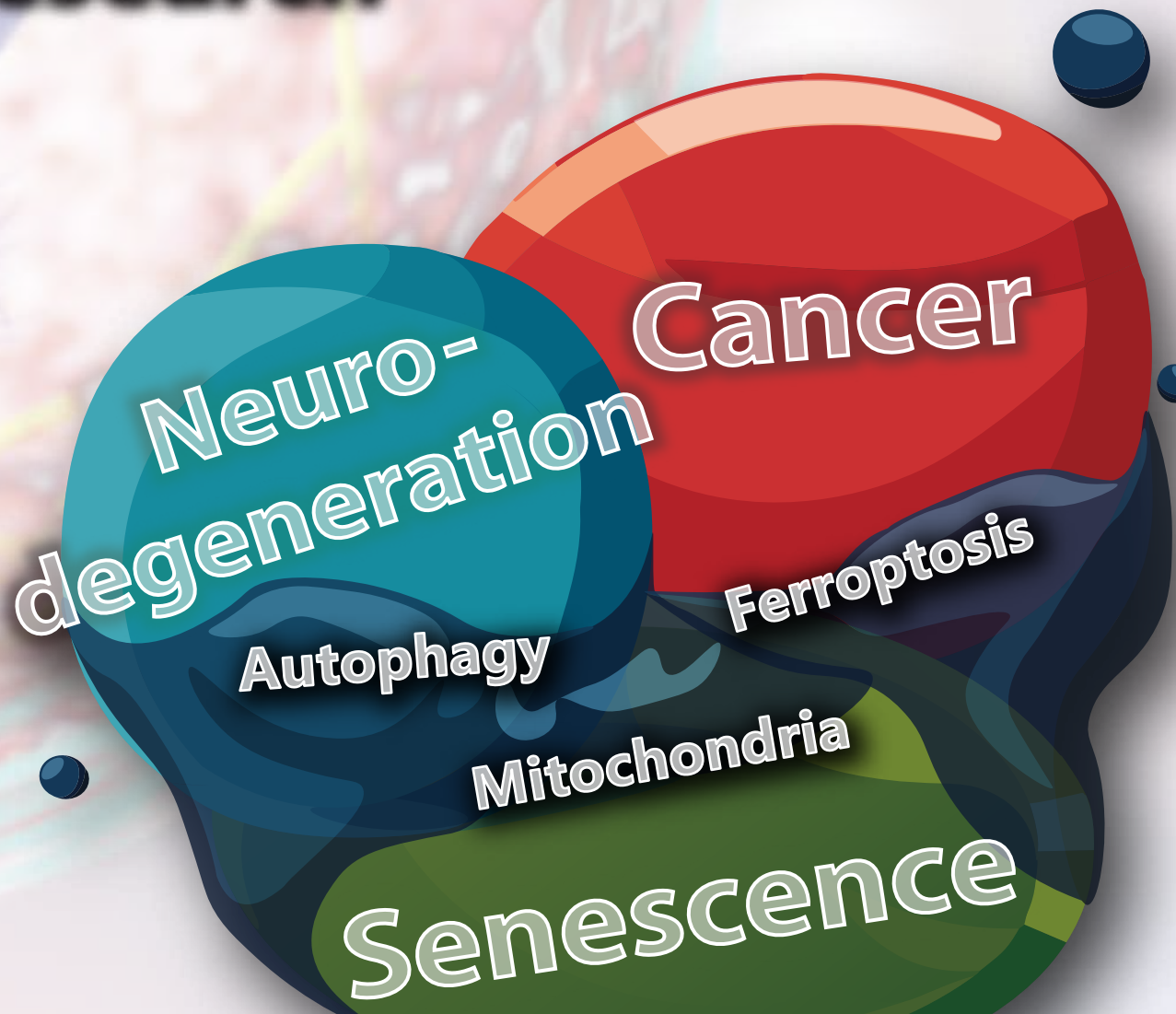
DOJINDO

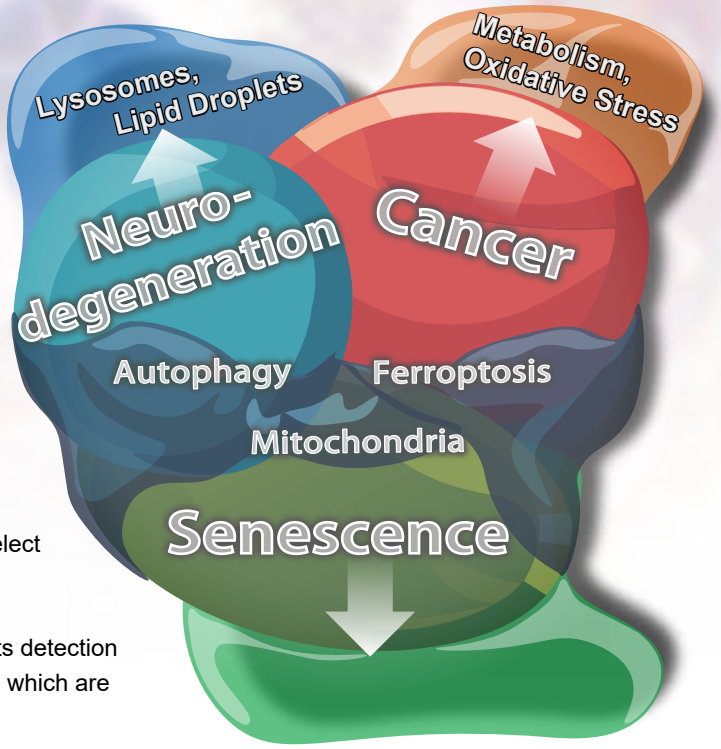
Cellular Function Analysis

6th Edition

for Cancer, Neurodegeneration, and Senescence, Research



Cancer, Neurodegeneration, and Senescence Research



Introduction

Cancer, Neurodegeneration, and Senescence are among the areas of greatest interest in recent medical research. Research in these fields is directly linked to the elucidation of disease mechanisms and the development of theraputic drugs or treatments.Since the mechanisms of pathological progression involve a variety of interrelated phenomena, it is important to select appropriate indicators that meets the research targets and the objectives.
Here we introduce the multiple cellular function indicators and its detection reagents focusing on autophagy, mitochondria, and ferroptosis, which are closely related to these three research areas.

Cancer

Mitochondria

Mitochondrial function is known to be altered in cancer cells. They gain energy through mitochondrial activity, while modulating to reduce ROS generation at the mitochondria to protect themselves.
It has also been reported that cancer cells use mitochondria to avoid the initiation of apoptosis.

Autophagy

Cancer cells activate the autophagy system under stress to obtain energy and cellular components necessary for their survival. Autophagy is also being investigated as a potential target for cancer treatment because excessive autophagy induces cell death.

Ferroptosis

Cancer cells are resistant to Ferroptosis by promoting expression of the anti-ferroptosis factors such as GPX4 or uptake of cystine as a source of the antioxidant glutathione. Inhibition of these pathways is attracting attention as a new approach to cancer treatment.

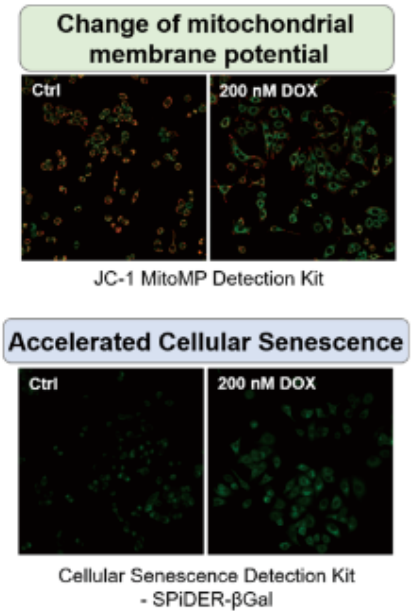


Figure 1. Changes in mitochondrial membrane potential associated with cellular senescence

Doxorubicin (DOX) inhibits cell proliferation during the G2/M phase of the cell cycle and induces cellular senescence. After addition of DOX to A549 cells, cellular senescence and mitochondrial membrane potential changes were detected using the Cellular Senescence Detection Kit - SPiDER-βGal (p.28) and JC-1 MitoMP Detection Kit (p.20), respectively.

Neurodegeneration

Mitochondria

Because neuronal cells have high energy demands, mitochondrial function is important. Mitochondrial dysfunction, caused by the accumulation of lipid droplets, has been observed in Parkinson's disease.

Autophagy

In neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, autophagy and lysosomal dysfunction causes the accumulation of abnormal proteins, which promotes neuronal cell death. Autophagy dysfunction is also known to provoke cell death by leading to the accumulation of lipid droplets, which are associated with mitochondrial dysfunction.

Ferroptosis

In Alzheimer's disease, a typical neurodegenerative disease, iron accumulation and lipid peroxidation have been observed. Therefore, neuronal ferroptosis has been focused as one of the factors of neuronal cell death.

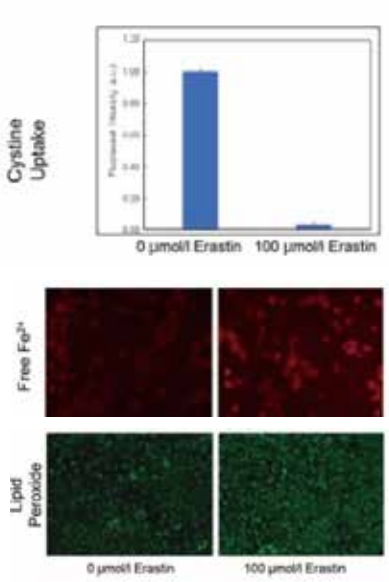


Figure 2. Changes in related indicators associated with ferroptosis induction

In A549 cells stimulated with elastin, a cystine uptake inhibitor, to induce ferroptosis, changes in cystine uptake, intracellular ferrous iron, and the lipid peroxides were detected using Cystine Uptake Assay Kit (p.16), FerroOrange (p.27), and Liperfluo (p.26) respectively.

Senescence

Mitochondria

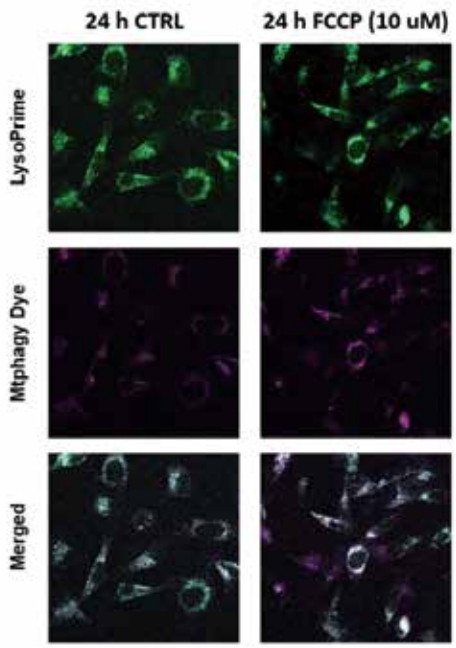
Mitochondrial function declines with age, resulting in reduced energy metabolism and increased oxidative stress. The decline in mitochondrial function suppresses fatty acid consumption, which promotes lipid droplet formation, causing further damage to the mitochondria.

Autophagy

Autophagy is also involved in the process of cellular senescence. Dysfunctional cells accumulate as autophagic activity decreases with age.

Ferroptosis

Iron accumulation and increase in oxidative stress associated with senescence promotes ferroptosis. It is particularly observed in tissues with high iron accumulation, such as the liver and brain.



SH-SY5Y human neuroblastoma cells stained with mitophagy, a selective autophagy of mitochondria, detection dye - Mtphagy dye (p.23) and lysosomal dye - LysoPrime Green (p.33) were stimulated with FCCP to induce mitophagy, then fluorescence imaging was performed.

Figure 3. Detection of mitophagy in neuroblastoma cell

References

Mitochondria

1. Wallace, D. C. (2012). Mitochondria and cancer. Nature Reviews Cancer, *12*(10), 685-698.
2. Nunnari, J., & Suomalainen, A. (2012). Mitochondria: in sickness and in health. Cell, *148*(6), 1145-1159.
3. Guo, Y., Cordes, K. R., Farese Jr, R. V., & Walther, T. C. (2009). Lipid droplets at a glance. Journal of Cell Science, *122*(6), 749-752.

Autophagy

1. Mizushima, N., & Komatsu, M. (2011). Autophagy: renovation of cells and tissues. Cell, *147*(4), 728-741.
2. Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell, *132*(1), 27-42.
3. Fujimoto, T., & Parton, R. G. (2011). Not just fat: the structure and function of the lipid droplet. Cold Spring Harbor Perspectives in Biology, *3*(3), a004838.

Ferroptosis

1. Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., ... & Stockwell, B. R. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell, *149* (5), 1060-1072.
2. Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., ... & Jiang, X. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell, *171*(2), 273-285.

Table of Contents

Each color icon indicates the major products used in each research area.

Cancer Neurodegeneration Senescence

Cell Proliferation / Cytotoxicity

Cell Proliferation / Cytotoxicity Assay

Cell Counting Kit-8

Plate Reader

This kit uses the WST method developed by Dojindo Laboratories to measure live cells using intracellular metabolic activity as an indicator. It is widely used in cell proliferation tests and cytotoxicity tests due to its ease of measurement, long-term stability of reagents, and high reproducibility.

DOJINDO MOLECULAR TECHNOLOGIES, INC.

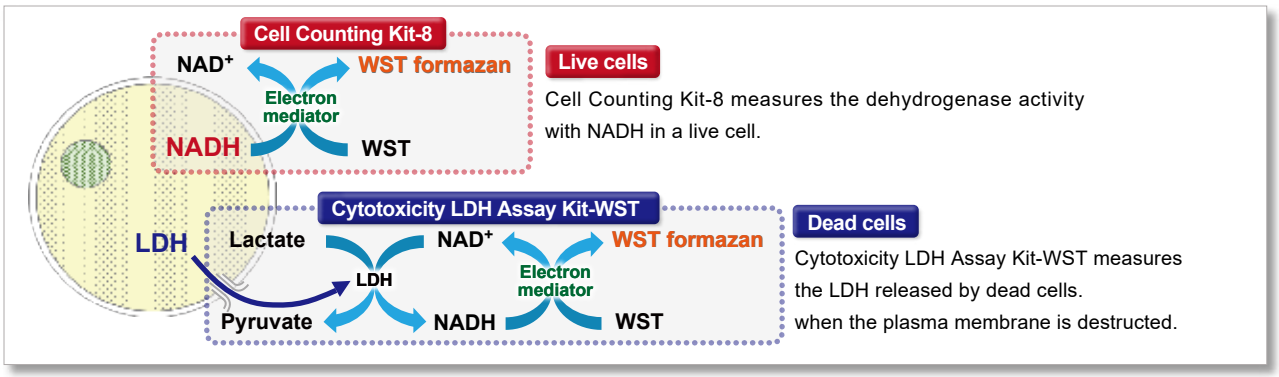
Cytotoxicity Assay

Cytotoxicity LDH Assay Kit-WST

Plate Reader

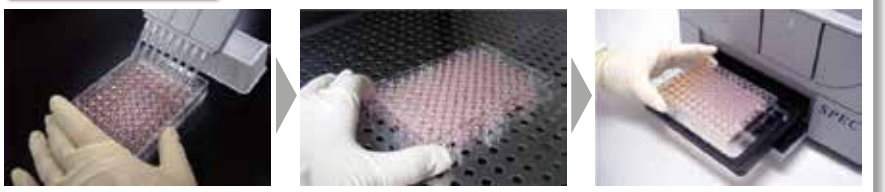
This kit measures cytotoxicity by measuring lactate dehydrogenase (LDH) activity released from cells into the medium. The LDH assay method is increasingly being used in conjunction with Cell Counting Kit-8 (WST method) and MTT method when measuring cytotoxicity.

Detection Principle



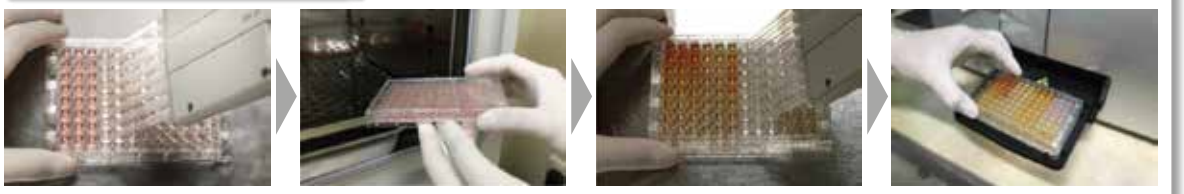
Procedure

Cell Counting Kit-8



Add CCK-8 solution → Incubate for 1-4 hours → Measure O.D. at 450 nm

Cytotoxicity LDH Assay Kit-WST



Add Working Solution → Colorimetric reaction → Add Stop Solution → Measure absorbance

Description	Unit	Code
Cell Counting Kit-8	1,000 tests	CK04
	3,000 tests	
	10,000 tests	
Cytotoxicity LDH Assay Kit-WST	100 tests	CK12
	500 tests	
	2,000 tests	

Cell Proliferation / Cytotoxicity Assay Kit

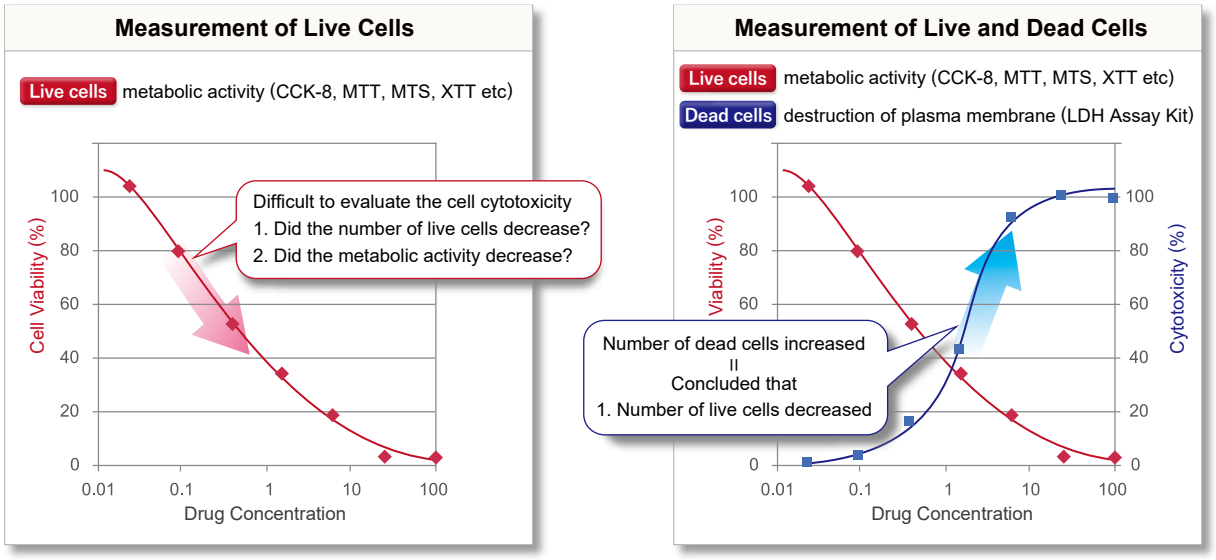
Viability/Cytotoxicity Multiplex Assay Kit

Plate Reader

A set of Cell Counting Kit-8 and Cytotoxicity LDH Assay Kit-WST (500 tests each)

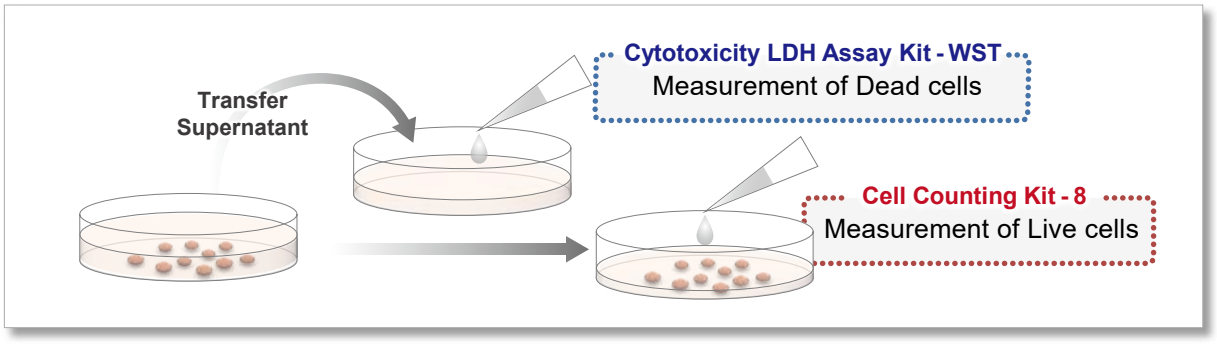
Purpose of Using Two Detection Methods

Since Cell Counting Kit-8 (CK04) measures the metabolic activity of live cells, the data does not specifically verify cell death. To ensure the data is reflecting the cell death instead of decreased metabolic activity, Cytotoxicity LDH Assay Kit-WST (CK12) is used to support the data reliability by measuring LDH released from dead cells.



The same cell sample can be applied

This kit includes CCK-8 for measuring live cells and LDH Assay Kit for measuring dead cells, both of which can be evaluated by plate assay at the same absorbance wavelength. And since same samples can be used for Cell Counting Kit-8 and Cytotoxicity LDH Assay Kit-WST, it is convenient and time-efficient.



Description	Unit	Code
Viability/Cytotoxicity Multiplex Assay Kit	500 tests	CK17

Cell Count Normalization

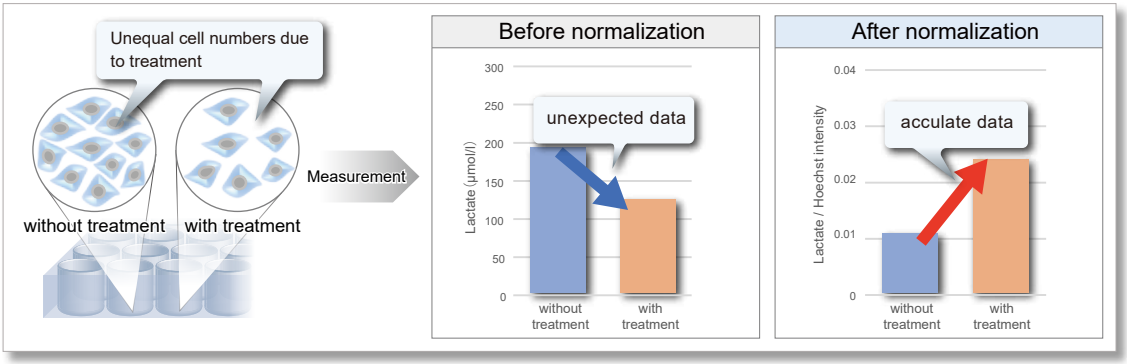
Cell Count Normalization Kit

Plate Reader

This kit contains a nuclear staining dye (Hoechst 33342) and an optimized buffer for plate assay, which together allow you to easily determine the cell number per well just by adding the reagent.

