit-Blue	0	×	0	1 hour *	λ <sub>ex</sub> :386 nm λ <sub>em</sub> :474 nm
Glucose Uptake Assay Kit-Green	0	0	0	1 hour *	λ <sub>ex</sub> :507 nm λ <sub>em</sub> :518 nm
Glucose Uptake Assay Kit-Red	0	0	0	1 hour *	λ <sub>ex</sub> :560 nm λ <sub>em</sub> :572 nm
2-NBDG	0	×	0	30 minutes or less *	λ <sub>ex</sub> :465 nm λ <sub>em</sub> :540 nm

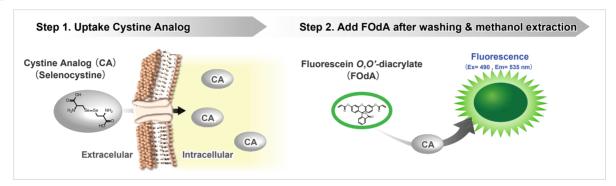
\*Result of A549 cells, the retention time for other cell lines may be different.

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

# **Cystine Uptake Assay Kit**

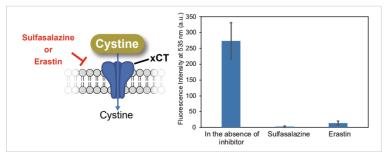
- Easier way to cystine uptake assay
- Applied for plate assay

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

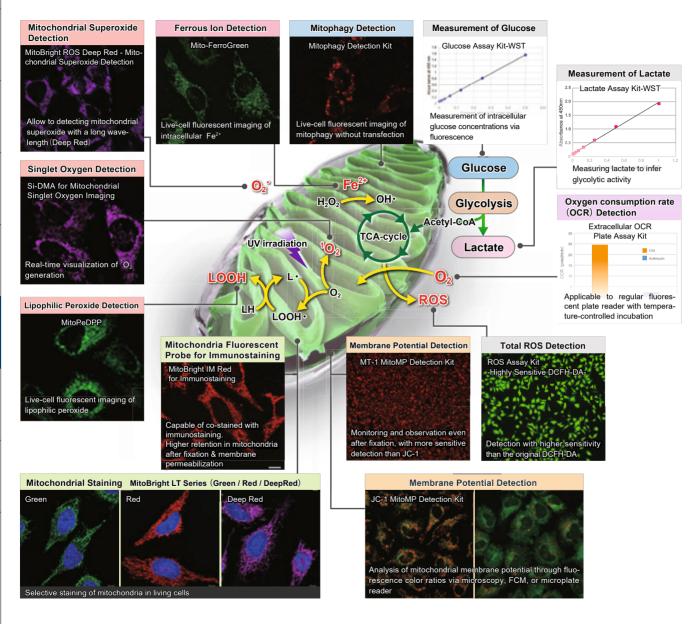
Instrument: Fluorescent Plate Reader

Filter: Ex=485 nm, Em=535 nm

Description	Unit	Code
Custing Untake Assaul Vit	20 tests	UP05-10
Cystine Uptake Assay Kit	100 tests	UP05-12

# **Mitochondrial Research**





### **Mitochondrial Research**

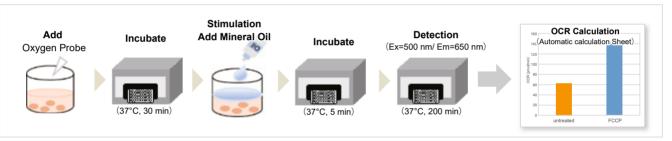
# **Extracellular OCR Plate Assay Kit**



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



### **Procedure**

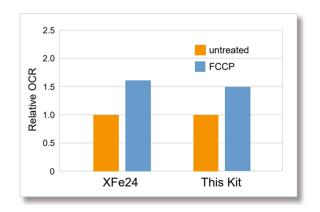


# **Comparison with Flux Analyzer**

Flux Analyzer (XFe24) and this kit were measured on the same day under the same conditions (cell type, cell number, and FCCP concentration).