Necessity of cell count normalization

When cells are analyzed in a microplate, the results obtained may differ depending on the number of cells per well. In such cases, normalization of the measured values will be necessary.



Description	Unit	Code
Cell Count Normalization Kit	200 tests 1,000 tests	C544

Cell Cycle Measurement

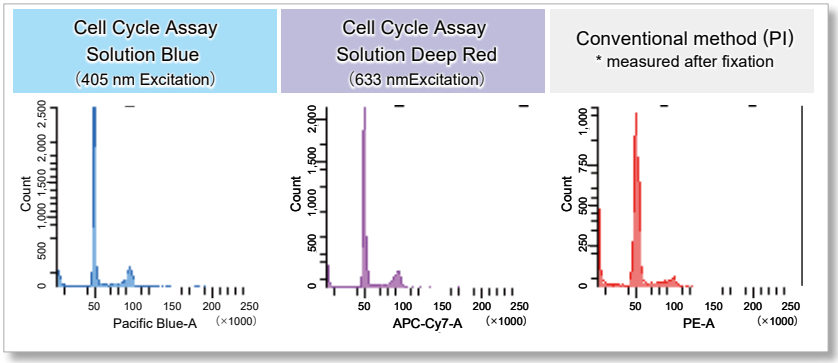
Cell Cycle Assay Solution Blue / Deep Red

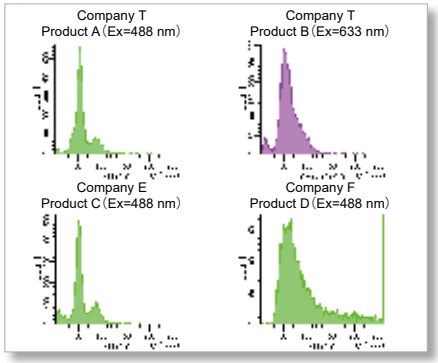
Plate Reader

Clearly Identifies Cell Cycle Stages

Live CHO cells stained by the Cell Cycle Assay Solution Blue and Deep Red were measured by flow cytometry. Similar experiments were performed using the existing reagent for cell cycle analysis and PI staining a widely used staining technique. The results obtained by the Cell Cycle Assay Solution were equivalent to PI staining results (shown below). Compared to four different products, our product obtained a sharp histogram peak in live cells.

Strong correlation between PI | **Applicable for both live and fixed cell** | **Sharp histogram**





Description	Unit	Code
Cell Cycle Assay Solution Blue	50 tests	C549
Cell Cycle Assay Solution Deep Red	50 tests	C548

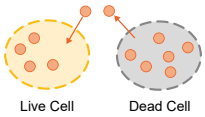
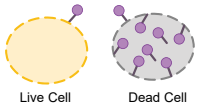
Dead Cell Staining

Dead Cell Makeup Blue / Deep Red - Higher Retention than PI

FCM

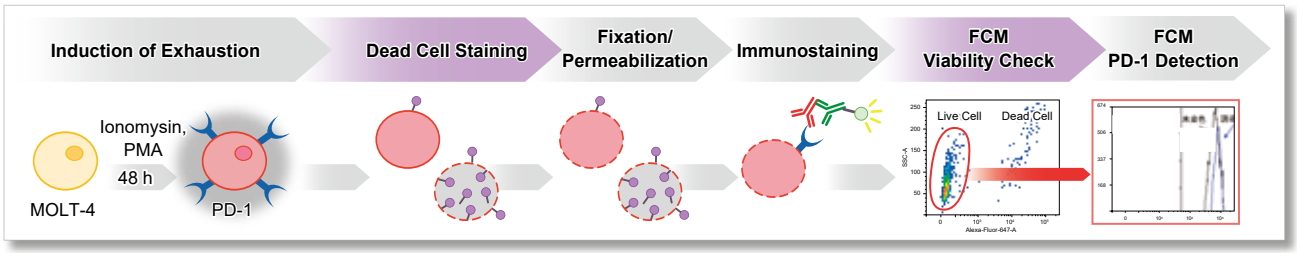
Propidium iodide (PI) is used to discriminate dead cells, but fixation or permeabilization of the membrane causes PI to leak from the cells, making it difficult to obtain accurate data. Dead Cell Makeup has the property of covalently binding to proteins on the cell surface and inside the cells, so the dye does not leak out even after fixation and permeabilization of the cells. In addition, there is a significant difference in fluorescence intensity between live and dead cells, allowing to easily distinguish dead cells and exclude them from the Flow Cytometry analysis.

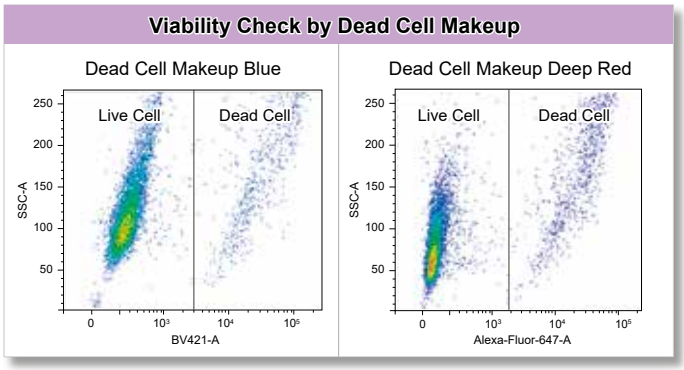
Comparison with PI

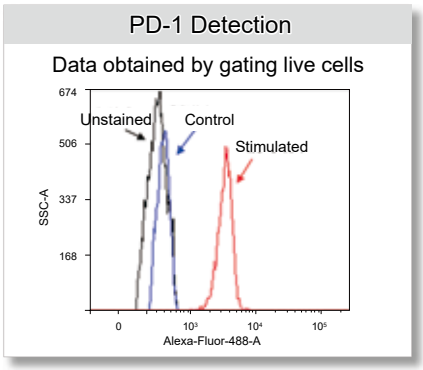
	PI	Dead Cell Makeup series Blue / Deep Red	
Principle	・Non-permeable Dye ・Intercalates into DNA/RNA in the nucleus	・Non-permeable Dye ・Covalently binds to cell-surface and intracellular protein	
Advantage	・Commonly used for dead cell staining ・Low cost	・Enter into dead cell and show high fluorescence intensity. ・Does not leak from cells	
FCM Excitation laser / Emission filter (example)	Excitation: 561nm / Emission: 586/15 nm（PE）	・Blue	Excitation: 405nm / Emission: 450/50nm (Pacific Blue, Brilliant Violet 421, Alexa Fluor 405)
		・Deep Red	Excitation: 640nm / Emission: 670/30nm (APC, Alexa Fluor 647, Cy5)
After Fixation/ Permeabilization	PI leaked from dead cells enters into live cells through its compromised membrane. 	Dead Cell Makeup Dyes covalently binds to the protein, hence does not leak even after permeabilization. 	

PD-1 detection after induction of MOLT-4 cell exhaustion

MOLT-4 cells were stimulated for 48 hours in RPMI medium containing Ionomycin (500 ng/ml) and PMA (Phorbol 12-myristate 13-acetate, 50 ng/ml). Cells were stained with Dead Cell Makeup, and PD-1 expression was detected by immunostaining (primary antibody: anti-PD-1 mouse antibody, secondary antibody: anti-mouse antibody-Alexa488). The results showed that dead cells and live cells could be clearly discriminated, and that PD-1 expression was predominantly elevated in the stimulated cell group when only live cells were gated.



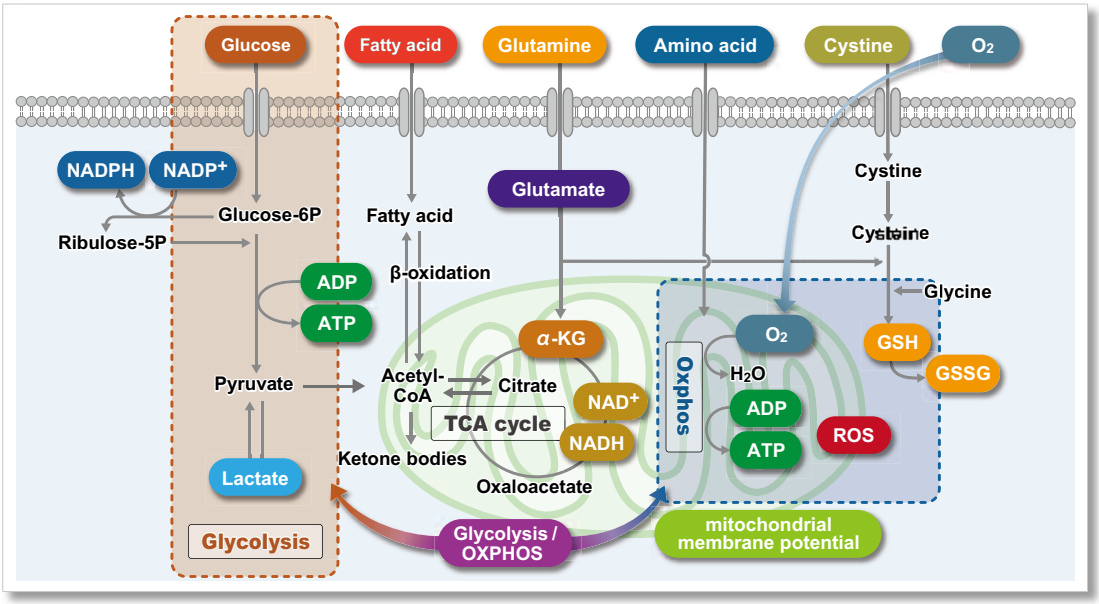




Description	Unit	Code
Dead Cell Makeup Blue - Higher Retention than PI	100 tests	C555
Dead Cell Makeup Deep Red - Higher Retention than PI	100 tests	C556

Intracellular Metabolism

Analyzing the various intracellular metabolic pathways [e.g., the glycolysis, the tricarboxylic acid (TCA) cycle, electron transport chain, etc.] is important when trying to understand cellular status. Metabolites and energy sources [e.g., glucose, lactate, and NAD(P)+/NAD(P)H] are the indicators used for analyzing intracellular metabolisms.
We offer a variety of assay kits to evaluate these indicators with necessary components for sample preparation and measurement.



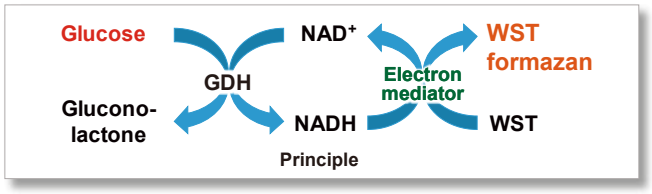
Description	Unit	Code
Starter Kit		
Glycolysis/OXPHOS Assay Kit	50 tests	G270
Glycolysis/JC-1 MitoMP Assay Kit	50 tests	G272
Quantification of Intracellular Metabolism		
ATP Assay Kit-Luminescence	50 tests	A550
	200 tests	A550
ADP/ATP Ratio Assay Kit-Luminescence	100 tests	A552
Glucose Assay Kit-WST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

Glucose

Glucose Metabolism Assay

Glucose Assay Kit-WST

Glucose Assay Kit-WST is a colorimetric assay kit for quantification of glucose, one of the major indicators of energy metabolism (lower limit: 0.02 mmol/l). Glucose quantification is used as an indicator of glucose metabolism in diabetes research, as well as to monitor the changes in cellular metabolism in cancer.

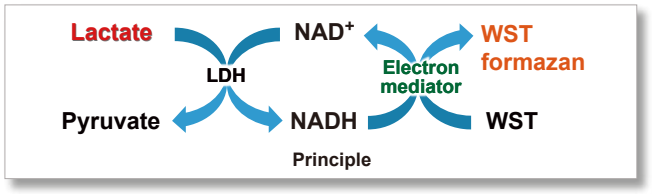


Lactate

Lactate Measurement

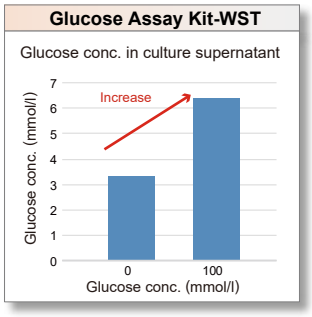
Lactate Assay Kit-WST

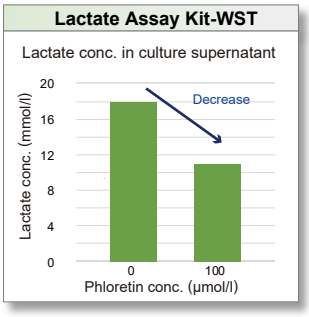
Lactate Assay Kit-WST is a colorimetric assay kit for quantification of lactate, a metabolite of glycolysis (lower limit: 0.02 mmol/l). Lactate quantification is widely used in cancer research as an indicator to monitor changes in cellular metabolic pathways. Recently, there are an increasing number of reports using lactate as an indicator in areas such as stem cell differentiation, diabetes research, and mitochondrial function analysis.



Evaluation of culture supernatant using two indicators

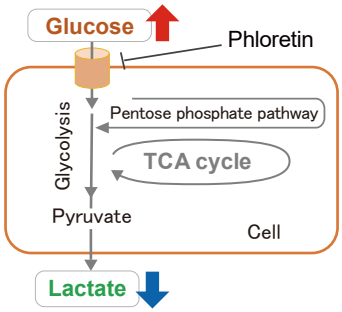
Phloretin, the glucose transporter inhibitor, was added to Jurkat cells and the intracellular metabolism change was evaluated using Glucose Assay Kit-WST and Lactate Assay Kit-WST.





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

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



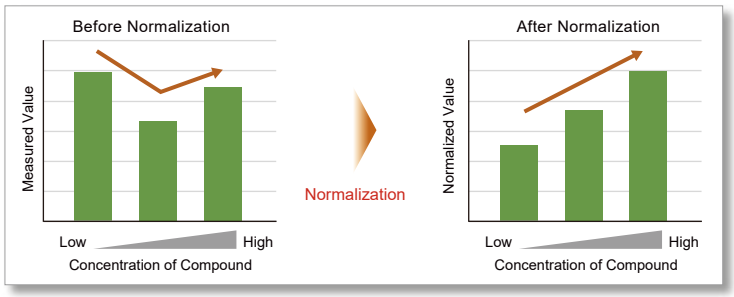
Description	Unit	Code
Glucose Assay Kit-WST	50 tests 200 tests	G264
Lactate Assay Kit-WST	50 tests 200 tests	L256

Improve the reliability of metabolic measurement data

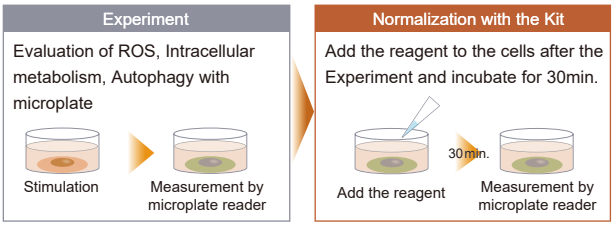
Plate Reader

Cell Count Normalization (p.7)

When cells are analyzed in a microplate, the results obtained may differ depending on the number of cells per well. In such cases, normalization of the measured values will be necessary.
With this kit, by simply adding the reagent to the cell culture medium, the nuclei within the cells are stained, and the number of cells can be easily evaluated from the obtained fluorescence intensity.



Simple Experiment procedure



Description	Unit	Code
Cell Count Normalization Kit	200 tests 1,000 tests	C544

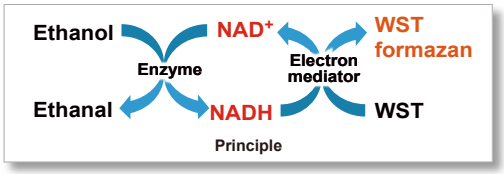
NAD+

NAD+/NADH Assay

NAD/NADH Assay Kit-WST

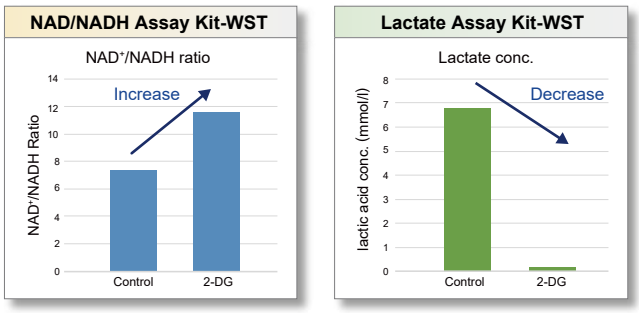
NAD/NADH Assay Kit-WST is a colorimetric assay kit for quantification of NAD+ and NADH, and measuring their ratio.

NAD is an important cofactor involved in redox reactions in the main metabolic pathways in cells such as glycolysis, electron transfer system and TCA cycle. Maintaining appropriate levels of its oxidized form NAD+ and its reduced form NADH is essential for cell function. Additionally, recent studies report the relations between the decreased level of NAD+ and Senescence.

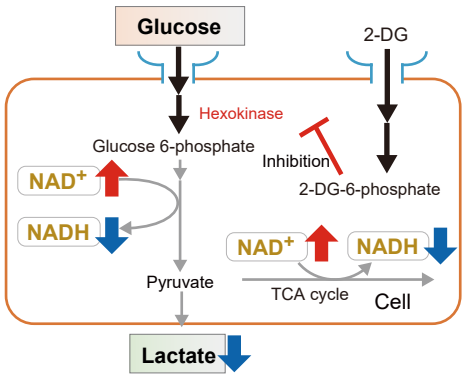


NAD+/NADH in combination with Lactate Assay Kit

2-Deoxy-D-glucose (2-DG) was added to HeLa cells. After 24 hours of incubation, lactate levels in the supernatant were quantified using the Lactate Assay Kit-WST (Code L256), and the NAD+/NADH ratio was determined with the cell pellet after removing the supernatant using the NAD/NADH Assay Kit-WST.



Conditions
Cell Line: HeLa cells (1x10^6 cells)
Stimulation condition: 2-DG (final conc.: 6mmol/l), 24 hrs
Sample: Culture supernatant (Lactate)
Cell (NAD+/NADH ratio)



As a result, intracellular glycolysis was inhibited by 2-Deoxy-D-glucose, which led to decreased lactate levels and an increase in the NAD+/NADH ratio.

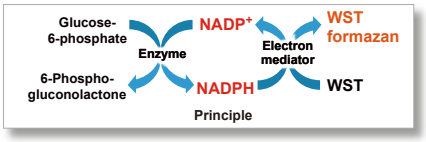
NADP+

NADP+/NADPH Assay

NADP/NADPH Assay Kit-WST

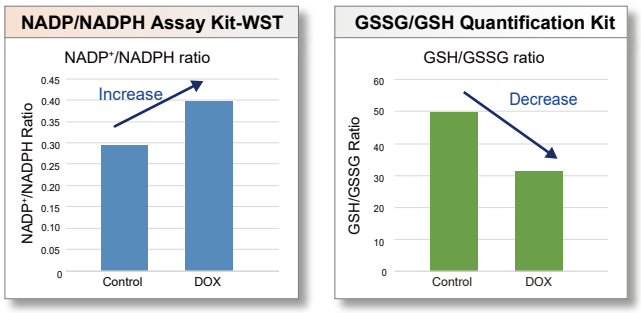
NADP+/NADPH Assay Kit-WST is a colorimetric assay kit that enables quantitation of the amount of total NADP+/NADPH, NADPH and NADP+, and measurement of their ratio.

NADP is an important cofactor involved in reactions in the pentose phosphate pathway, one of the metabolic pathways in cells. NADP exists as an oxidized form NADP+ and a reduced form NADPH in cells and involved in biosynthesis of fatty acids and cholesterol as well as generation of reduced glutathione (GSH). In addition, recent study suggests that NADP+/NADPH is associated with the extension of life span by carbohydrate restriction.

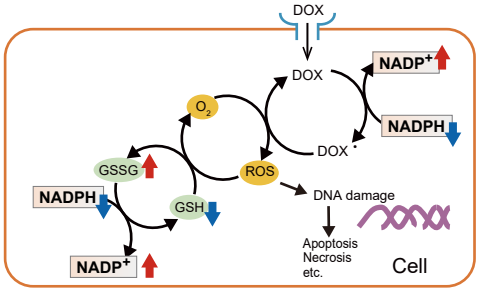


Measurement of NADP+/NADPH in combination with Glutathione (GSH) Quantification Kit

Doxorubicin (DOX) was added to Jurkat cells. After 24 hours of incubation, NADP+/NADPH ratio and reduced/oxidized glutathione (GSH/GSSG) ratio were determined using the NADP/NADPH Assay Kit-WST and the GSSG/GSH Quantification Kit (Code G257).



Conditions
Cell Line: Jurkat cells (3x10^6 cells)
Stimulation condition: DOX (final conc.: 500 nmol/l), 24 hrs
Sample: Cell



The results shown above are likely to be explained by the following mechanism. When DOX (doxorubicin) was added to cells, DOX radicals, along with NADP+, were generated by enzymatic reaction. DOX radicals form reactive oxygen species (ROS), which induces DNA damage and apoptosis. In the meantime, to eliminate ROS formed in cells, GSH is consumed and GSSG is increased. Moreover, NADPH is used to reduce GSSG to GSH, resulting in an increase in NADP+.

Description	Unit	Code
NAD/NADH Assay Kit-WST	100 tests	N509
NADP/NADPH Assay Kit-WST	100 tests	N510

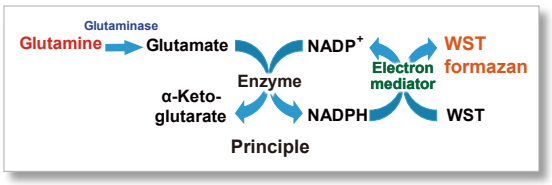
Glutamine

Glutamine Assay

Glutamine Assay Kit-WST

Glutamine Assay Kit-WST is a colorimetric assay kit that enables quantification of glutamine present in cell culture supernatant or intracellular glutamine (Lower limit: 5μmol/l).

Glutamine is a source of α-ketoglutarate (one of the TCA cycle intermediates). It is reported that Glutaminolysis, the process by which glutamine as a substrate is converted to α-ketoglutarate, is upregulated in cancer cells and largely contributed to scavenge the reactive oxygen species (ROS) and reduction of oxidized glutathione.



Glutamate

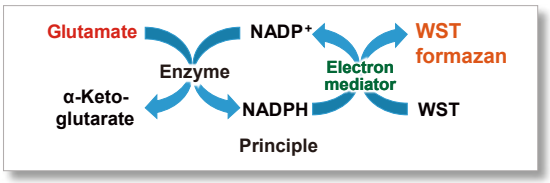
Glutamate Assay

Glutamate Assay Kit-WST

Glutamate Assay Kit-WST is a colorimetric assay lit that enables you to quantify glutamate present in cell culture supernatant or intracellular glutamate (Lower limit: 5μmol/l).

Glutamate contributes to the biosynthesis of protein and glutathione. It also functions as important neurotransmitter, and an excess glutamate is considered to be a cause of neurodegenerative disorders, including Alzheimer's disease.

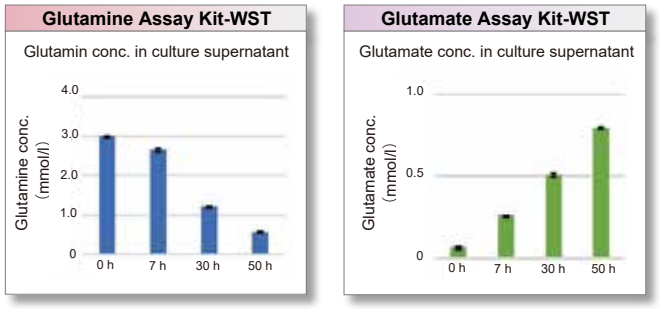
A recent study showed that an iron-dependent cell death called "ferroptosis" is induced by inhibition of cystine/glutamate antiporter (xCT) responsible for cystine uptake and glutamate release. As such, cancer research which targets xCT has also been conducted in recent years.



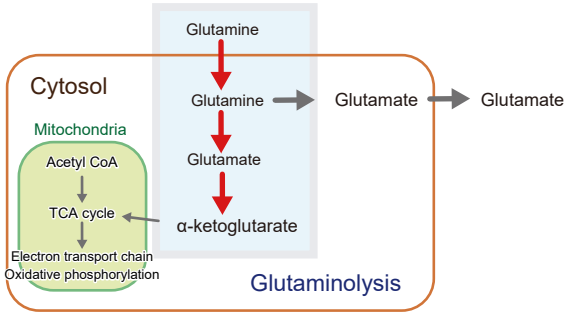
Measurement of Glutamine/Glutamate level

A549 cells were seeded in 6-well plates and the concentration of glutamine and glutamate in culture supernatant were measured over time using the Glutamine Assay Kit-WST and Glutamate Assay Kit-WST and Glutamate Assay Kit-WST.

The results showed that the concentration of glutamine in culture supernatant decreased with time, while the concentration of glutamate increased with time.



Conditions : Cell Line: A549 cells (5x10^5 cells)
Incubation: 50 hrs

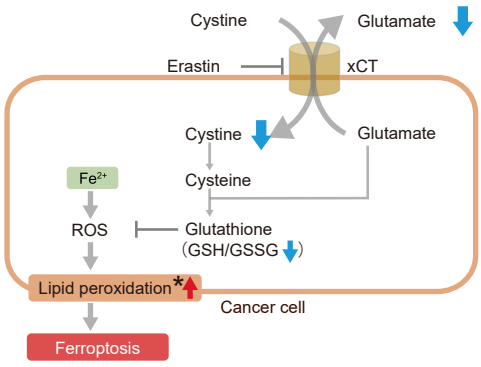


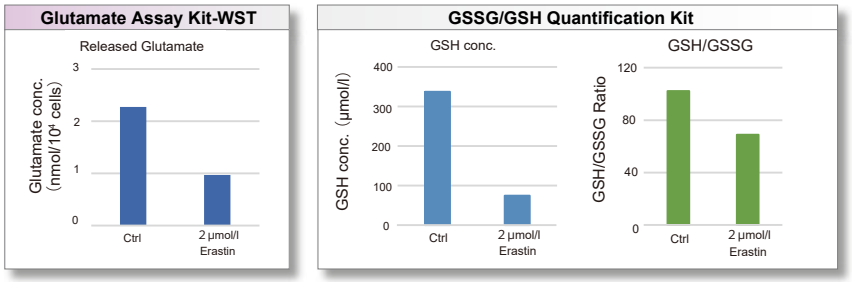
Induction of Ferroptosis by Erastin

Erastin is a known inducer of ferroptosis by inhibiting the Cystine/Glutamate transporter (xCT).

Released Glutamate and intracellular Glutathione in erastin-treated A549 cells were measured using Glutamate Assay Kit-WST and GSSG/GSH Quantification Kit (Code G257).

As a result, decrease in release of Glutamate and intracellular Glutathione by inhibition of cystine uptake was obseved in erastin treated cells .





Conditions
Cell Line: Jurkat cells (3x10^6 cells)
Stimulation condition: DOX (final conc.: 500 nmol/l), 24 hrs
Sample: Culture supernatant (Glutamate)
Cell (GSH/GSSG)

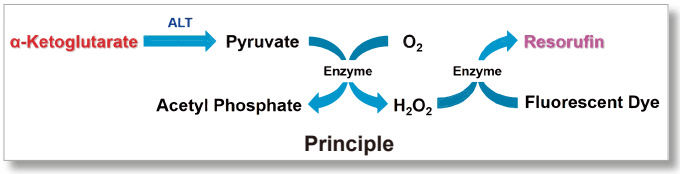
Description	Unit	Code
Glutamine Assay Kit-WST	100 tests	G268
Glutamate Assay Kit-WST	100 tests	G269

α-KG

α-Ketoglutaric Acid Measurement

α-Ketoglutarate Assay Kit-Fluorometric

α-Ketoglutarate Assay Kit-Fluorometric is a fluorometric assay kit for quantification of intracellular α-ketoglutarate (α-KG) .
α-KG is an important intermediate of the TCA cycle. It is used as an indicator of enhanced uptake of glucose metabolites into TCA cycle and of enhanced glutaminolysis, a pathway that supplies α-KG using glutamine as a substrate.
α-KG has been attracting attention as a metabolic indicator since it plays an essential role in the production of glutamate and γ-aminobutyric acid (GABA), and contributes scavenging the reactive oxygen species.

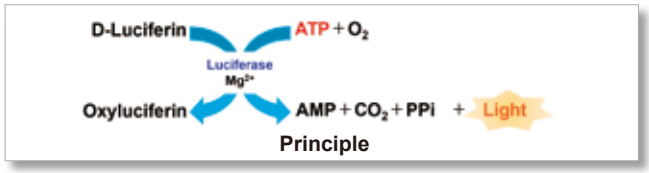


ATP

ATP Measurement

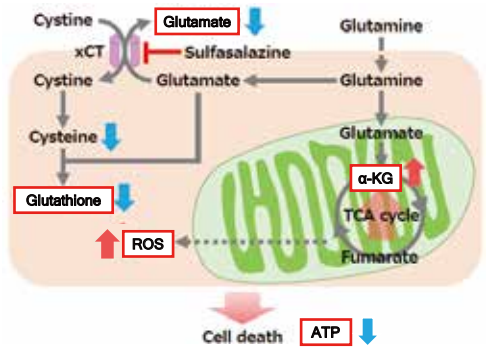
ATP Assay Kit-Luminescence

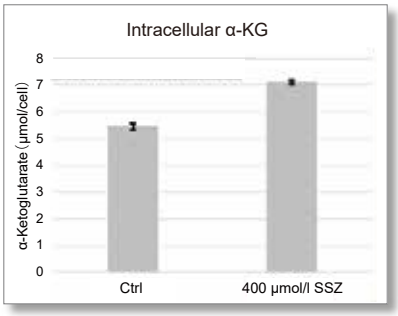
ATP Assay Kit-Luminescence is a luciferase luminescence assay kit for quantification of intracellular ATP. ATP is an important energy source of living cells that is synthesized in both glycolysis and mitochondrial oxidative phosphorylation.
Mitochondrial dysfunction reduces ATP levels in cells and the 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 also 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.

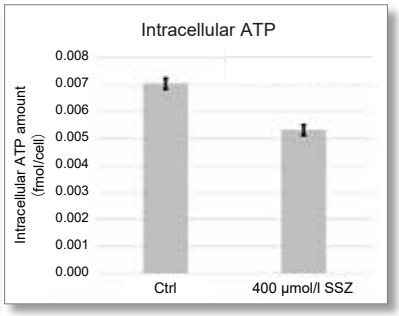


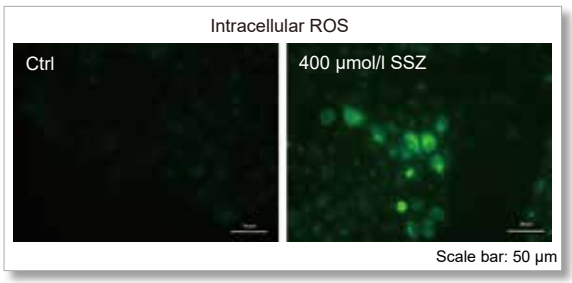
Mesurement of α-KG and ATP

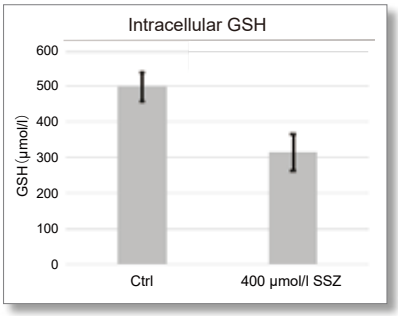
Sulfasalazine (SSZ), a known inhibitor of cystine/glutamate transporter (xCT), was added to A549 cells and the changes in intracellular α-ketoglutarate (α-KG), ATP, glutathione (GSH), ROS and glutamate release were observed.
The results showed that the addition of SSZ decreased intracellular ATP, glutathione (GSH) and glutamate release, and increased intracellular α-ketoglutarate and ROS.

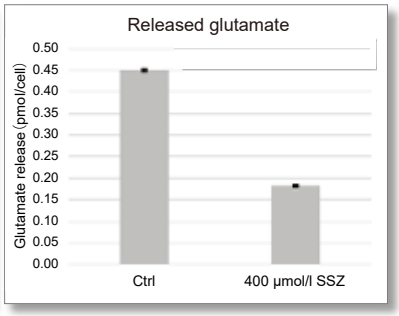












Experimental Condition
Cell Line: A549 (5x10^6 cells) Stimulation: 48hrs

Description	Unit	Code
α-Ketoglutarate Assay Kit-Fluorometric	100 tests	K261
ATP Assay Kit-Luminescence	50 tests 200 tests	A550

Related product

Description	Unit	Code
ROS Assay Kit -Highly Sensitive DCFH-DA	100 tests	R252
Glutamate Assay Kit-WST	100 tests	G269
GSSG/GSH Quantification Kit	200 tests	G257

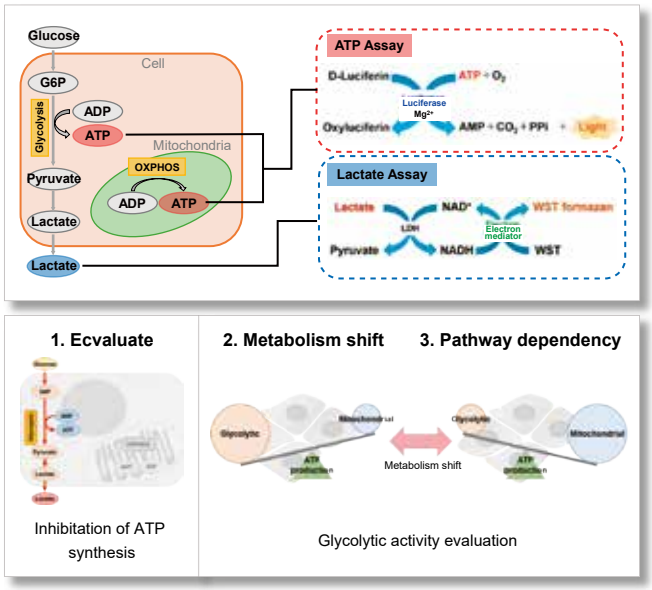
Glycolysis/OXPHOS

Glycolysis/Oxidative phosphorylation Assay

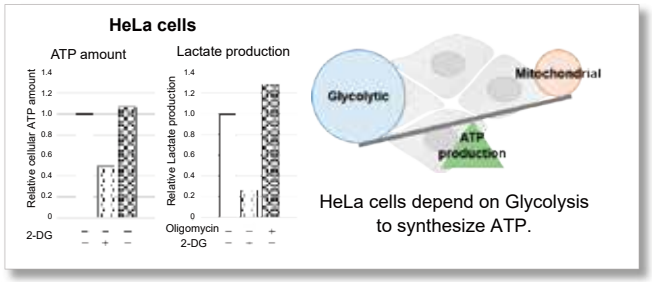
Glycolysis/OXPHOS Assay Kit

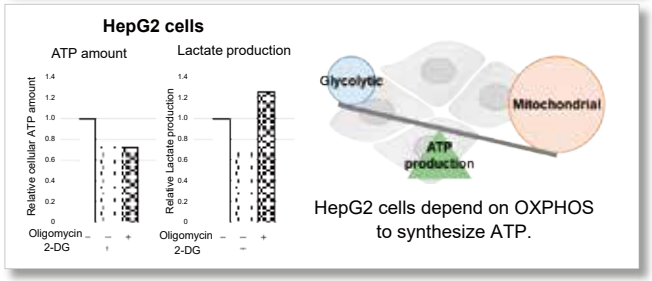
Many cancer cells produce ATP through energy metabolism that depends on the glycolytic pathway. On the other hand, it has been recently reported that cancer cells whose glycolytic pathway is suppressed, survive by shifting their energy metabolism to oxidative phosphorylation(OXPHOS). These phenomena have attracted much attention because it will not only help elucidate the mechanisms of anticancer drugs but also lead to the development of therapeutic strategies in various diseases including aging and neurodegenerative disorders.

This kit evaluates glycolytic capacity, metabolism shift, and whether cells rely on the glycolytic system or oxidative phosphorylation for energy production using a plate reader. This ready-to-use kit includes all the reagents required.



Comparison of metabolic pathway dependence in different cell lines





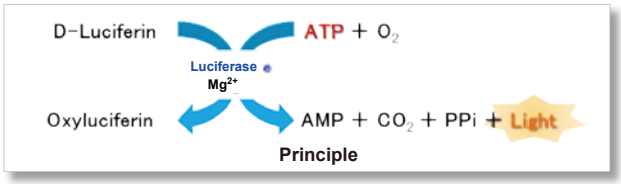
ATP ADP

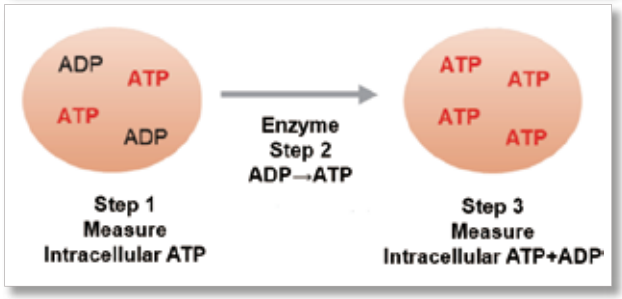
ADP/ATP Ratio Assay

ADP/ATP Ratio Assay Kit-Luminescence

Normally, when the intracellular ATP production decreases, the degradation product ADP will be resynthesized to ATP to maintain the intracellular ATP concentrations. However, when the metabolism of ATP production is disrupted, ATP cannot be resynthesized from ADP, but intracellular ATP is converted to ADP and the ADP/ATP ratio increases. The change in ADP/ATP ratio is not only related to apoptosis and autophagy, but also intracellular energy metabolism, and is used as one of the indicators of metabolic activity.

This kit can measure the ratio of ADP to ATP in cells. First, measure intracellular ATP by the luciferase luminescence method.

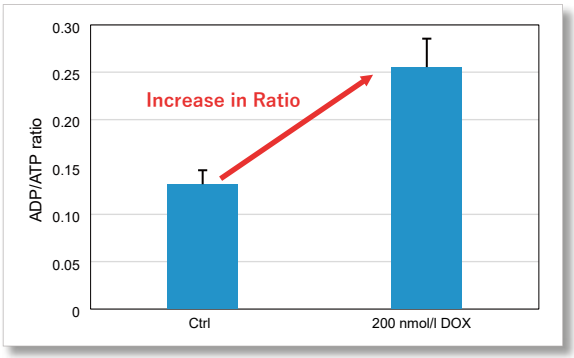




ATP/ADP change in senescence-induced cells

We measured the ADP/ATP ratio of A549 cells treated with Doxorubicin (DOX) and untreated A549 cells using this kit.

As a result, it was confirmed that the ADP/ATP ratio of DOX-treated A549 cells increased predominantly, and it was possible to observe the variation of ADP/ATP ratio by senescence induction using this kit.



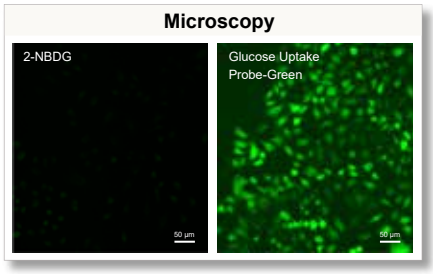
Description	Unit	Code
Glycolysis/OXPHOS Assay Kit	50 tests	G270
ADP/ATP Ratio Assay Kit-Luminescence	100 tests	A552

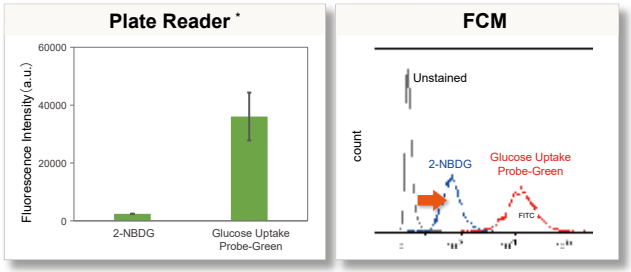
Glucose

Glucose Uptake Assay

Glucose Uptake Assay Kit-Blue,Green,Red

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, compared to the existing method (2-NBDG), the measurement time can be significantly reduced.





* Only Green and Red can be detected with a plate reader.

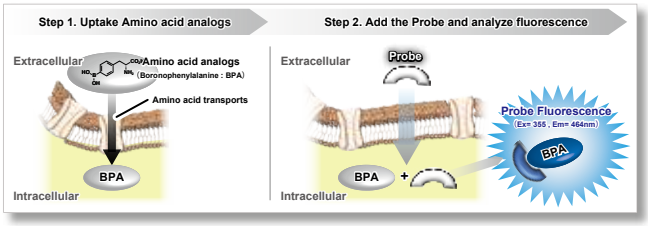
Amino acid

Amino Acid Uptake Assay

Amino Acid Uptake Assay Kit

Amino acids are essential for intracellular protein and nucleic acid synthesis, Especially for cancer cells which are proliferating continually. Since the supply of acetyl CoA from the glycolytic pathway is decreased in cancer cells, they have a furthermore huge demand for amino acids which are an important nutrient source for the TCA cycle. A research has shown that cancer cells increase the expression of the amino acid transporter LAT1 (L-type amino acid transporter [1)] and take up a large number of amino acids, which is expected to be a new target for anti-Cancer drugs' discovery.

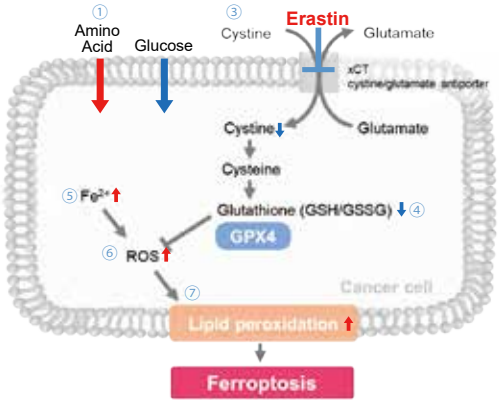
This product is suitable for fluorescence imaging, plate reader, and flow cytometry, making it possible to visualize and quantify the amino acid uptake ability of cells and is useful for evaluation of amino acids uptake ability and screening of amino acids transporter inhibitors.

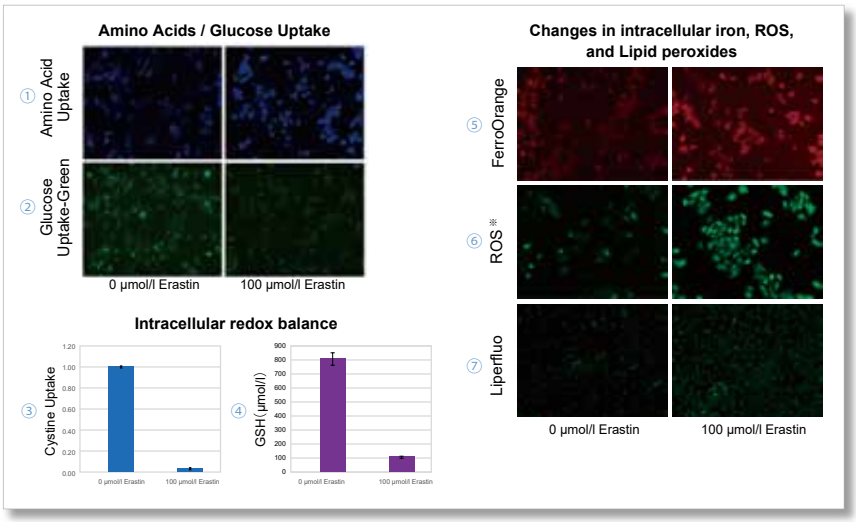


Induction of Ferroptosis by Erastin

The following experimental examples show changes in each aforementioned index as a consequence of erastin stimulation. Measurements were made using Dojindo reagents.

Using erastin-treated A549 cells, we measured intracellular Fe^{2+}, ROS, lipid peroxide, glutathione, glutamate release into the extracellular space, and cystine uptake. As a result, inhibition of xCT by elastin was observed and also the release of glutamate and uptake of cystine were decreased. Furthermore, elastin treatment decreased intracellular glutathione while it increased intracellular Fe^{2+} , ROS, and lipid peroxides.