As a result, correlated data of oxygen consumption rate changes were obtained for XFe24 and this kit.

Cells: HepG2
Cell Number: 5×10<sup>4</sup> cells/well
Stimulation: FCCP (Carbonyl cyanide
4-(trifluoromethoxy) phenylhydrazone)
FCCP Concentration: 2 µmol/l



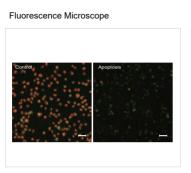
	Description	Unit	Code
Extracellular OCR Plate Assay Kit		100 tests	E297-10

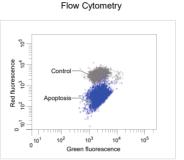
# Mitochondrial Membrane Potential Detection

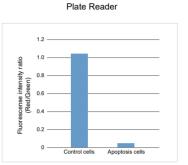
# **JC-1 MitoMP Detection Kit**



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.







De	escription	Unit	Code
JC-1 MitoMP Detection Kit		1 set	MT09-10

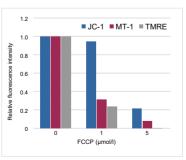
# **Mitochondrial Membrane Potential Detection**

# **MT-1 MitoMP Detection Kit**

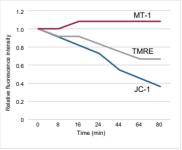


JC-1 dye, TMRE, and TMRM are widely used to monitor MMP, however, these dyes have some limitations, such as low photostability and poor retention after aldehyde fixation. These limitations result in poor reproducibility of experiments. Dojindo's MT-1 MitoMP Detection Kit overcomes these limitations. 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







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

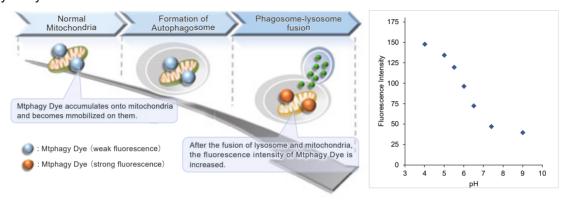
Metabolism

### Mitochondrial Research

# **Mitophagy Detection Kit**

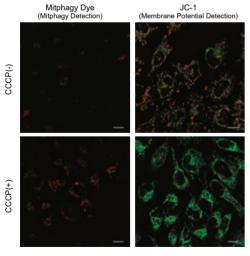


This kit is composed of Mtphagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtphagy Dye accumulates in intact mitochondria, is immobilized on it with chemical bond and exhibits a weak fluorescence from the influence of surrounding condition. When Mitophagy is induced, the damaged mitochondria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

Autophagy

# **Mitochondrial Superoxide Detection**

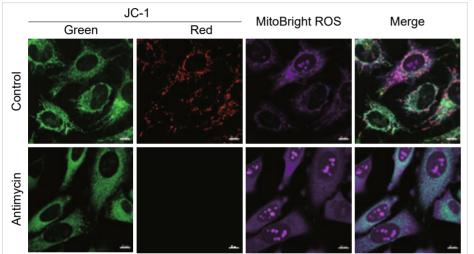
# MitoBright ROS Deep Red - Mitochondrial Superoxide Detection



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

# Experimental Example

Simultaneously Evaluation of Mitochondrial Superoxide and Membrane Potential



<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 um

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
Mita Prinkt DOC Deep Dad Mitach and rial Compresside Detection	100 nmol $\times$ 1	MT16-10
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection	100 nmol × 3	MT16-12