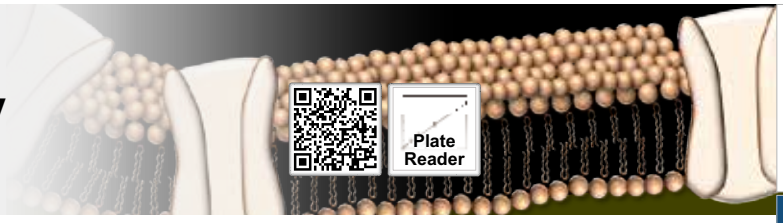
Description	Unit	Code
Glucose Uptake Assay Kit-Blue	1 set *1	UP01
Glucose Uptake Assay Kit-Green	1 set *1	UP02
Glucose Uptake Assay Kit-Red	1 set *1	UP03
Amino Acid Uptake Assay Kit	20 tests *2 100 tests *2	UP04

*1 ＜Approximate number of uses per 1 set＞ 35 mm dish x 10, 96-well plate x 1 *2 < Approximate number of uses per 100 tests> 35 mm dish x 10, 96-well plate x 1

Cystine Uptake Assay

Cystine

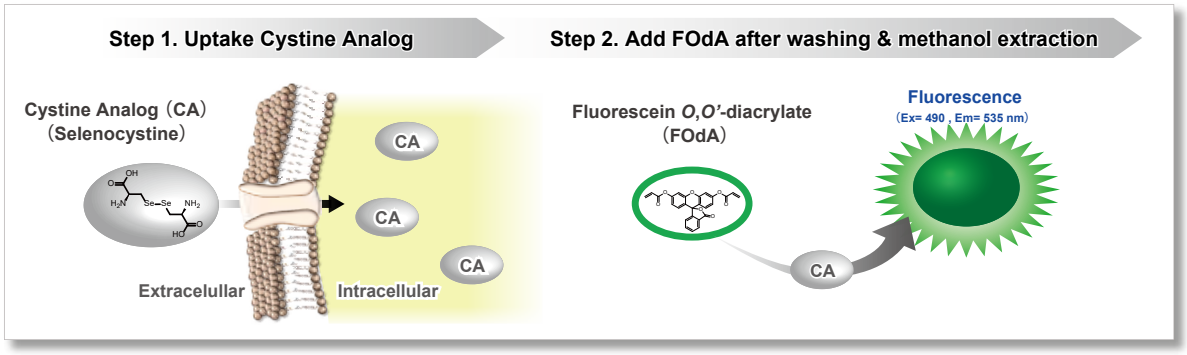
Cystine Uptake Assay Kit



This kit uses Selenocystine as a Cystine Analog (CA) and can measure the cystine uptake ability of cells by a plate reader in a short time.

What you can do with the kit

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 pending]



The relevant technicals are published in the following journal:

Shimomura T, Hirakawa N, Ohuchi Y, Ishiyama M, Shiga M, Ueno Y, Simple Fluorescence Assay for Cystine Uptake via the xCT in Cells Using Selenocystine and a Fluorescent Probe. *ACS Sensors*, 2021, *6*(*6*), 2125-2128

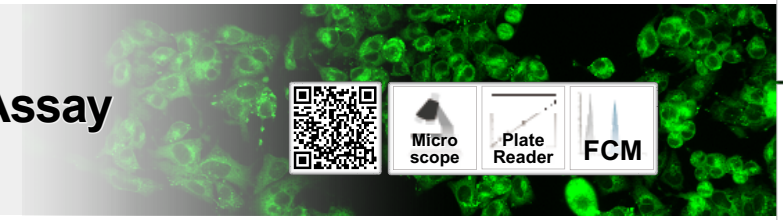
Description	Unit	Code
Cystine Uptake Assay Kit	20 tests * 100 tests *	UP05

*<Approximate number of uses per 100 tests> 96-well plate x 1

Fatty Acid Uptake Assay

Fatty acid

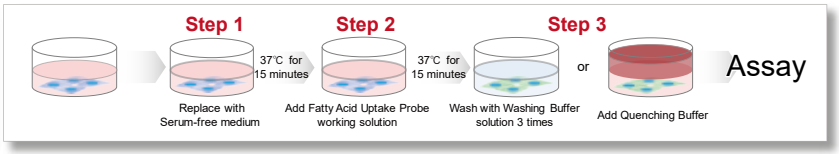
Fatty Acid Uptake Assay Kit



This kit contains a fatty acid analog (Fatty Acid Uptake Probe) which can be taken up by cells via fatty acid transporters, and fatty acid uptake ability can be detected by a fluorescence method (Principle). The Quenching Buffer enables detection without cell-washing steps (Protocol).

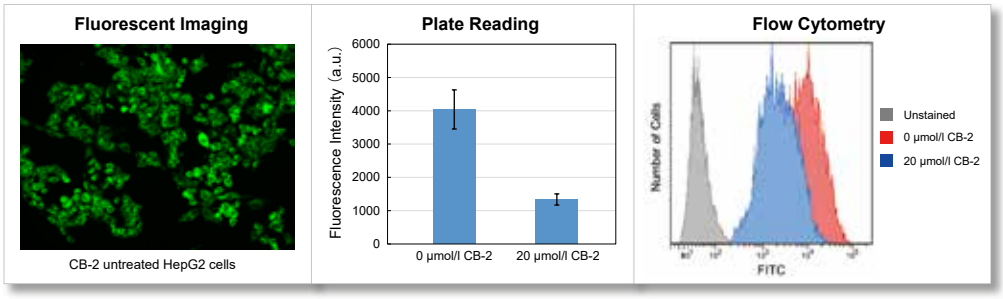
Principle

Protocol



Supported Devices

We were able to observe and quantify the inhibition of fatty acid uptake in HepG2 cells by a fatty acid transporter inhibitor (FATP2 inhibitor: CB-2).



Description	Unit	Code
Fatty Acid Uptake Assay Kit	100 tests *	UP07

* <Approximate number of uses per 100 tests> 35 mm dish x 10, 96-well plate x 1

Glycolysis / Mitochondria Membrane Potential Assay

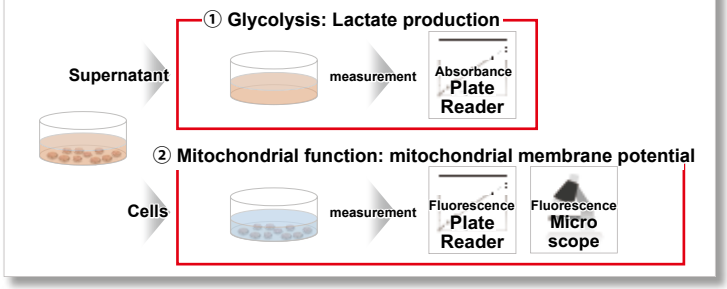
Glycolysis/JC-1 MitoMP Assay Kit

Fluorescence Micro scope | Absorbance / Fluorescence Plate Reader

Glycolysis/JC-1 MitoMP Assay Kit enables the measurement of lactate production (via a lactate assay) to detect changes in the glycolysis, as well as the mitochondrial membrane potential (via a JC-1 assay) to assess mitochondrial function, from the same samples, using cell culture supernatant and the cells respectively. The kit includes all necessary reagents for the assays, and a combined protocol is available.

Simultaneous measurement of the same sample

Measure two indicators in same sample



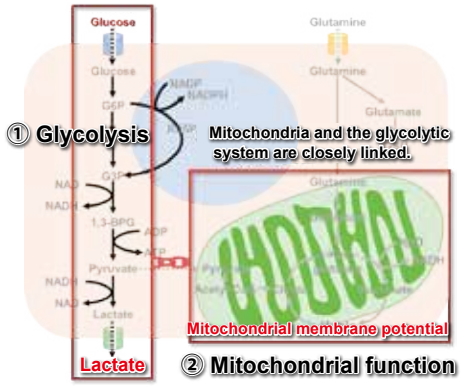
All reagent acquired is included, ready-to-use kit

Easy-to-understand, detailed protocols are included

Why monitor glycolysis and mitochondrial function?

The link between mitochondrial function and cellular metabolism is well-known, with implications for a range of diseases including cancer, aging, and neurodegenerative diseases. It has been found that aging cells often rely on the glycolytic system for survival, rather than drawing on mitochondrial energy sources. Conversely, cancer cells, which typically depend heavily on glycolysis, activate mitochondrial function to ensure their survival even when the glycolytic system is inhibited. Given these observations, there is a growing necessity to investigate both mitochondrial function and the glycolytic pathway to enhance our understanding of intracellular metabolic alterations.

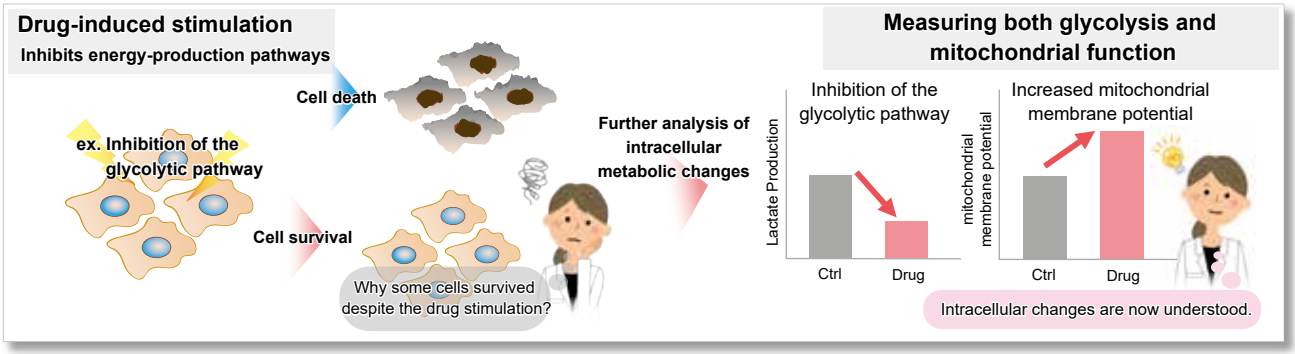
Energy production pathways



Intracellular metabolic changes induced by drug stimulation

Simultaneous monitoring of glycolysis and mitochondrial function

In certain instances, cells manage to survive despite sustaining damage to their glycolysis 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. Simultaneously monitoring both the glycolysis and mitochondrial function, as detailed below, can give an insight into what is happening inside the cell.



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

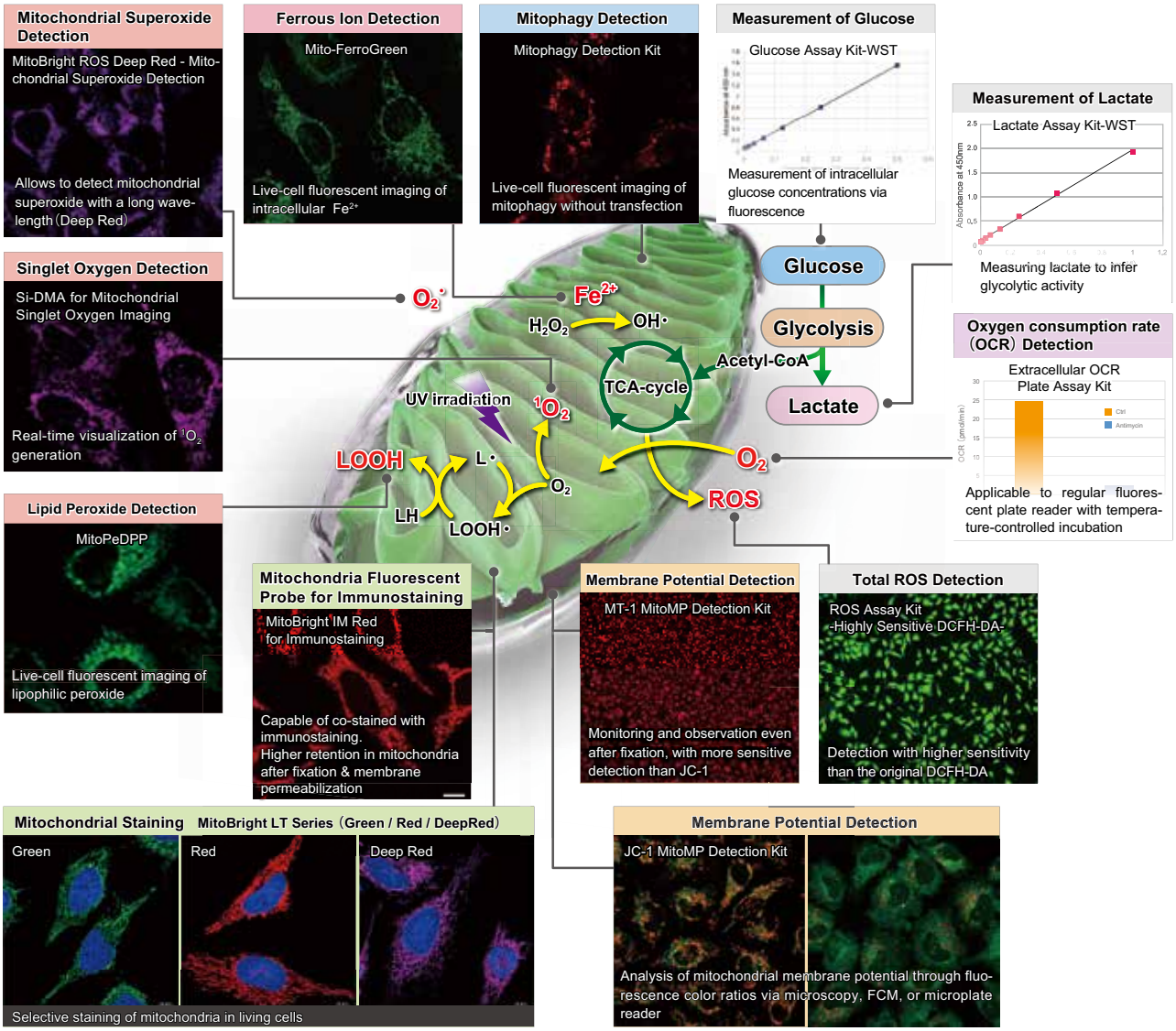
* <Approximate number of uses> 96-well plate x 0.5

Mitochondria Research

Mitochondria, which have important functions in cells, are the place for ATP production by oxidative phosphorylation, and their activity and dysfunction are closely related to cancer, senescence, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Therefore, understanding the state of mitochondria is particularly important.

Product lineup for Mitochondrial research

Mitochondrial research products include the detection of Mitophagy, which is a quality control system for damaged mitochondria, the detection of ROS: reactive oxygen species (Si-DMA, MitoBright ROS), and the detection of peroxidation caused by oxidation by ROS. Detection of lipid peroxides (MitoPeDPP), as well as various fluorescent probes for mitochondrial staining and membrane potential dependent fluorecent probes (MitoBright LT series Green/Red/Deep Red), MitoBright IM, JC-1, MT-1) are available.



Mitochondrial Staining

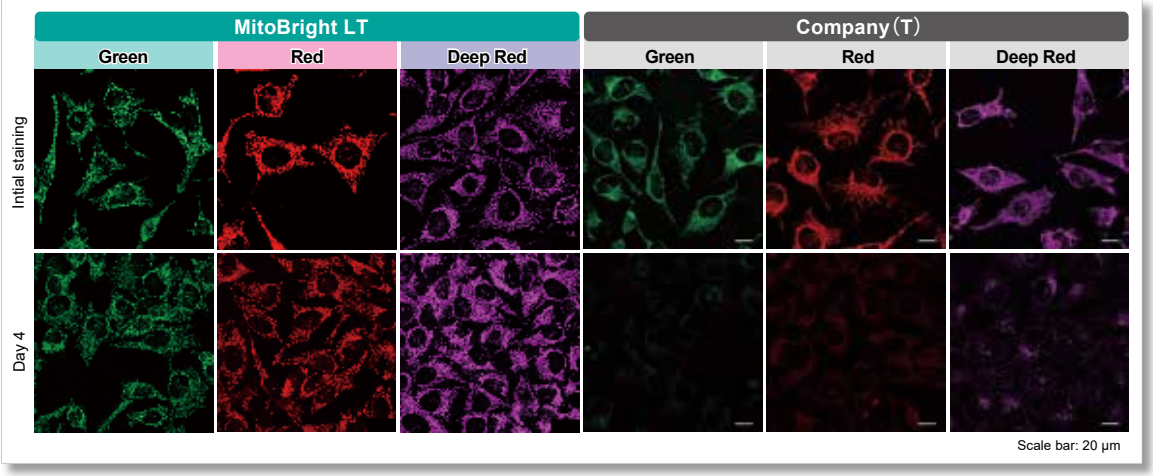
MitoBright LT Green / Red / Deep Red

Micro scope FCM

MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared to 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.

Long-time observation

HeLa cells were washed with HBSS and subsequently stained with each of MitoBright LTs or an existing reagent. The culture medium was replaced with serum-containing medium, and mitochondria were observed after 4 days of incubation. Fluorescence intensity of an existing reagent decreased significantly, while that of MitoBright LT remained unchanged and mitochondria were clearly observable.



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

*<Estimated number of use per 400μl> 35 mm dish x 200

Mitochondria Fluorescent Probe

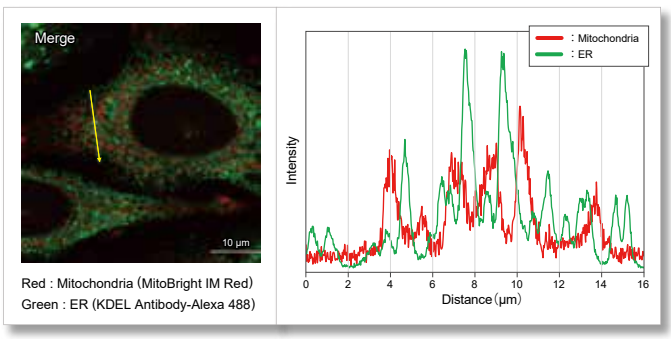
MitoBright IM Red for Immunostaining

Micro scope

MitoBright IM has a structure that allows to be easily retained in mitochondria even during the immunostaining process after staining live cells. MitoBright IM is a new reagent that overcomes the problem of combined use with immunostaining.

Clear observation of various organelles

Mitochondria of Hela cells were stained with MitoBright IM, then fixed and permeabilzed. After that, cells were co-stained with immunostaining using KDEL antibody, a marker protein of the endoplasmic reticulum(ER). The fluorescence intensity was measured in the area indicated by the blue arrow in the fluorescence image (right). As a result, the morphology of the mitochondria and the neighboring endoplasmic reticulum was clearly observed.



Description	Unit	Code
MitoBright IM Red for Immunostaining	20 μl * 20 μl ×3 *	MT15

*<Approximate number of uses per 20 μl> 35 mm dish x 10

JC-1 Mitochondrial Membrane Potential Detection

JC-1 MitoMP Detection Kit

Micro scope | FCM | Plate Reader

MT-1 Mitochondrial Membrane Potential Detection

MT-1 MitoMP Detection Kit

Micro scope | FCM

Mitochondria is an important organelle that uses oxygen to synthesize ATP, producing the necessary energy for live cells to thrive. Decreased mitochondrial activity and mitochondrial dysfunction are associated with cancer, aging, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Therefore, mitochondrial membrane potential (MMP) has been widely studied as a promising target for mitochondria-related diseases.

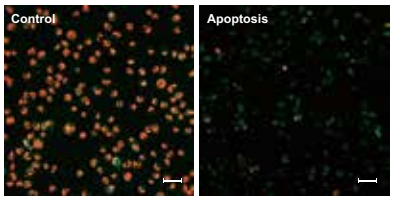
Product	Features	Sensitivity	Fixation	Monitoring	Applicable Instruments
JC-1 MitoMP Detection Kit	Recomended for starting-up	✓			Micro scope, FCM, Plate Reader
MT-1 MitoMP Detection Kit	Recommended for more detailed analysis	✓ (High)	✓	✓	Micro scope, FCM

Applicable to various instruments — **JC-1** MitoMP Detection Kit

Jurkat cells treated with Staurosporine, an apoptosis inducing reagent, were stained with JC-1 MitoMP Detection Kit.

Fluorescence Microscopy

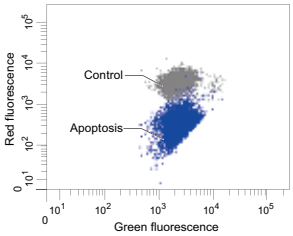
Clear imaging of the change in mitochondrial membrane potential caused by induced apoptosis



Imaging Condition Green：Ex=488 nm , Em=500-550 nm
Red ：Ex=561 nm , Em=560-610 nm
Scale Bar：80 μm

Flow Cytometer

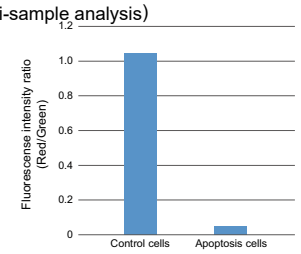
Quantitative analysis of change in mitochondrial membrane potential in each cells.



Detecting Condition Green：Ex=488 nm , Em=515-545 nm
Red ：Ex=488 nm , Em=564-604 nm

Plate Reader

Analysis of the change in mitochondrial membrane potential of all cells in a well (useful for multi-sample analysis)

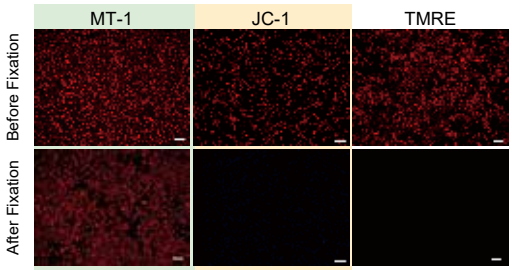


Detecting Condition Green：Ex=485 nm , Em=525-545 nm
Red ：Ex=535 nm , Em=585-605 nm

More detailed analysis — **MT-1** MitoMP Detection Kit

Applicable to fixation after staining

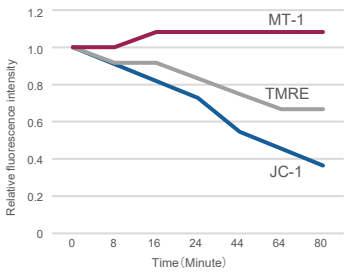
MT-1 dye is retained in cells even fixed with PFA after staining. It enables to conduct a reproducible experiment.



Imaging Condition Ex=530-560 nm, Em=570-640 nm
Scale Bar：100 μm

Allows to monitor MMP

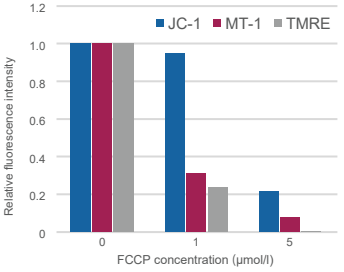
Fluorescence intensity of MT-1 is retained after staining.



Detecting Condition
Ex=530-560 nm, Em=570-640 nm

High sensitivity

Demonstrated high sensitivity equivalent to TMRE.



Detecting Condition
Ex=530-560 nm, Em=570-640 nm

FCCP：carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone

Description	Unit	Code
JC-1 MitoMP Detection Kit	1 set *1	MT09
MT-1 MitoMP Detection Kit	1 set *2	MT13

*1 ＜Approximate number of uses（Reagent concentration: 2 μmol/l)＞ 96 well plate x 5, 35 mm dish x 25, *2 ＜Approximate number of uses＞ 35 mm dish x 30

Mitochondrial Lipid Peroxide Detection

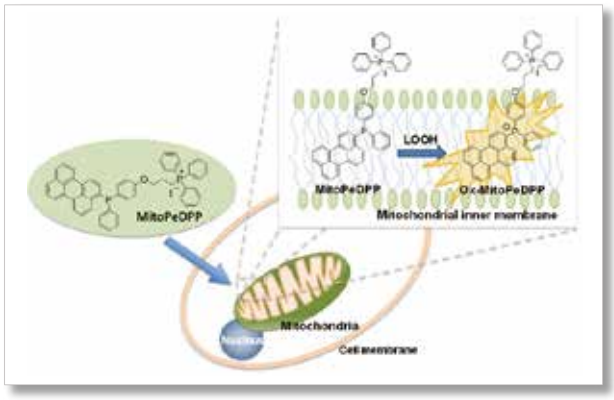
MitoPeDPP

Micro scope

MitoPeDPP is a fluorescent dye which penetrates cell membrane and accumulates in mitochondria. It is oxidized specifically by the lipid peroxide in mitochondrial membrane and emits strong fluorescence. MitoPeDPP enables the imaging of lipid peroxide that were oxidized under oxidative stress conditions (ROS, etc.) in mitochondrial membrane. It is important to evaluate the reactive oxygen species (ROS) in mitochondria where oxygen for energy production is consumed. MitoPeDPP is also used to monitor lipid peroxidation in ferroptosis research.

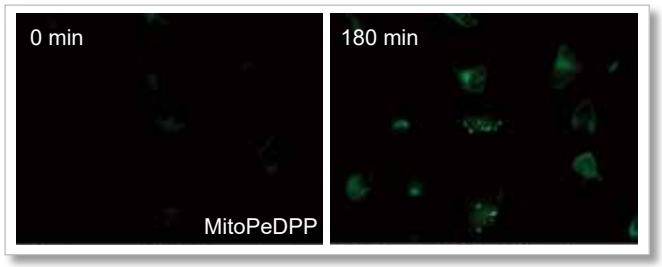
*This probe has been developed by Dr. Shioji et al., Fukuoka University, Department of Chemistry

Principle



Detection of mitochondrial lipid peroxide using rotenone

Additional information is available on our website.



Description	Unit	Code
MitoPeDPP	5 μg ×3 *	M466

* ＜Approximate number of uses per 5 μg＞ 96 well plate x 1-5, 35 mm dish x 5-25

Oxygen Consumption Rate(OCR) Plate Assay Kit

Extracellular OCR Plate Assay Kit

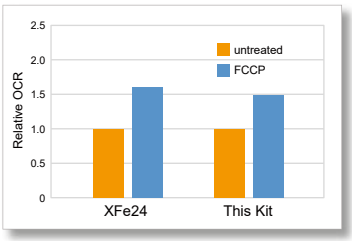
* OCR：Oxygen Consumption Rate

Fluorescent Plate Reader

Comparison with Flux Analyzer

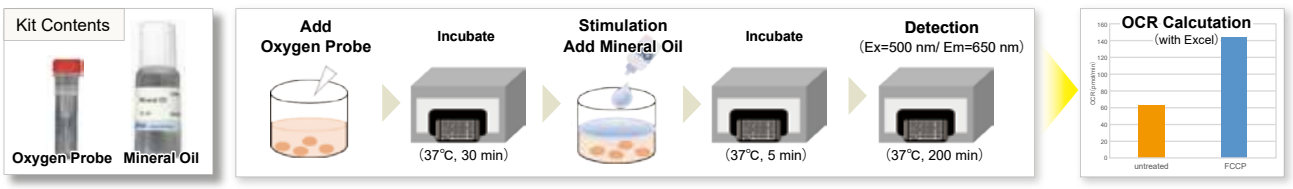
Flux Analyzer (XFe24) and this kit were measured on the same day under the same conditions (cell type, cell number, and FCCP concentration).
As a result, correlated data of oxygen consumption rate changes was obtained for XFe24 and this kit.

* This product was commercialized under the guidance of Dr. Toshitada Yoshihara, Gunma University.



Cell Type : HepG2
Cell Number : 5×10^4 cells/well
FCCP Conc. : 2 μmol/l

The Extracellular OCR Plate Assay Kit includes an Oxygen Probe, which has the property of increasing phosphorescence intensity as the oxygen concentration in the medium decreases, and Mineral Oil blocks the influx of oxygen from the air.
After measuring the phosphorescence intensity according to the extracellular oxygen concentration with a fluorescent microplate reader, the OCR of the cells is calculated (automatic calculation sheet) based on the Stern-Volmer equation.



Description	Unit	Code
Extracellular OCR Plate Assay Kit	100 tests	E297

Mitochondrial Superoxide Detection

MitoBright ROS Deep Red - Mitochondrial Superoxide Detection

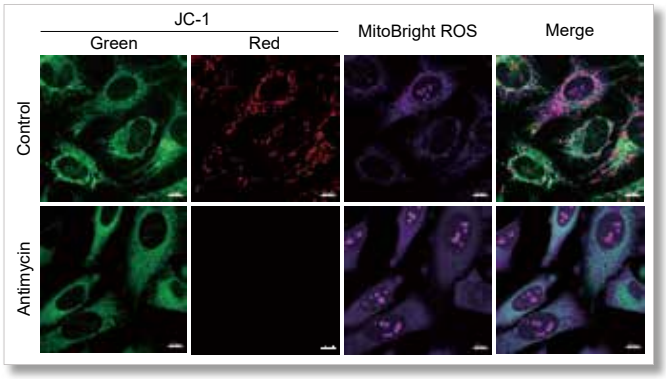
*Same product as Product code 'MT14'

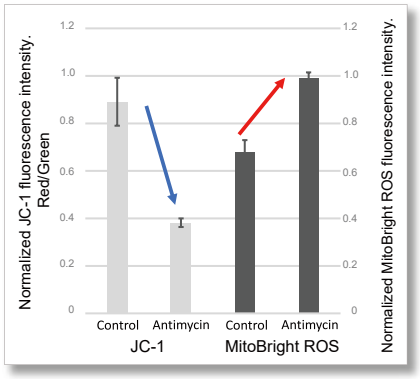
Micro scope | Plate Reader | FCM

MitoBright ROS Deep Red selectively reacts with superoxide within mitochondria and emits fluorescence with a longer wavelength than existing products.

Simultaneous evaluation of mitochondrial superoxide and membrane potential

HeLa cells were washed with HBSS, co-stained with MitoBright ROS Deep Red and mitochondrial membrane potential staining dye (JC-1: code MT09 or MT-1: code MT13), and the generated mitochondrial ROS and membrane potential were observed simultaneously. As a result, the decrease in mitochondrial membrane potential and the generation of mitochondrial ROS were simultaneously observed.





Description	Unit	Code
MitoBright ROS Deep Red - Mitochondrial Superoxide Detection *1	100 nmol *2	MT16

*1. Same product as Product code 'MT14'. *2. ＜Approximate number of uses (Reagent concentration: 2 μmol/l)＞ 96 well plate x 5, 35 mm dish x 25

DOJINDO MOLECULAR TECHNOLOGIES, INC.

Mitochondrial Singlet Oxygen Imaging

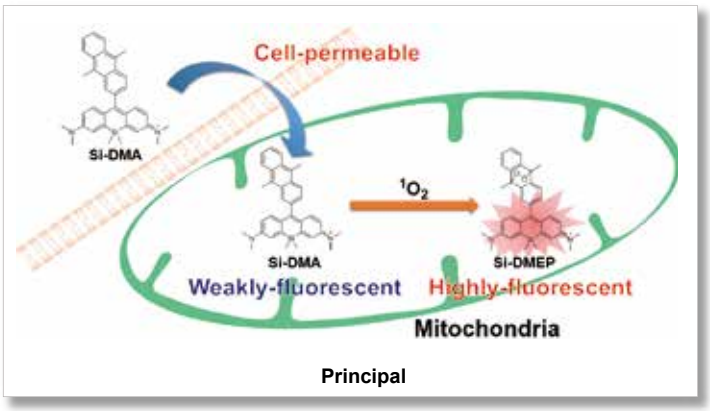
Si-DMA for Mitochondrial Singlet Oxygen Imaging

Micro scope

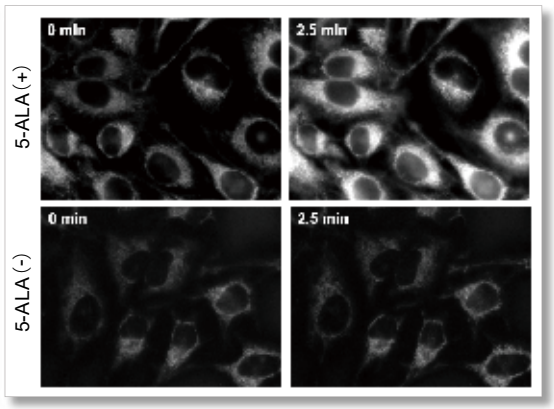
Majima et al. synthesized a new far-red fluorescence probe, "Si-DMA", composed of silicon-containing rhodamine and anthracene moieties. Under the presence of $^1O_2$, fluorescence of Si-DMA increases 17 times due to endoperoxide formation at the anthracene moiety. Among seven different ROS, Si-DMA can selectively detect the $^1O_2$. In addition, Si-DMA was able to visualize the real-time generation of $^1O_2$ from protoporphyrin IX in mitochondria with 5-aminolevulinic acid (5-ALA).

* This probe was developed by Dr. Tetsurou Majima et al., Osaka University

Product Description / Singlet oxygen detection after the addition of 5-ALA



Principal



Description	Unit	Code
Si-DMA for Mitochondrial Singlet Oxygen Imaging	2 μg *	MT05

* ＜Approximate number of uses＞ μ-Slide 8 well x 22 to 90

DOJINDO MOLECULAR TECHNOLOGIES, INC.

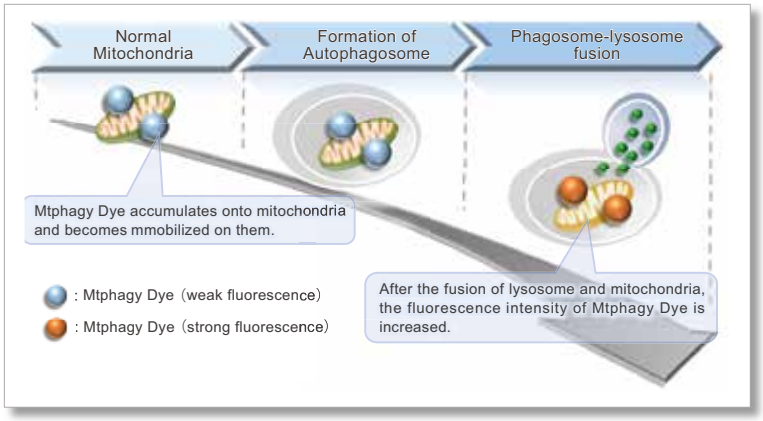
Mitophagy Detection

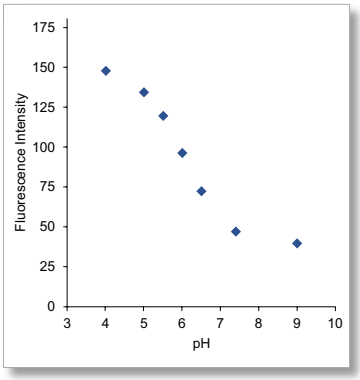
Mitophagy Detection Kit

Micro scope

Increased fluorescence intensity during mitophagy

This kit is composed of Mtphagy Dye and Lyso Dye. Mtphagy Dye accumulates to intact mitochondria, and 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 for co-staining.





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

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

* <Approximate number of uses> 96 well plate x 5, 35 mm dish x 25 (Reagent concentration: 2 µmol/l)

Mitochondrial Iron Detection

Mito-FerroGreen

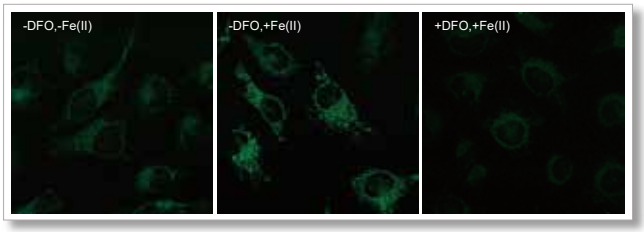
Micro scope

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

* This product was commercialized under the advisory of Dr. Hideko Nagasawa and Dr. Tasuku Hirayama (Gifu Pharmaceutical University).

Detection of mitochondrial Fe^{2+}

By adding Mito-FerroGreen to HeLa cells cultured in MEM growth medium containing serum, intrinsic iron in mitochondria was detected by fluorescence (left figure). On the other hand, strong fluorescence of Mito-FerroGreen was observed in HeLa cells treated with iron (iron (II) ammonium sulfate) (middle figure), while Mito-FerroGreen fluorescence was not observed in cells treated with iron chelating reagent (DFO: deferoxamine) and iron. Thus, difference in the amount of mitochondrial iron were confirmed as difference in fluorescence intensity. The protocol of this experiment is discribed in the manual (available on our website).

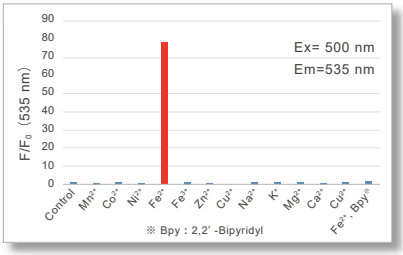


Ex=488 nm, Em=500-565 nm

High selectivity of Fe^{2+}

High selectivity of Fe^{2+} and high signal are obtained.

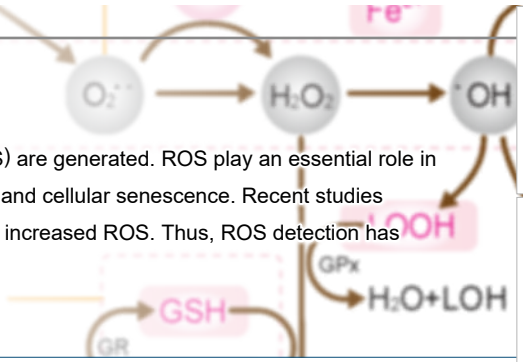
Add 2 µl of 1 mmol/l Mito-FerroGreen, 2 µl of 10 mmol/l various metals, and 20 µl of 1 mg/ml esterase to 1 ml of 50 mmol/l HEPES Buffer (pH 7.4) and fluorescence intensity was measured after 1 hour reaction at room temperature.



Description	Unit	Code
Mito-FerroGreen	50 µg ×2 *	M489

* <Approximate number of uses per 50 µg> µ-silde 8 well x 6

Oxidative Stress



Oxygen is used for energy production and along the process, reactive oxygen species (ROS) are generated. ROS play an essential role in signaling pathways and the immune system, while excess ROS is associated with diseases and cellular senescence. Recent studies suggested that ferroptosis is a new type of cell death characterized by iron dependency and increased ROS. Thus, ROS detection has been attracting considerable interest in ferroptosis research.

Oxidative stress related products

Reactive oxygen species (ROS) Detection

ROS Assay Kit -Highly Sensitive DCFH-DA-
(Code R252)

ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-
(Code R253)

Intracellular Iron Measurement

FerroOrange
(Code F374)

Mitochondrial Iron Detection

Mito-FerroGreen
(Code M489)

Mitochondrial Superoxide Detection

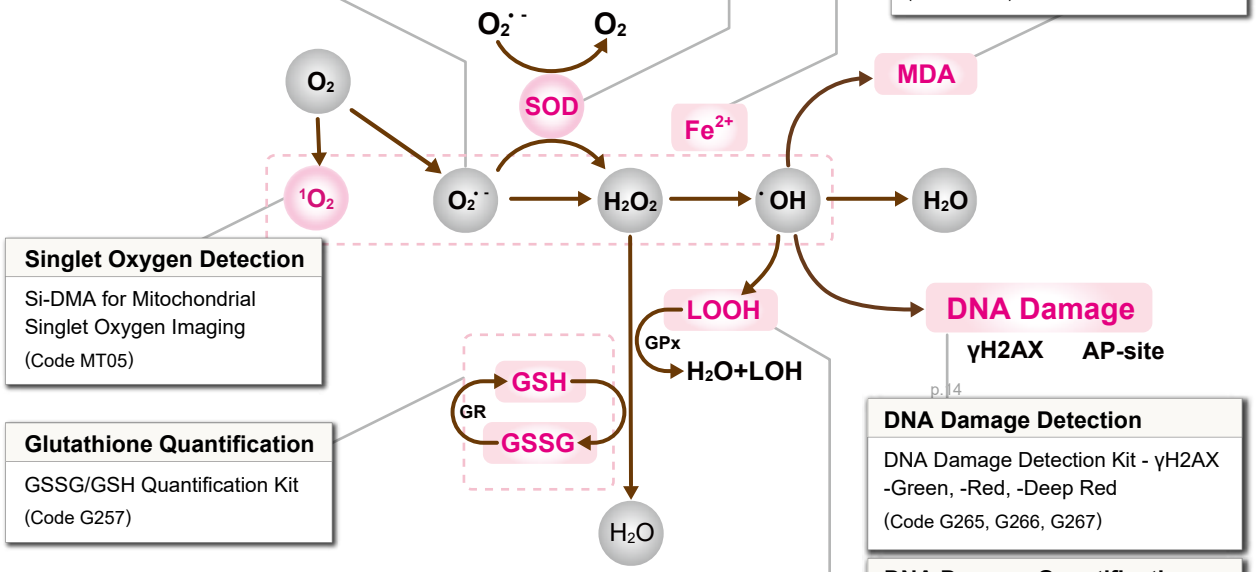
MitoBright ROS Deep Red
- Mitochondrial Superoxide Detection
(Code MT16)

SOD Activity Assay

SOD Assay Kit - WST
(Code S311)

MDA (malondialdehyde) Assay

MDA Assay Kit
(Code M496)



Singlet Oxygen Detection

Si-DMA for Mitochondrial Singlet Oxygen Imaging
(Code MT05)

Glutathione Quantification

GSSG/GSH Quantification Kit
(Code G257)

DNA Damage Detection

DNA Damage Detection Kit - γH2AX -Green, -Red, -Deep Red
(Code G265, G266, G267)

DNA Damage Quantification

-Nucleostain- DNA Damage Quantification Kit -AP Site Counting-
(Code DK02)

Mitochondrial Lipid Peroxide Detection

MitoPeDPP
(Code M466)

Lipid Peroxide Detection

Liperfluo
(Code L248)

Lipid Peroxidation Assay

Lipid Peroxidation Probe -BDP 581/591 C11-
(Code L267)

Oxidative Stress

Photo-oxidation Resistant Detection of total ROS

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

Micro scope | Fluorescent Plate Reader | FCM

The dye that is employed in this kit allows ROS detection with higher sensitivity than DCFH-DA; It does not leak from cells because the fluorescent dye is immobilized to protein via a chemical bond, and it is resistant to photo-oxidation compared with DCFH-DA.

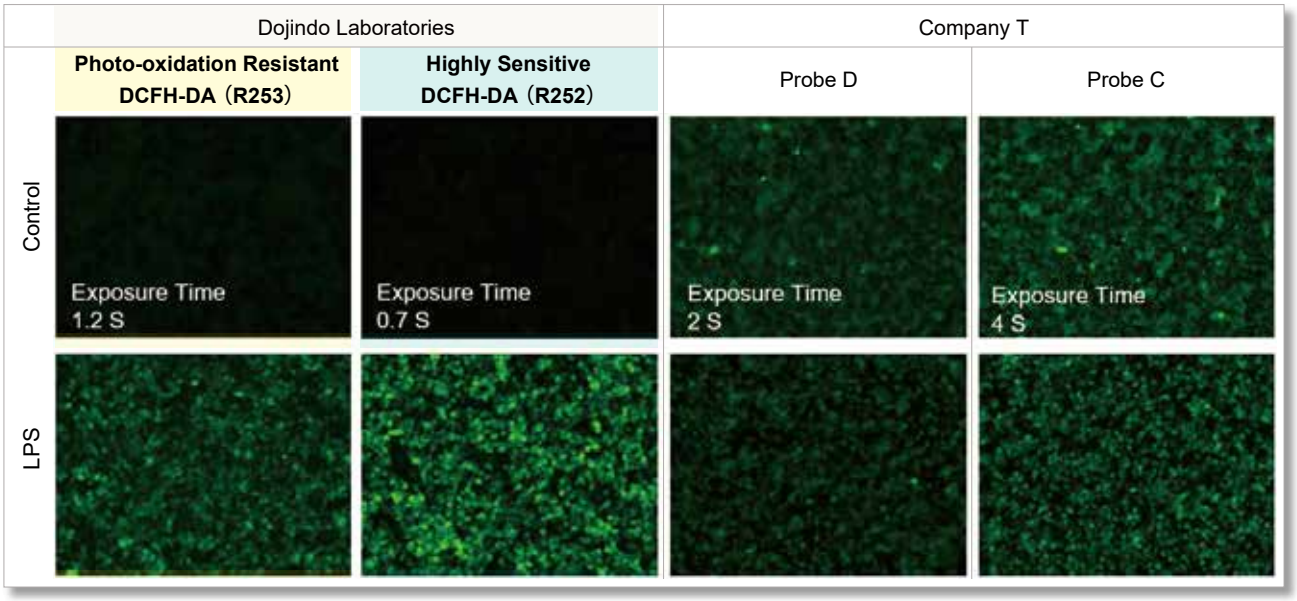
Highly Sensitive total ROS detection

ROS Assay Kit -Highly Sensitive DCFH-DA-

Micro scope | Fluorescent Plate Reader | FCM

The dye employed in the kit allows ROS detection with higher sensitivity than DCFH-DA. Moreover, the Loading Buffer included in this kit maintains cellular health during the assay.