Autophagy

### **Mitochondrial Superoxide Detection**

# Mito-FerroGreen

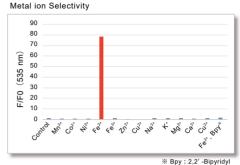




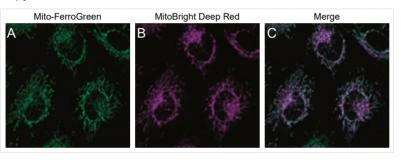
Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion (Fe<sup>2+</sup>) in mitochondria where Fe-S clusters and heme proteins are synthesized, and enables live cell fluorescent imaging of intracellular Fe<sup>2+</sup>. Mito-FerroGreen has no no chelating ability. Mito-FerroGreen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

# Double staining with mitochondrial staining probe

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







Double staining with 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 $\mu$ g $ imes$ 2)	M489-10
Forms Orange		1 tube	F374-10
FerroOrange		3 tube	F374-12

# **MitoBright LT Series**



Senescence

Proliferation Cytotoxicity

Autophagy Se

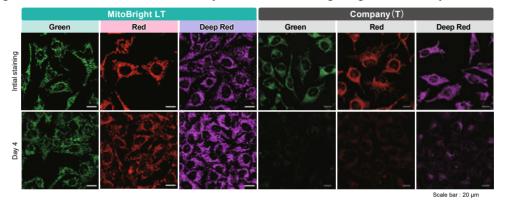
Oxidative Stress

Metabolism

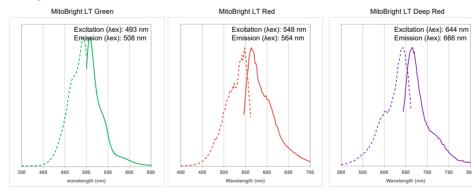
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color 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 μΙ	MT12-12

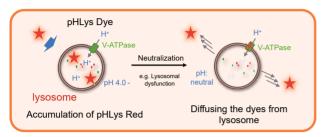
**Lysosomal Analysis** 

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

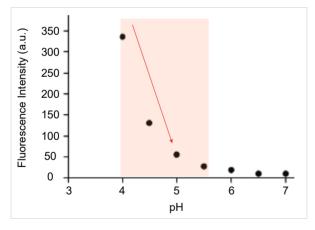




### Lysosomal pH-dependent Fluorescent Probe

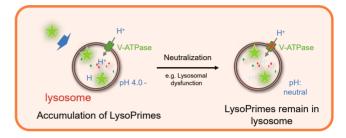


# pH dependence of pHLys Red

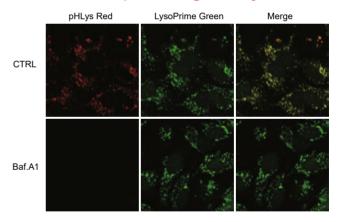


The fluorescence intensity of pHLys Red at each pH was confirmed in vitro, and it was confirmed that the fluorescence intensity changed sensitively within the range of lysosomal pH (pH 4.0-5.5).

### Lysosomal pH-independent Fluorescent Probe



# Resistance to pH changes in lysosomes



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

# Lysosomal Acidic pH Detection Kit

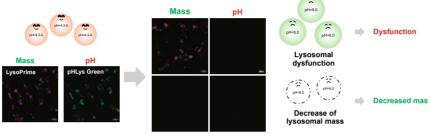


Proliferation Cytotoxicity (pH-independent). The pHLvs and LvsoPrime dves accumulate in the intact lvsosomes. The fluorescence intensity

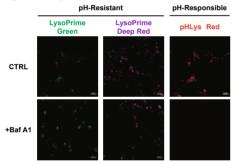
of pHLvs dves are enhanced as the acidity increases, and weak fluorescence is observed when lysosomes are neutralized due to the lysosomal dysfunction. On the other hand, LysoPrime dyes gives stable emissions even lysosomes are neutralized. Lysosomal pH and lysosomal mass can be measured by combining these pHLys and

The kit includes lysosome staining dyes, pHLvs Red/Green (pH dependent), and LysoPrime Green/Deep Red

LvsoPrime dves.