Comparison of Fluorescent Sensitivity



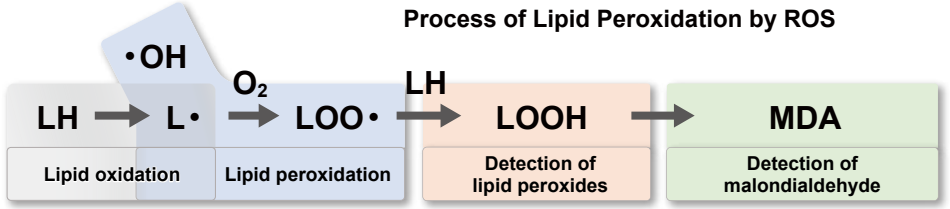
Comparison with Existing Reagents

	Dojindo Laboratories		Company T	
Product Name	**ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-**	**ROS Assay Kit -Highly Sensitive DCFH-DA-**	Probe D	Probe C
Photo-oxidation Resistant	◎ Highest resistant ability	× Photo-oxidation	× Photo-oxidation	△ Photo oxidation
Cell fixation	◎ Highest retention ability	× Leakage from cell	× Leakage from cell	○ OK for fixation
Sensitivity (Intracellular)	○ Better sensitivity	◎ Highest sensitivity	△ Lower sensitivity	△ Lower sensitivity
Code	R253	R252	—	—

Description	Unit	Code
ROS Assay Kit -Highly Sensitive DCFH-DA-	100 tests	R252
ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-	100 tests	R253

Reactive Oxygen Species (ROS) refers to a variety of reactive molecules derived from molecular oxygen. Various ROS are generated *in vivo* and each of them differs in its properties, reactivities and interactions. Therefore, it is essential to select an appropriate reagent to detect and analyze the ROS molecules accurately.

Process of Lipid Peroxidation by ROS

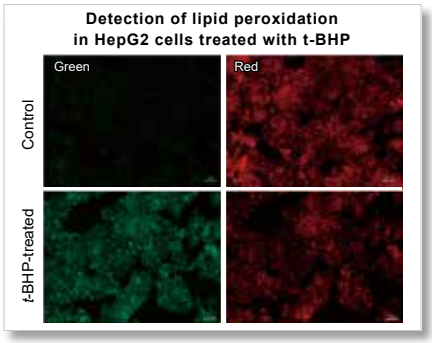


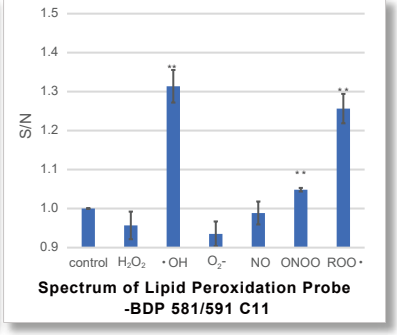
LH : Lipid
L• : Lipid radical
•OH : Hydroxyl radical
LOO• : Lipid peroxide radical
LOOH : Lipid peroxide
MDA : Malondialdehyde

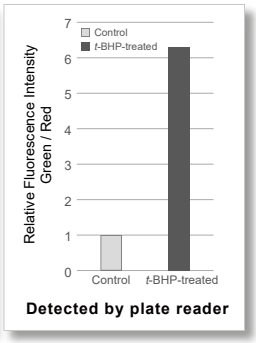
Lipid Peroxidation Detection — Lipid Peroxidation Probe -BDP 581/591 C11-

Micro scope | Fluorescent Plate Reader | FCM

Lipid Peroxidation Probe reacts with lipid radicals formed during lipid peroxidation by reactive oxygen species with high sensitivity.



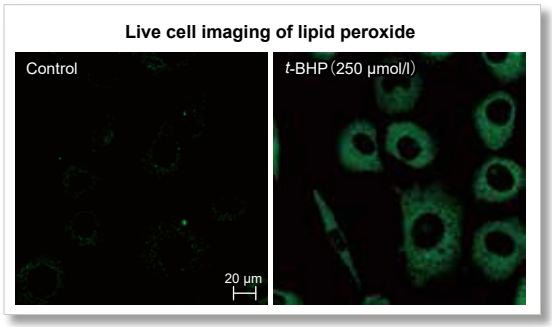


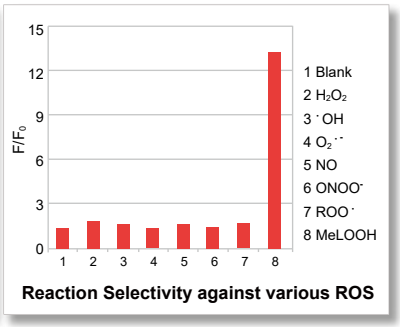


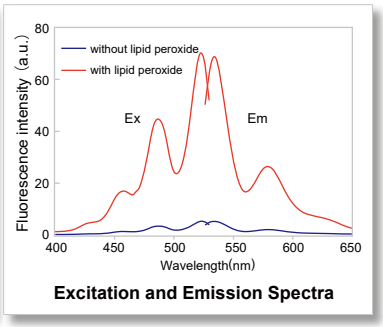
Lipid peroxide Detection — Liperfluo

Micro scope

Liperflow can be applied to lipid peroxide imaging by a fluorescence microscopy and a flow cytometric analysis of live cells and is used to monitor lipid peroxidation in ferroptosis research.



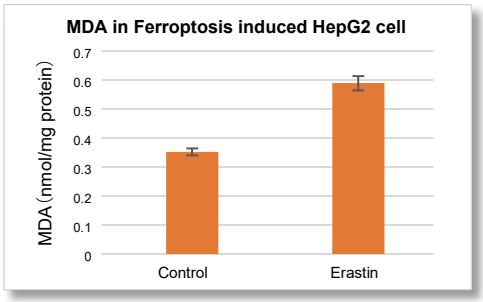


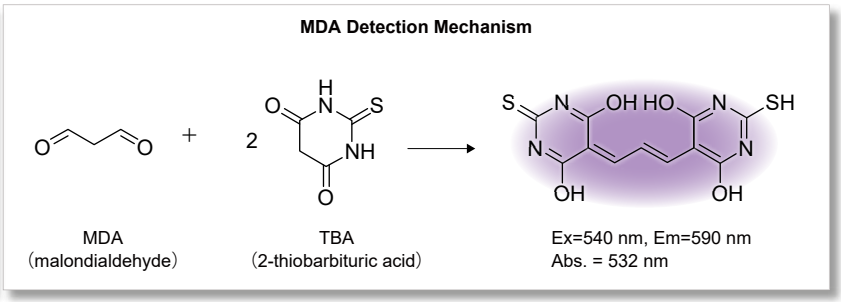


Malondialdehyde (MDA) Detection — MDA Assay Kit

Fluorescent Plate Reader

MDA Assay Kit uses the TBARS method to detect MDA in cells or tissues by measuring the fluorescence or absorbance of the MDA-TBA adduct.





Description	Unit	Code
Lipid Peroxidation Probe -BDP 581/591 C11-	200 tests *1	L267
Liperfluo	1 set (50 μg×5) *2	L248
MDA Assay Kit	100 tests *3	M496

*1 <Approximate number of uses> 96 well plate x 2, *2 <Approximate number of uses> 5-50 / 50 μg (cannot be stored), *3 <Approximate number of uses> 96-well plate x 1

Fluorescent Probe for Intracellular Iron Detection

FerroOrange

Micro scope | Plate Reader

FerroOrange is a novel probe that enables live-cell fluorescent imaging of intracellular Fe^{2+}. By simply adding it to cultured cell, it penetrates the cell membrane and selectively interacts with intracellular Fe^{2+}. FerroOrange is used in ferroptosis research.

* This product was commercialized under the advisory of Dr. Hideko Nagasawa and Dr. Tasuku Hirayama (Gifu Pharmaceutical University).

Live-cell imaging of intracellular iron

The fluorescence intensity of FerroOrange was increased in HeLa cells treated with Ammonium iron (II) sulfate compared to untreated cells; conversely, its fluorescence intensity was decreased in cells treated with Bpy, the chelator of iron.

＜Detection conditions＞ Ex: 561 nm、Em: 570-620 nm

Scale bars：20 μm

Description	Unit	Code
FerroOrange	1 tube * 3 tubes *	F374

*＜Approximate number of uses per 1 tube (24 μg)＞ μ-Slide 8 well x 20

DOJINDO MOLECULAR TECHNOLOGIES, INC.

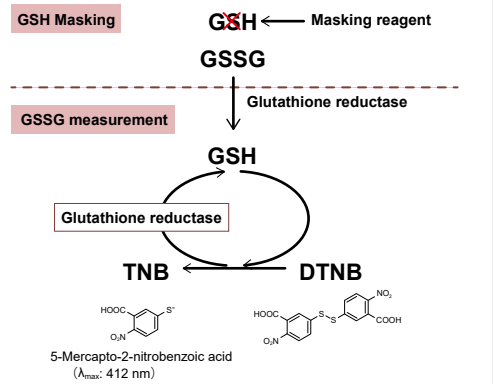
Glutathione Quantification

GSSG/GSH Quantification Kit

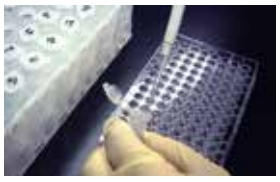
Plate Reader

GSSG/GSH Quantification Kit contains Masking Reagent of GSH. GSH can be deactivated in the sample by simply adding the Masking Reagent. Therefore, GSSG is quantified by measuring the absorption (λ max = 412 nm) of DTNB (5,5 Edithiobis (2-nitrobenzoic acid) using the enzymatic recycling system. GSH amount can also be determined by subtracting GSSG from the total amount of glutathione.

Assay principle and procedure



Principle of total glutathione quantification



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

3) Incubate at 37℃ for 1h.

4) Add Substrate working solution and Enzyme/ Coenzyme working solution to each well.

5) After incubating at 37℃ for 10 minutes, measure the absorbance of each well with a microplate reader.

Description	Unit	Code
GSSG/GSH Quantification Kit	200 tests	G257

Highly Sensitive Detection of Cellular Senescence

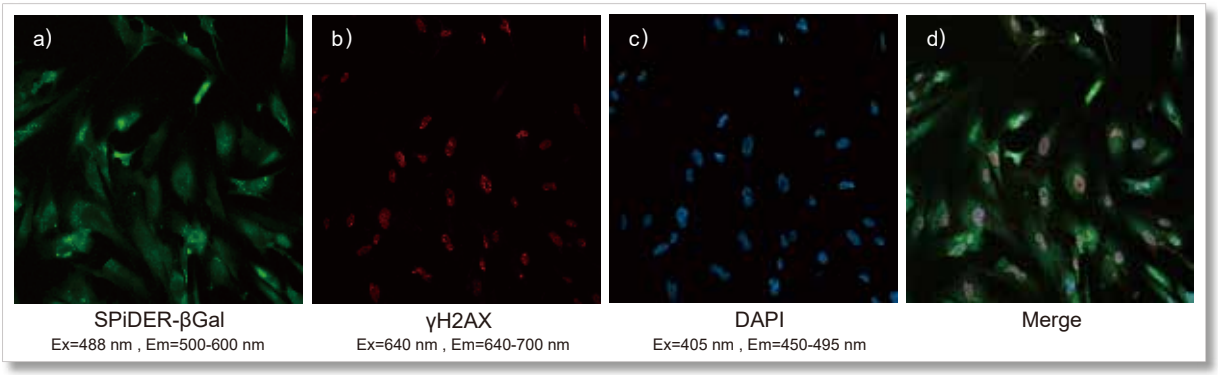
Cellular Senescence Detection Kit - SPiDER-βGal

Micro scope FCM

Cellular Senescence Detection Kit – SPiDER-βGal is a fluorometric assay kit to detect SA-β-gal with high sensitivity and ease of use. SPiDER-βGal is a reagent to detect β-galactosidase which possesses a high cell-permeability and a high intracellular retention enabling the use in both live and fixed cells. This kit can be applied to quantitative analysis by flow cytometry, and co-staining with SA-β-Gal and other markers.

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

WI-38 cells (passage 10) were stained with this kit, DNA damage marker γH2AX as one of the senescence markers, and DAPI for indicating the location of nucleus. Details of this experiment are available on our website.



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

* <Approximate number of uses> 35 mm dish x 10

Senescent Cell Detection（for Plate Assay）

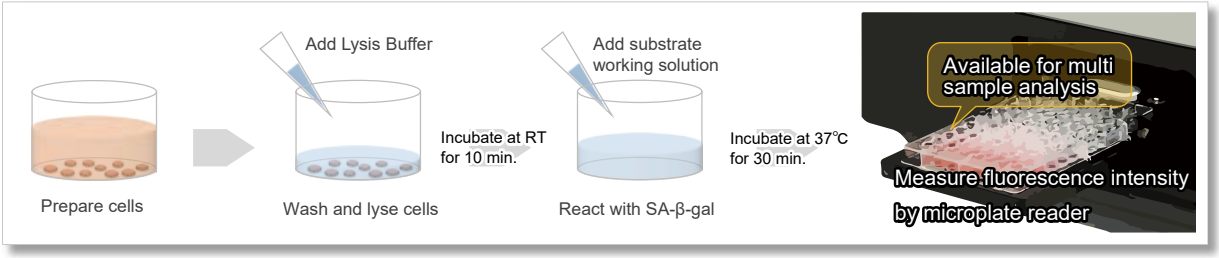
Cellular Senescence Plate Assay Kit - SPiDER-βGal

Plate Reader

This product is a simple detection kit that can be used by plate assay to measure senescence-associated β-galactosidase（SA-β-gal）activity. By simply adding SPiDER-βGal, a reagent for detection of β-galactosidase, to a 96 well plate, you can quantify SA-β-gal activity and evaluate multiple samples.

Simple and easy protocol - Just add the reagents into well -

Cells prepared in advance are lysed with the Lysis Buffer included in this kit. Fluorescence intensity according to the SA-β-gal activity are obtained by simply adding the fluorescent substrate SPiDER-βGal to the cell lysate.



* Precautions when using this kit
Cell counts may need to be normalized. When cells are analyzed in a microplate, the results obtained may differ depending on cell numbers per well. In such cases, normalization of the measured values obtained from cell counting and total protein will be necessary. In the Cell Count Normalization Kit (code: C554), cell numbers can be easily measured by the fluorescence intensity induced by a reagent added to the cell culture medium for staining nuclei. (Protocol for combined analysis is available on our website.)

Description	Unit	Code
Cellular Senescence Plate Assay Kit - SPiDER-βGal	20 tests 100 tests	SG05

γH2AX Detection Kit

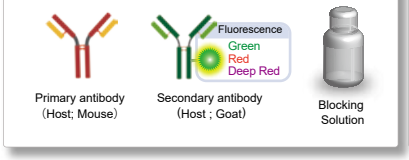
DNA Damage Detection Kit - γH2AX Green / Red / Deep Red

Micro scope

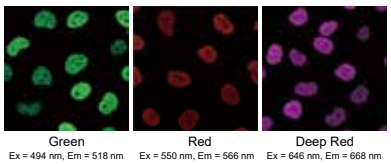
DNA Damage Detection Kit is an all-in-one kit for the detection of γH2AX, an indicator of DNA Damage, using primary and secondary antibodies.

Visualizing DNA Damage

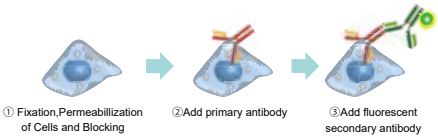
All reagents included in a Kit



Three color options



Simple protocol



Co-staining with Cellular Senescence Marker

γH2AX and SA-β-gal （senescence-associated β-galactosidase）of WI-38 (Passage 19) cells were detected with this kit and Cellular Senescence Detection Kit - SPiDER- βGal (code: SG03).

As a result, increased fluorescence derived from γH2AX and enhanced SA-β-gal activity were observed.

Comparison with WI-38 (Passage 1) cells is available on our website.

Description	Unit	Code
DNA Damage Detection Kit - γH2AX - Green	1 set *	G265
DNA Damage Detection Kit - γH2AX - Red	1 set *	G266
DNA Damage Detection Kit - γH2AX - Deep Red	1 set *	G267

* 5ml of staining solution can be prepared / 1 set

Nucleolus Fluorescent Staining

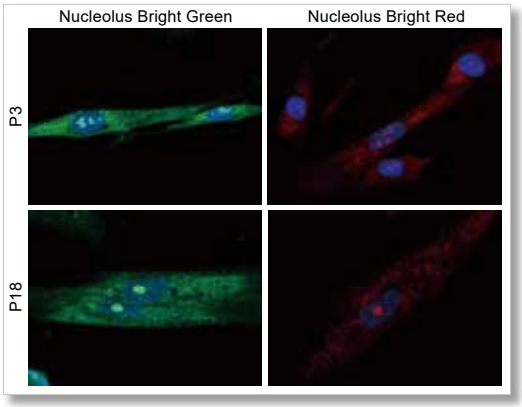
Nucleolus Bright Green/Red

Micro scope

Nucleolus Bright dyes are small molecules that bind to RNA in the nucleolus and emit fluorescnece. The nucleolus can be observed without any washing steps after staining with Nucleolus Bright dyes.

Detection in Senescent Cells

Different passage of WI-38 cells were fixed with 4% PFA and washed with PBS, then membrane permeabilized with 1% Triton X-100. Nucleolus Bright Green (N511) or Nucleolus Bright Red (N512) and nuclear staining dye, DAPI were added and were imaged using a confocal microscope.



As a result, most passages 3 cells (P3) had multiple nucleoli in one nucleus, whereas passages 18 cells (P18) had a single enlarged nucleolus.

Staining Condition

Cells were immersed in 4% PFA for 5 minutes, Triton X-100 for 20 minutes.
Then incubated in each fluorescent probe for 5 minutes.

Detection Condition

Nucleolus Bright Green： Ex=488 nm , Em=500-600 nm
Nucleolus Bright Red ： Ex=561 nm , Em=565-650 nm
DAPI ： Ex=405 nm , Em=450-495 nm

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

*＜Approximate number of uses per 60 nmol＞ 35 mm dish x 30 (Reagent concentration: 1 μmol/l)

Lipid Droplet

Fluorescent reagent for Lipid Droplet Staining

Lipi series　Blue / Green / Red / Deep Red

Micro scope

Lipi probes are small molecules that emit strong fluorescence in a hydrophobic environment such as Lipid droplets, which can be observed without any washing steps after staining with Lipi probes. It can be used with both live and fixed cells.

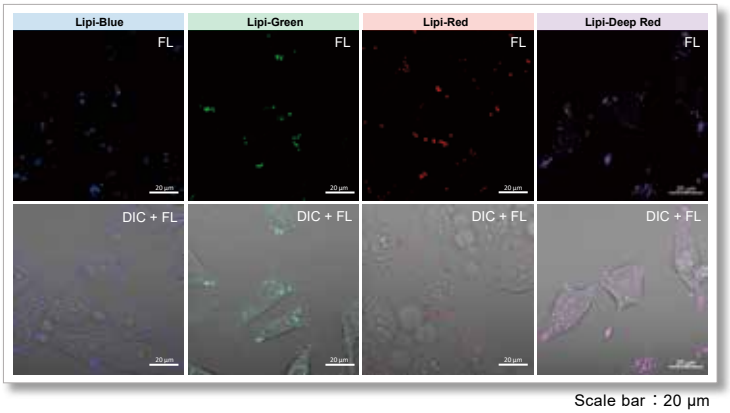
Live cell imaging

Oleic-acid-treated HeLa cells were stained with each Lipi series probe.

Staining condition

HeLa cells were cultured overnight in a medium containing oleic acid. The cells were washed with PBS and stained with each Lipi product working solution (Lipi-Blue / Green / Deep Red: 0.1 µmol/l, Lipi-Red: 1 µmol/l) for 15 minutes.

Upper row: fluorescence image, lower row: merged image with phase contrast image.
Detection conditions are shown on our website.



Scale bar：20 µm

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

* ＜Approximate number of uses＞ 35 mm dish x 10-50

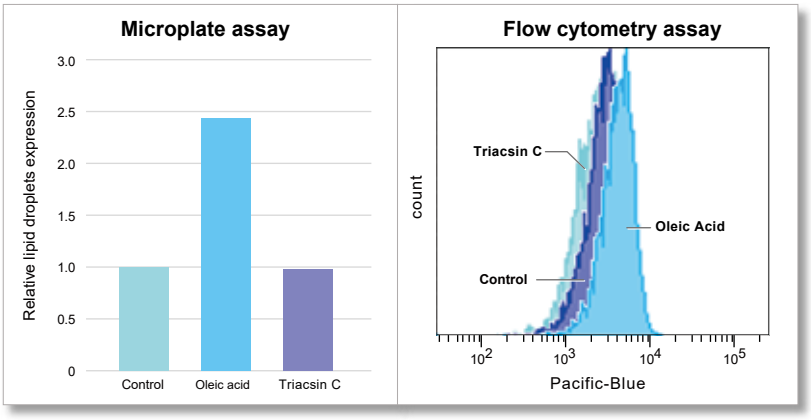
Lipid Droplet Assay Kit for Quantification

Lipid Droplet Assay Kit　Blue / Deep Red

Plate Reader　FCM

Lipid Droplet Assay Kit is a fluorometric assay kit for quantification of lipid droplets. This kit considerably shortens the entire process compared to the colorimetric method and can be used for both live and fixed cells.

Microplate / Flow cytometry assay　Lipid Droplet Assay Kit - Blue



Changes in lipid droplets by the addition of oleic acid or Triacsin C (acyl-CoA synthetase inhibitor, an inhibitor of lipid droplet formation) to HeLa cells (plate assay) and A549 cells (flow cytometry) were examined using the Lipid Droplet Assay Kit-Blue. As a result, the oleic acid-treated cells showed an increase in the number of LDs, compared to control and Triacsin C-treated cells.

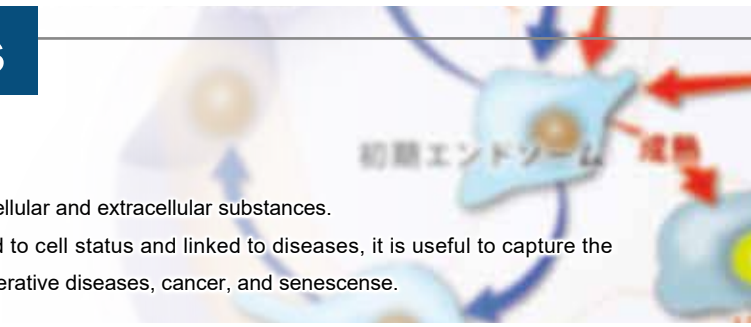
Detection Conditions

Microplate assay　：Ex=376-386 nm, Em=435-455 nm
Flow cytometry assay：Ex=405 nm, Em=425-475 nm

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

* ＜Approximate number of uses＞ 96 well plate x 1, 40 assays for flow cytometry

Cell Membrane Dynamics



The cell membrane plays an important role in selective transport of intracellular and extracellular substances.
Since cell membrane dysfunction or disruption of Endocytosis is related to cell status and linked to diseases, it is useful to capture the cell membrane dynamics in various research areas, such as neurodegenerative diseases, cancer, and senescense.

Cell Membrane related products

Endosomal Traficking

AcidSensor Labeling Kit - Endocytic Internalization Assay (Code A558)

- pH sensor Labeling
- Co-staining with Endocytosis Detection Dye

Visualizing Endocytosis

ECGreen-Endocytosis Detection (Code E296)

- Visualizing phagocytosis
- Tracking the virul infection pathway

Isolating Exosomes

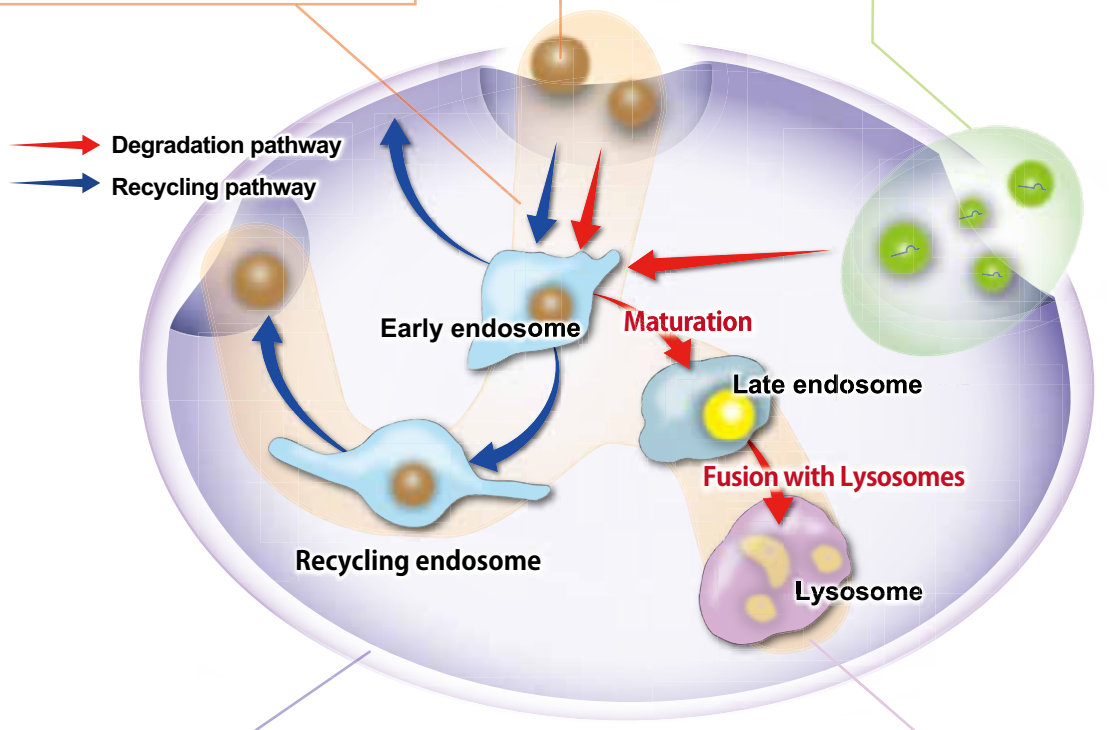
Exosome Isolation Kit (Code EX10/11)

- Easy to use, no technique required

Visualizing Exosomes

ExoSparkler Exosome Membrane/Protein Labeling Kit (Code EX01/02/03/04/05/06)

- Tracking of internalized exosome
- Flow Cytometry Analysis



Visualizing Cell Membrane

PlasMem Bright Series (Code P504/505)

- Neurotoxicity
- Membrane labeling for counting cell number

Visualizing Lysosomes

LysoPrime (Code L261/264), **pHLys Red** (Code L265)

- Lysosomal function (pH) and localization
- Quantification of lysosomal mass

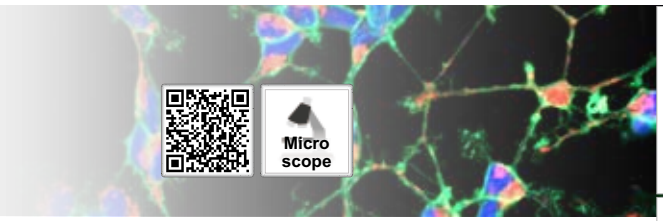
Lysosomal pH Detection

Lysosomal Acidic pH Detection Kit (Code L268)

- High sensitivity for lysosomal pH change
- Including positive control, convenient for first time users

Cell Membrane Staining

PlasMem Bright Green / Red



PlasMem Bright dyes are designed to overcome the problems with conventional small molecule dyes, enabling to stain plasma membranes for over a day. Furthermore, PlasMem Bright dyes are more water-soluble and can be diluted with culture medium.

A solution for plasma membrane staining

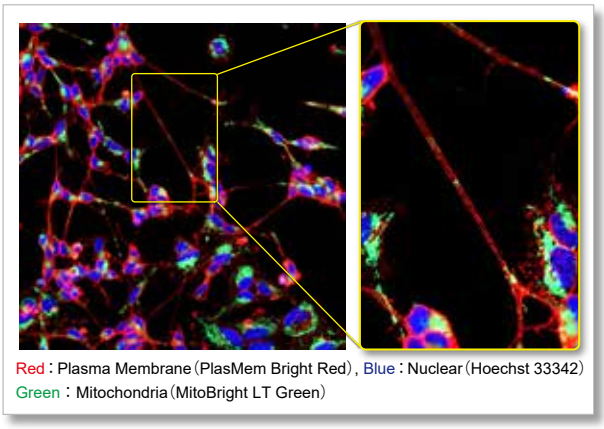
Small-molecule fluorescent dyes are widely used as a method for plasma membrane staining. However, short-lived stain retention in the plasma membrane and low water solubility are often problematic. Our PlasMem Bright dyes offer solutions to these problems.

Features

- Applicable to live cells and fixation after staining
- Higher retention with low toxicity
- Simple procedure: just add reagents to culture medium

Clear visualization of plasma membrane

Prolonged Retention in the Plasma Membrane



Red：Plasma Membrane（PlasMem Bright Red）, Blue：Nuclear（Hoechst 33342）
Green：Mitochondria（MitoBright LT Green）

Detection Conditions PlasMem Bright Red: Ex = 561 nm , Em = 560-700 nm

Plasma membrane staining inside an ES cell colony

Mouse ES cells were cultured in a gelatin-coated glass bottom dish for 4 days, and the colonies were stained with PlasMem Bright Green (200x dilution) for 15 minutes and observed under a confocal microscope (Zeiss: LSM710) after medium exchange.
As a result, the membranes of the cells inside the colonies could be visualized with PlasMem Bright Green.

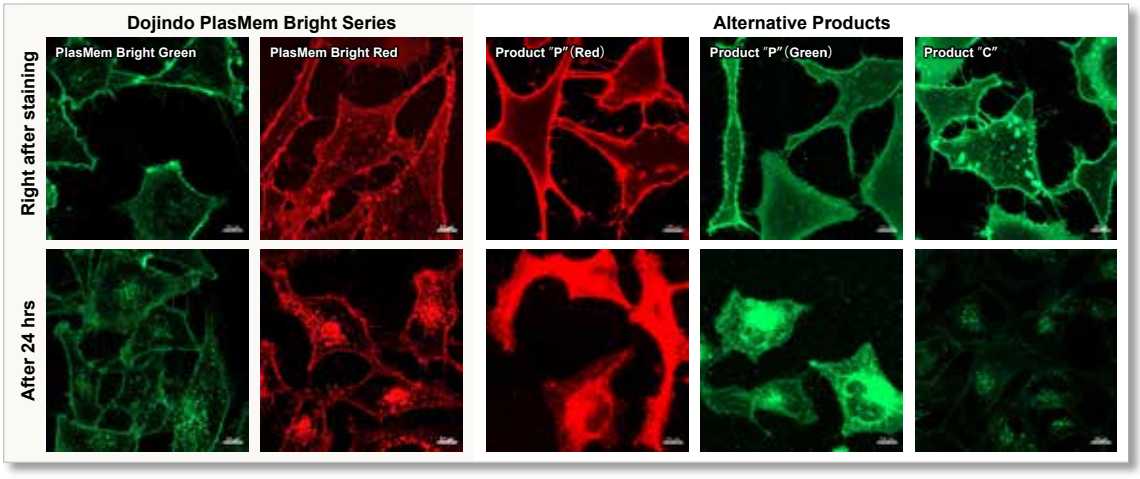
Detection Conditions PlasMem Bright Green: Ex = 488 nm , Em = 500-560 nm

* This data was kindly provided by Dr. Otsugu Ishizu, Keio University School of Medicine.

High retention on plasma membrane

HeLa cells stained with each plasma membrane staining reagent were incubated for 24 hrs and their fluorescent image was compared. PlasMem Bright series had higher retention on plasma membrane than other products.

* Retention on plasma membrane may vary depending on the cell type. (Difference was observed between Hela cells and SH-SY5Y cells.)



Scale bar：10 μm

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

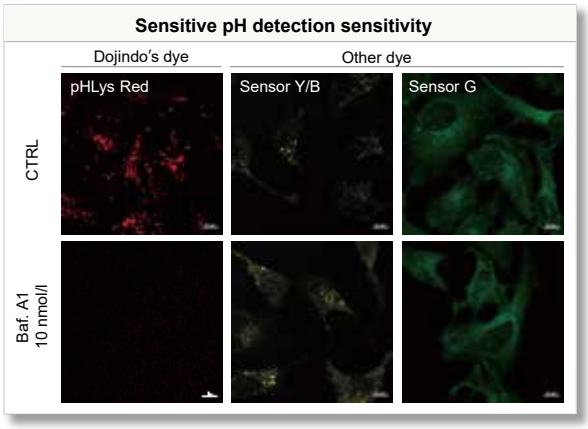
* ＜Approximate number of uses per 100 μl＞ 35 mm dish x 10 / μ-Slide 8 well x 10

The lysosomal staining dye series includes pHLys Red, which shows lysosomal pH-dependent fluorescence change, and pH-resistant LysoPrime Green and Deep Red. Combining these dyes and simultaneously analyzing lysosomal mass and pH, enables more detailed analysis of lysosomal function.

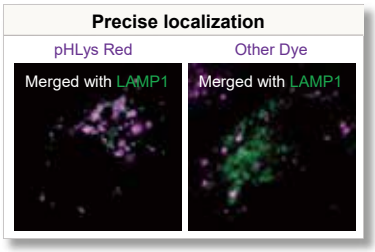
Plate Reader | Micro scope

Lysosomal pH Detection ①

pHLys Red – Lysosomal Acidic pH Detection

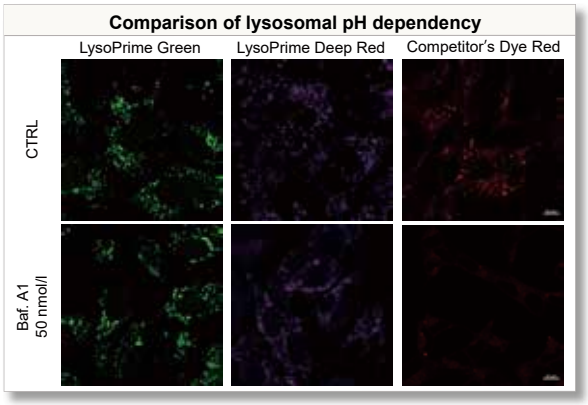


Live imaging using small molecule fluorescent probes has been widely used for lysosomal live cell analysis, but the low specificity and retention ability due to pH change have been cited as issues. The pHLys Red is a small molecule fluorescent probe with high lysosomal specificity and sensitivity to pH changes, enabling a more accurate analysis of lysosomal pH in live cells. It is also applicable to experiments that require long-term imaging due to its high retention ability.

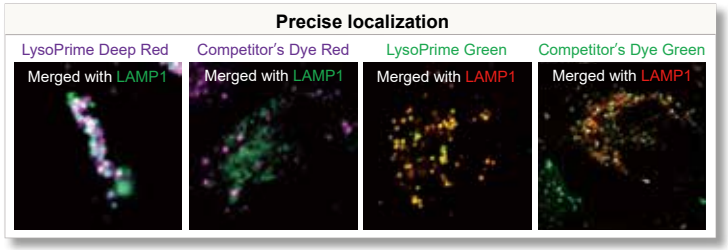


Lysosome Staining Dye ②

LysoPrime Green / Deep Red – High Specificity and pH Resistance



Dojindo's LysoPrime Green overcomes known problems with existing fluorescent lysosome probes, such as lack of specificity for lysosomes and staining dependent on the lysosomal pH. In addition, the high-retentivity of LysoPrime Green enables long-term imaging experiments.



Lysosomal Acidic pH Detection Combination of ① and ②

Lysosomal Acidic pH Detection Kit

All-in-one Kit including Lysosome Acidification Inhibitor

- Lysosome staining Dye : LysoPrime Green
- Lysosomal pH Detection Dye : pHLys Red
- Inhibitor of lysosomal pH acidification : Bafilomycin A1

For more information
L266 dojindo Search

Description	Unit	Code
① Dye for lysosomal pH detection		
pHLys Red – Lysosomal Acidic pH Detection	1 tube *1 3 tubes *1	L265
② Dye for lysosome mass and localization detection		
LysoPrime Green – High Specificity and pH Resistance	10 µl ×1 *1 10 µl ×3 *1	L261
LysoPrime Deep Red – High Specificity and pH Resistance	1 tube *1 3 tubes *1	L264
Accurate pH detection of lysosomes		
Lysosomal Acidic pH Detection Kit	1 set *2	L266

*1 <Approximate number of uses per 1 tube> 35 mm dish x 10, 96-well plate x 2 * 2 <Approximate number of uses per 1 set> 35 mm dish x 10, 96-well plate x 2

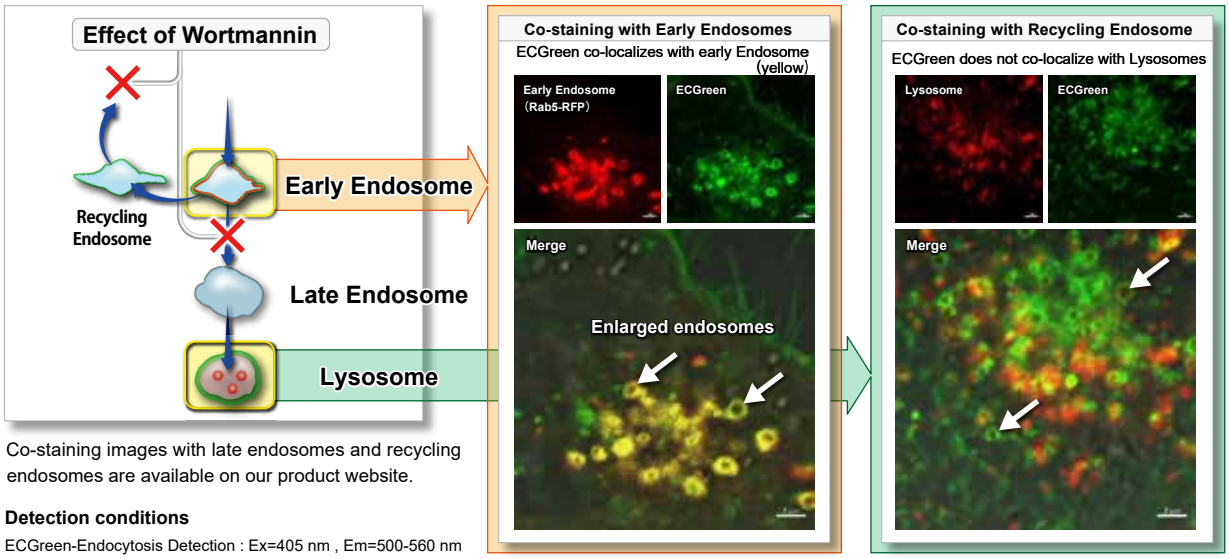
Endocytosis Detection

ECGreen-Endocytosis Detection

Micro scope

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

Clear visualization of intracellular vesicular trafficking



* Co-staining images with late endosomes and recycling endosomes are available on our product website.

Detection conditions

ECGreen-Endocytosis Detection : Ex=405 nm , Em=500-560 nm

Description	Unit	Code
ECGreen-Endocytosis Detection	40 µl *	E296

* ＜Approximate number of uses per 40 µl＞ 35mm dish x 20, µ-Slide 8 well x 20

pH Sensor Labeling Kit

AcidSensor Labeling Kit – Endocytic Internalization Assay

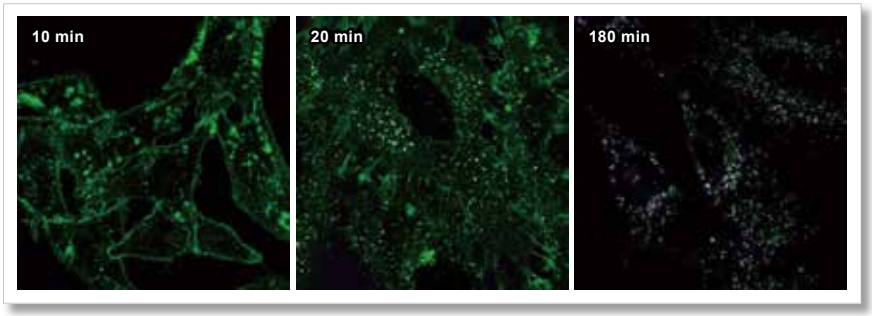
Micro scope

This kit is an all-in-one kit that allows visualization of the endocytic 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).

Co-staining for Observation with Endosomes - Cellular uptake of labeled IgG over time

AcidSensor-labeled mouse IgG stained with this kit and Dojindo's endocytosis detection dye, ECGreen -Endocytosis Detection ((code: E296))were added to HeLa cells, and observed at 10, 20, and 180 minutes after staining. The results showed that the AcidSensor (Deep Red) and endosomal membranes (Green) were localized at the same place, indicating that mouse IgG was taken up by the cells via the endocytic pathway.



Detection conditions

Green：ECGreen Ex = 405 nm, Em = 500-550 nm

Purple：AcidSensor Ex = 633 nm, Em = 650-700 nm

Endocytosis Detection
ECGreen-Endocytosis Detection
E296 dojindo Search

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

* ＜Approximate number of uses per 1 sample＞ Labels 50-200 µg of protein/antibodies. * Protein/Antibody is not included.

Lysosomal Acidic pH Detection Kit

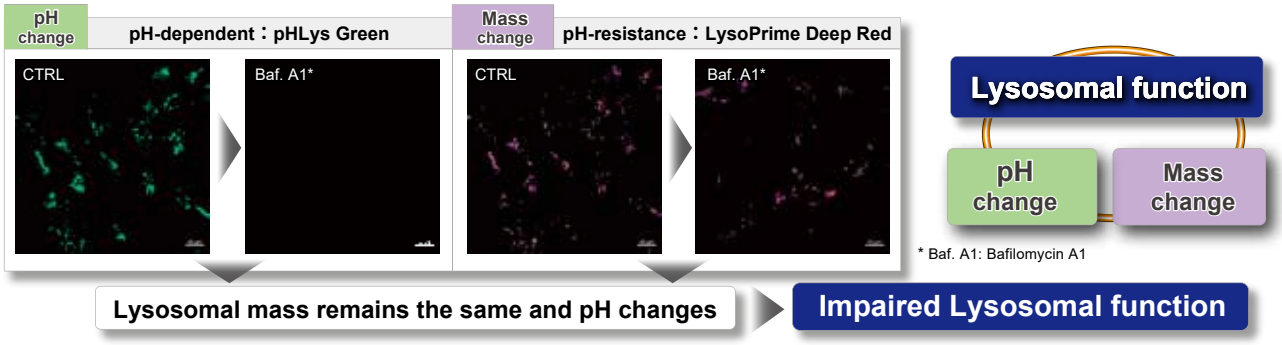
Lysosomal Acidic pH Detection Kit-Green/Deep Red

Micro scope FCM

Since lysosomal dysfunction is deeply involved in the onset and progression of neurodegenerative diseases and other disorders, detailed analysis of lysosomes has attracted much attention in elucidating pathological conditions and developing therapeutic agents. Furthermore, recent studies have shown that a decrease in acidity inside lysosomes in mouse models of the neurodegenerative disease Alzheimer's disease results in the inability to degrade waste products inside autophagosomes and the accumulation of toxic amyloid-β*, thus increasing the need to confirm the pH of lysosomes. * *Nature Neuroscience*, **2022**, *25*, 688–701.

Lysosomal pH and mass - accurate analysis of lysosomal function

With existing reagents, it was difficult to examine whether lysosomal mass or their function (pH) fluctuated because the discussion was based on changes in the fluorescence brightness of a single dye. This kit contains pHLys Green, which is highly specific to lysosomes and shows pH-dependent fluorescence change, and pH-resistant LysoPrime Deep Red (code: L264). Using these two dyes, lysosomal pH and mass can be measured from the same sample enabling the detailed analysis of lysosomal function.



Comparison with existing reagents

Compared to existing staining dyes, Dojindo's lysosomal detection dyes selectively accumulate on lysosomes and continue to stain for more than 24 hours. These dyes can be used in combination, and lysosomal mass and pH can be more accurately confirmed.