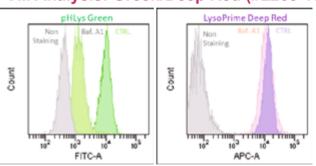


# Imaging Analysis: Green/Red (#L266-10)



<Experimental Conditions> LysoPrime Green: Ex = 488 nm, Em = 490 - 550 nm pHLys Red: Ex = 561 nm, Em = 560 - 620 nm

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



<Experimental Conditions> pHLys Green: FITC Filter (Ex = 488 nm, Em = 515 - 545 nm) LysoPrime Deep Red: APC Filter (Ex = 640 nm, Em = 650 - 670 nm)

Description	Unit	Code
Lysosomal Acidic pH Detection Kit – Green/Red *1	1 set	L266-10
Lysosomal Acidic pH Detection Kit – Green/Deep Red *2	1 set	L268-10
LysoPrime Green – High Specificity and pH Resistance	10 μl × 1	L261-10
	10 μl × 3	L261-12
Luca Dairea Dana Dadi Ulinh Caraiffaith and all Dariatenas	1 tube	L264-10
LysoPrime Deep Red - High Specificity and pH Resistance —	3 tube	L264-12
nUl va Pad Lyacomal Acidia nU Detaction	1 tube	L265-10
pHLys Red - Lysosomal Acidic pH Detection —	3 tube	L265-12

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

Senescence

Autophagy

Oxidative Stress

Metabolism

Mitochondria

Lysosome

Endocytosis

Other Organelles Exosome, Lipid Droplet, etc.

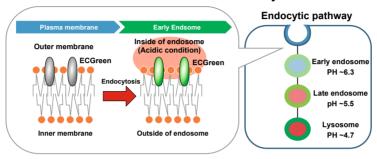
# **Endocytosis**

# **ECGreen-Endocytosis Detection**



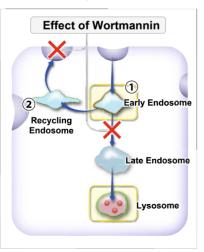
ECGreen-Endocytosis Detection is a pH dependent fluorescence dye that localizes to vesicle membrane. The visualization of endocytosis using the ECGreen is a more direct method than fluorescent analogs and allows visualization endocytosis from the stage of early endosomes.

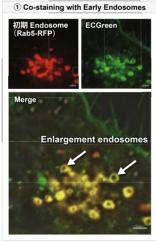
# The detection mechanism of endocytosis

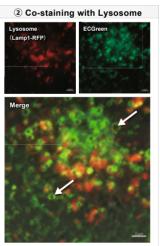


# Clear visualization of intracellular vesicular trafficking

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







Description	Unit	Code
ECGreen-Endocytosis Detection	40 µl	E296-10

# Proliferation Cytotoxicity

Senescence

Autophagy

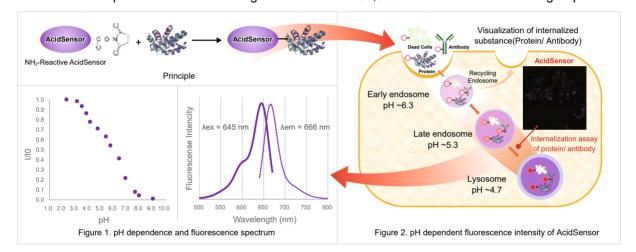
Oxidative Stress

AcidSensor Labeling Kit

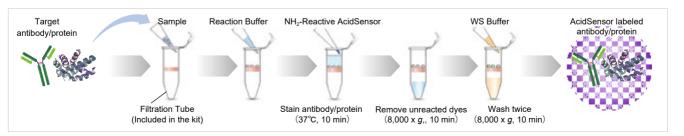
– Endocytic Internalization Assay

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).\*Notice:

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



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



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

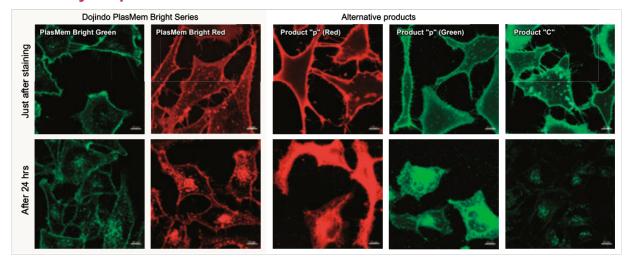
## **Cell Membrane Staining**

# PlasMem Bright Green / Red

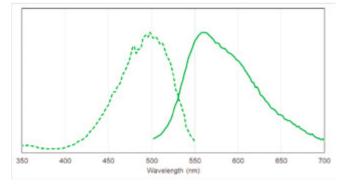


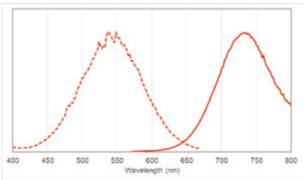
PlasMem Bright dyes overcome these limitations. PlasMem Bright dyes are designed to stain PMs for over a day. Furthermore, the PlasMem Bright dyes are more water-soluble compared with other commercially available dyes and can be diluted with culture medium. The PlasMem Bright dyes offer two different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

# High retentivity on plasma membrane



# **Excitation and emission spectra of PlasMem Bright dyes**

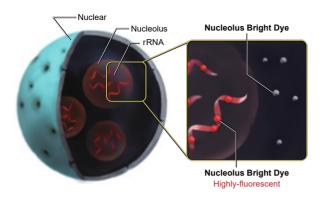




Description	Unit	Code
PlasMem Bright Green	100 µl	P504-10
PlasMem Bright Red	100 µl	P505-10

Proliferation Cytotoxicity

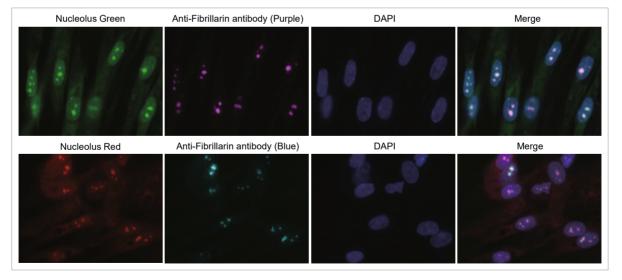
**Nucleolus Bright Green / Red** 



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

	Maximum Excitation Wavelength	Maximum Emission Wavelength	Fluorescence of MeOH fixed cells	Fluorescence of PFA fixed cells
Nucleolus Bright Green	513 nm	538 nm	0	0
Nucleolus Bright Red	537 nm	605 nm	0	0

### **Nucleolus Localization**



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

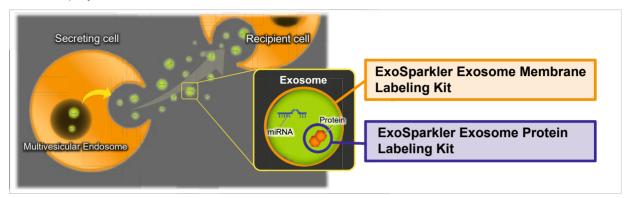
Endocytosis

## **Exosome Staining**

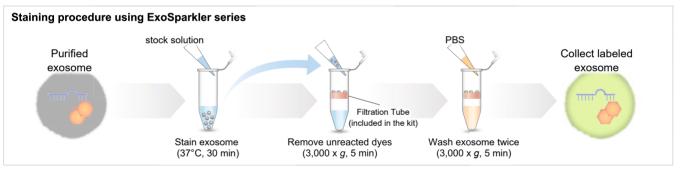
# **Exosome Labeling Kits**



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



# **Labelling Procedure**



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

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

# Exolsolator Exosome Isolation Kit

Senescence

Proliferation Cytotoxicity

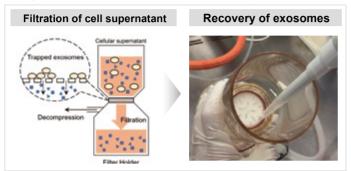
Autophagy

Oxidative Stress

Metabolism

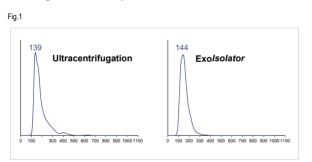
Exo*lsolator* Exosome Isolation Kit can collect exosomes from cell supernatants with a recovery rate equivalent to the ultracentrifugation(UC) method. Science Exo*lsolator* Exosome Isolation Kit requires only the filtration procedure, unlike the UC, exosomes are obtained quickly without any complicated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

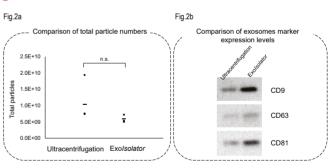
# Easy to Use no Technique Required



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

# **Recovery Rate Equivalent to Ultracentrifugation**





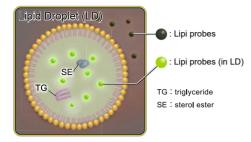
Ultracentrifugation is the most commonly used method to isolate exosomes. We isolated the exosomes from the supernatant of HEK293S using both of ultracentrifugation method and the Exo*lsolator* method. The particle size distribution (Fig. 1), the number of particles (Fig. 2(a)) and the expression level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

Description	Unit	Code
Exolsolator Exosome Isolation Kit	3 tests	EX10-10
Exolsolator Isolation Filter	10 pieces	EX11-10

# **Lipid Droplet Staining**

# Lipi-Blue / Green / Red / Deep Red

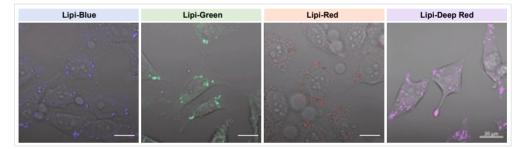




Lipi probes are small molecules that emit strong fluorescence in a hydrophobic environment such as LDs, which can be observed without any washing steps after staining with Lipi probes.

A medium that contained oleic acid (200  $\mu$ mol/I) was added and incubated overnight. Then, the supernatant was removed and the cells were washed with PBS. Each Lipi product series (1  $\mu$ mol/I) 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	✓	✓	1	✓	✓	1
Selectivity towards Lipid Droplet (Level of Background)	✓	<b>✓</b>	1			
General Filter Accommondation*1	✓	✓	✓* <sup>2</sup>	n.d.	*3	✓
Retention in Live Cells	1	1		n.d.		

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

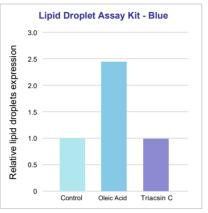
<sup>\*3</sup> Leaks in GFP filter (500 ∼ 540 nm)

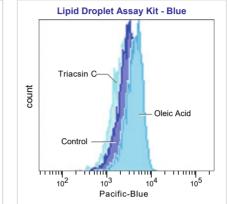
	Description	Unit	Code
Lipi-Blue		10 nmol $\times$ 1	LD01-10
Lipi-Green		10 nmol × 1	LD02-10
Lipi-Red		100 nmol × 1	LD03-10
Lipi-Deep Red		10 nmol × 1	LD04-10

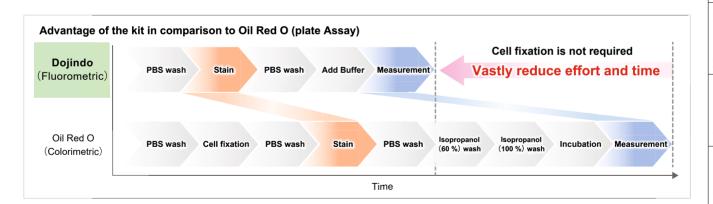
<sup>&</sup>lt;sup>\*2</sup> When co-staining with a green fluorescent dye, a green fluorescent emission filter less than 550 nm is recommended.

# 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



For inquiries, please contact Dojindo Europe GmbH.

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