	Lysosomal Acidic pH Detection Kit -Green/Deep Red (L268)		Lysosomal Acidic pH Detection Kit (L266)		Company T	Company T
Dye/ Wave length	**pHLys Green** Ex=488 nm Em=490-550 nm	**LysoPrime Deep Red** ※ Ex=633 nm Em=640-700 nm	**pHLys Red** ※ Ex=561 nm Em=560-650 nm	**LysoPrime Green** ※ Ex=488 nm Em=500-600 nm	Lysosomes pH sensor	Lysosomes Staining dye
Purpose	pH	Mass	pH	Mass	pH	Mass
lysosome pH sensitivity	✓	Resistant to pH change	✓	Resistant to pH change	Less sensitivity	Less sensitivity
lysosome Specificity	✓	✓	✓	✓	✓	✓
lysosome retention	✓	✓	✓	✓	✓	✓
Supported Devices	Microscopy・FCM		Microscopy・Plate Reader		Microscopy	Microscopy

Indicator	Detection Color	Description	Unit	Code
pH / Mass	Green / Purple	Lysosomal Acidic pH Detection Kit -Green/Deep Red	1 set*	L268

* ＜Estimated number of use per 1 set＞ 35 mm dish x 10, μ-Slide 8 well x 10, 96-well Plate x 2

Related product

Indicator	Detection Color	Description	Unit	Code
pH / Mass	Red / Green	Lysosomal Acidic pH Detection Kit	1 set	L266
pH	Red	pHLys Red- Lysosomal Acidic pH Detection	1 tube 3 tubes	L265
Mass	Purple	LysoPrime Deep Red - High Specificity and pH Resistance	1 tube 3 tubes	L264
	Green	LysoPrime Green - High Specificity and pH Resistance	10 μl 10 μl×3	L261

Autophagy

Autophagosome Detection

DAPGreen - Autophagy Detection

DAPRed - Autophagy Detection

Autolysosome Detection

DALGreen - Autophagy Detection

Micro scope FCM Plate Reader

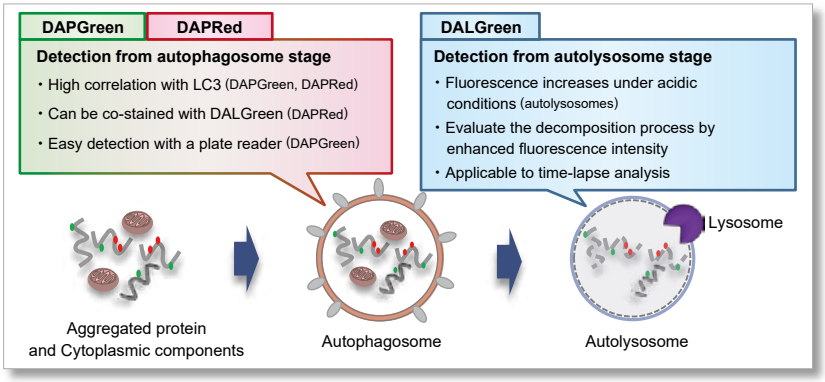
Micro scope

Micro scope FCM

Autophagy is an intracellular degradation system, where dysfunctional proteins and organelles are degraded.
DAPRed, DAPGreen, and DALGreen are fluorescent reagents that can easily detect autophagy by simply adding it to cultured cells.

Principle

DAPRed and DAPGreen, are incorporated inside of the membrane when an autophagosome membrane is formed. Its fluorescence is enhanced under lipophilic condition.On the other hand, DALGreen emits enhanced fluorescence under acidic condition after the autophagosome is fused with the lysosome. In this way, DAPRed, DAPGreen, and DALGreen enables to monitor the process of autophagosome by simply adding reagents.

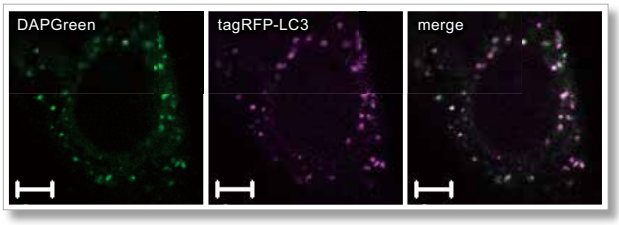


Related Product Information

	Applicable instruments			Fluorescent properties	Volume / the number of usable assays	Existing methods
	Fluorescent Microscopy	Flow cytometer	Microplate reader			
DAPGreen	○	○	○	Ex = 425-475 nm Em = 500-560 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	20 nmol x 1 / 35 mm dish: 10 (when used in 1.0 μmol/l)	LC3-GFP-RFP etc.

High Correlation with LC3 DAPGreen

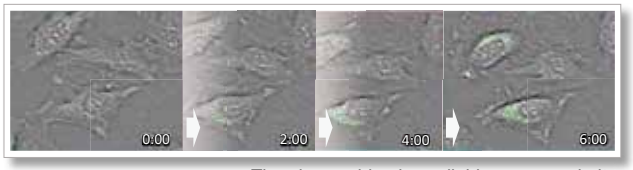
After adding DAPGreen to the RFP-LC3 expressed Hela cells, cells were treated with rapamycin to induce autophagy.
Fluorescent imaging was conducted by confocal microscopy



Imaging Condition
Ex=488 nm , Em=500-563 nm
Scale bar：10 μm

Time-lapse imaging DALGreen

After staining with DALGreen, HeLa cells were cultured in amino acid-free medium, and changes in cells and fluorescence images were observed for up to 6 hours.
As a result, an increase in DALGreen fluorescence was confirmed in cells in which autophagy was induced.



Time-lapse video is available on our website

Imaging Condition
Ex=405 nm, Em=500-550 nm
Confocal Imaging Cytometer（Yokogawa Electric Corporation：CQ1）

Description	Unit	Code
DAPGreen - Autophagy Detection	5 nmol	D676
DAPRed - Autophagy Detection	5 nmol	D677
DALGreen - Autophagy Detection	20 nmol	D675

Substrate for Immunofluorescence

CLAMP F405-Signal Boosting

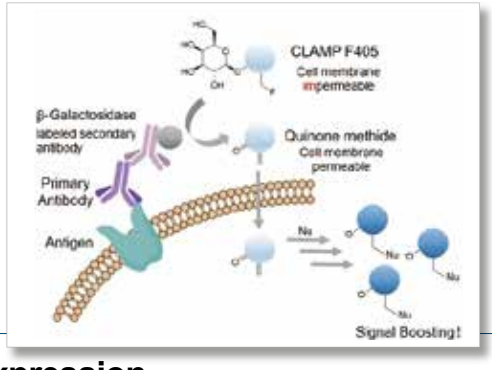
Micro scope | FCM | Plate Reader

A highly sensitive CLAMP method (quinone methide-based catalyzed signal amplification) can be applied to live/fixed cells or tissue sections. In this method, using primary antibody, β-galactosidase-labeled secondary antibody and newly developed fluorescent substrate CLAMP F405, the cells expressing a specific low-expressed cell surface protein can be selectively detected with high sensitivity.

Note: This method cannot confirm the localization of surface antigens.

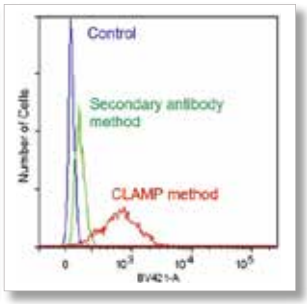
*This product was developed with technical guidance and information provided by Prof. Yoshiki Katayama at Kyushu University.

Reference：Noguchi, K. et al., "β-Galactosidase-Catalyzed Fluorescent Reporter Labeling of Living Cells for Sensitive Detection of Cell Surface Antigens", *Bioconjugate Chem.*, **2020**, 31(7), 1740–1744.



Highly sensitive detection of cell surface antigen expression

A fluorescence detection method using a fluorescence-labeled antibody is widely known as a specific detection method for cell surface proteins (fluorescence immunostaining method). However, it is difficult to apply this method for low expressed surface proteins due to low sensitivity. CLAMP F405-Signal Boosting allows you to detect these proteins.



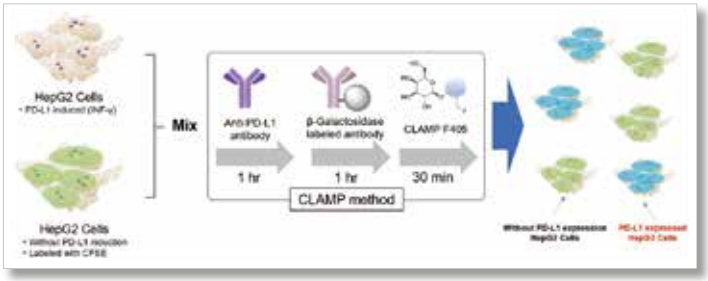
Cell Line：HeLa Cells
Antigen：CD44
Instrument：
(Left) Fluorescent microscope
Ex=340–380 nm, Em=435–485 nm
(Right) Flow Cytometer
Ex=405 nm, Em=425–575 nm

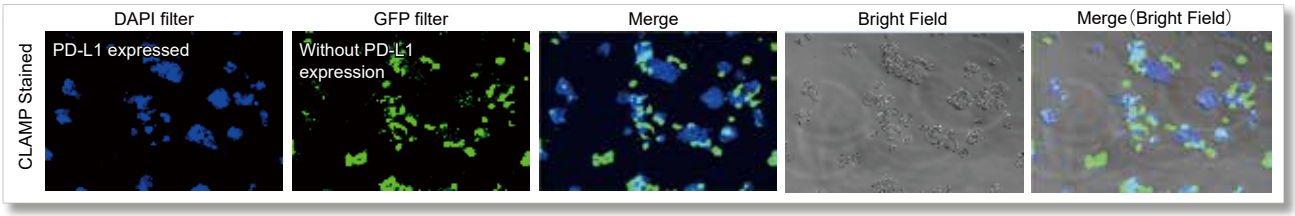
Application Data

highly sensitive, high selectivity, and high retention

PD-L1 expressed HepG2 cells and CFSE-stained control cells were prepared and mixed. The results showed the CLAMP stained cells did not localize in the CFSE stained cells, indicating that the CLAMP method accurately differentiates PD-L1 expressed HepG2 cells, which was difficult using the secondary antibody method.

* CFSE: 5- or 6-(*N*-Succinimidyloxycarbonyl)fluorescein 3′,6′-diacetate





FFPE Tissue Sections - Human Small Intestine

Using the CLAMP method and TSA (Tyramide signal amplification) method detected the αSMA (α-smooth muscle actin) and keratin on FFPE tissue sections of human small intestine samples. The results showed that the CLAMP method can be used for co-staining with other staining method. Please refer to the reference for a detailed protocol on the CLAMP method for tissue sections.

Reference: Hirata, M., et al. "Galactosidase-catalyzed fluorescence amplification method (GAFAM): sensitive fluorescent immunohistochemistry using novel fluorogenic β -galactosidase substrates and its application in multiplex immunostaining." *Histochem Cell Biol* 159, 233–246 (2023)

* The data was kindly provided by Dr. Masahiro Hirata, Department of Diagnostic Pathology, Kyoto University Hospital.

Description	Unit	Code
CLAMP F405-Signal Boosting	10 µl *	C554

* Primary antibody and β-Galactosidase labeled secondary antibody is not included in this kit.

Autophagic Flux Assay

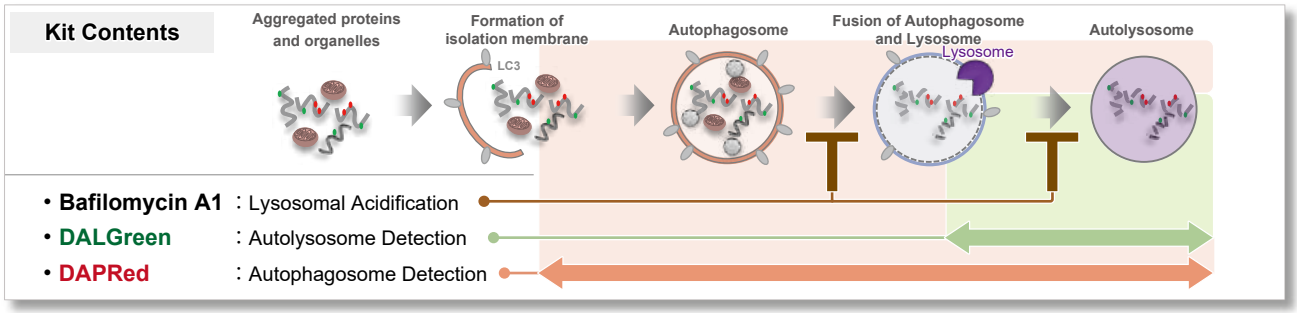
Autophagic Flux Assay Kit

Autophagic Flux Assay Kit contains autophagosome and autolysosome detection dye (DAPRed), autolysosome detection dye (DALGreen), and lysosomal acidification inhibitor (Bafilomycin A1). This kit allows the accurate evaluation of autophagic flux by monitoring autophagosome formation through autolysosome formation[1] in live cells.

1) H. Sakurai, *et al.*, *iScience*, **2023**, *26*, 107218.

What this kit can do

This all-in-one kit includes DAPRed (code: D677) for the detection of autophagosomes and autolysosomes, DALGreen (code: D675) for the detection of autolysosomes, and Bafilomycin A1 for inhibiting lysosome acidification. By simply adding the reagents, you can monitor the process from autophagosome formation to autolysosome formation [2),3)]。



Analyzing the autophagic flux by fluorescence changes

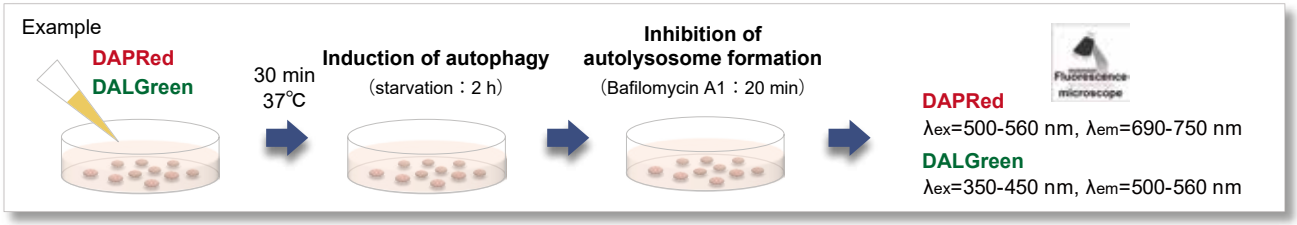
Autophagic flux can be analyzed more in detail by using this kit in conjunction with autophagy induction or inhibition. It enables to evaluate the stages in the autophagic flux by observing the fluorescence change of DAPRed and DALGreen simultaneously.

Fluorescence change relative to control		Indication from observed fluorescence changes	Typical inhibitors [4),5)]
DAPRed	DALGreen		
↑	↑	Autophagy induction or autolysosome accumulation	E64d/Pepstatin A
↓	↓	Inhibition in autophagosome formation step	3-MA
↑ or →	↓	Inhibition of autolysosome formation	Bafilomycin A1

2) X. Chen, *et al., Am J Transl Res.*, **2020**, *12*(9):4902-4922.
3) C. Oh, *et al., J Neurosci.*, **2022**, *42*(14), 3011-3024.
4) N. Mizushima, *et al., Cell*, **2010**, *140*(3), 313-326.
5) D. J. Klionsky, *et al., Autophagy*, **2021**, *17*(1), 1-382.

Simple Operation

Add DAPRed and DALGreen working solution to cells at the same time, then induce/inhibit the autophagy. No operations such as plasmid transfection are required.



*Since the autophagic capacity differs depending on the cell type, we recommend optimizing the measurement conditions in advance using the co-staining protocol described above.
*This kit includes Bafilomycin A1 for evaluation of the condition.

Description	Unit	Code
Autophagic Flux Assay Kit	1 set *	A562

Related product

* 〈Approximate number of uses per 1 set〉 35 mm dish x 5, μ-Slide 8 well x 5, 96-well plate x 1

	Description	Unit	Code
Autophagosome detection dye	DAPGreen - Autophagy Detection	5 nmol *1	D676
	DAPRed - Autophagy Detection	5 nmol *1	D677
Autolysosome detection dye	DALGreen - Autophagy Detection	20 nmol *2	D675
Lysosomal pH detection kit	Lysosomal Acidic pH Detection Kit -Green/Deep Red	1 set *3	L268
	Lysosomal Acidic pH Detection Kit	1 set *3	L266

〈Approximate number of uses〉 *1 : 35 mm dish x 25, *2 : 35 mm dish x 10, *3 : per 1 set - 35 mm dish x 10, μ-Slide 8 well x 10, 96-well plate x 2

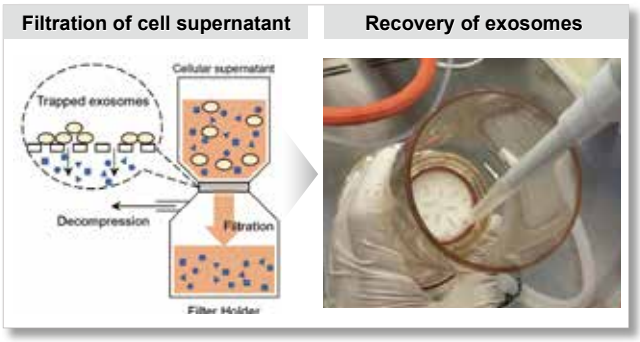
Exosome

Exosome Isolation

Exo*Isolator* Exosome Isolation Kit ／ Exo*Isolator* Isolation Filter

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

Simple and easy operation



Exo*Isolator* Exosome Isolation Kit includes Filter Holder and Isolation Filter that can collect exosomes from cell supernatant by adding PBS to the filter surface after filtration. Its recovery rate is high and easy to use, no complicated technique is required throughout the process.

* Sample processing volume: 25 ml / filter is recommended for culture supernatant,

Video instructions

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*Isolator* 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*Isolator* recovered exosomes with equivalent particle size distribution and the number of particles to the ultracentrifugation method, and the amount of exosome marker expression per protein was higher, indicating that Exo*Isolator* recovered exosomes with higher purity than the ultracentrifugation method.

Comparison of ultracentrifugation and ExoIsolator

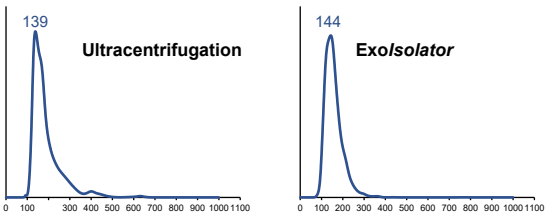


Fig. 1 Nanoparticle size of isolated exosomes

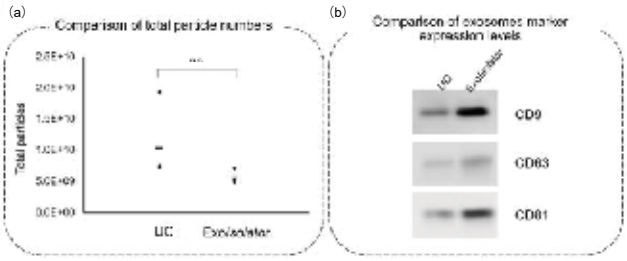


Fig 2. Nanoparticle number(a) and expression level of exosome markers(b)

Kit contents

For first-time users

Exo*Isolator* Exosome Isolation Kit

Code : EX10

This kit contains

The Filter Holder can be reused after autoclaving.

To purchase additional filters

Exo*Isolator* Isolation Filter

Code : EX11

The Isolation Filter is also sold separately in a pack of 10 pieces.

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

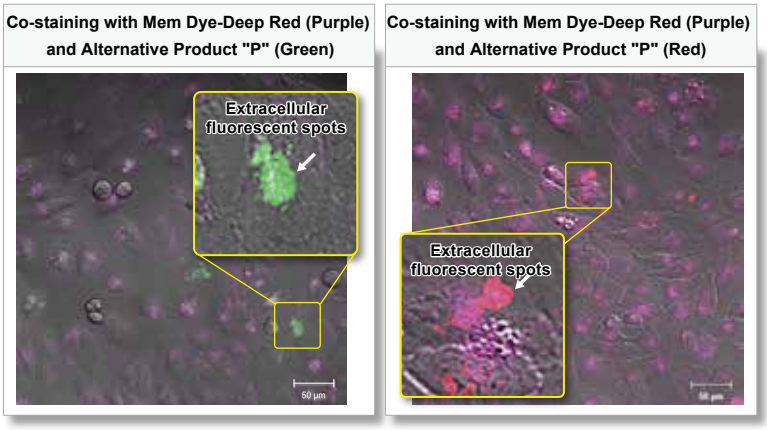
Exosome Membrane / Protein Fluorescent Staining

ExoSparkler Exosome Membrane / Protein Labeling Kit　Green / Red / Deep Red

Micro scope

ExoSparkler series can be used for staining the membrane or proteins of the purified exosomes and enables the imaging of labeled exosomes taken up by cells.

ExoSparkler series does not cause extracellular aggregation



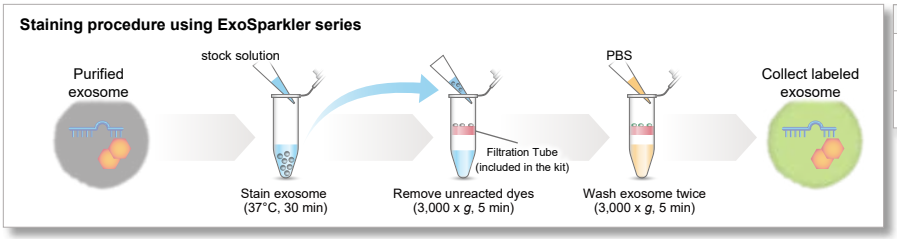
Exosomes stained with ExoSparkler's Mem Dye-Deep Red or an alternative product (green or red) were added to each well containing HeLa cells. The labeled exosomes taken into HeLa cells were observed by fluorescent microscopy. As a result, extracellular fluorescent spots suspected of dye aggregations were observed in each well containing the exosomes stained with the alternative product (green or red).

Detection conditions

Mem Dye-Deep Red (Purple): Ex 640nm/Em 640-760nm
Alternative Product "P" (Green): Ex 561nm/Em 560-620nm
Alternative Product "P" (Red): Ex 640nm/Em 650-700nm

All-in-one kit for preparing the labeled exosomes

ExoSparkler series contains filtration tubes for the removal of unreacted dyes 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.

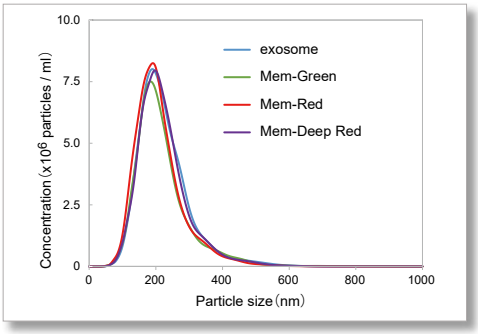


	Recovery rate
Filtration tube (included in the kit)	About 50%
Gel filtration method	About 10%

Note: Compared the number of exosome particles before and after purification with NTA

ExoSparkler Mem Dyes have little effect on exosome properties

NTA (nanoparticle tracking analysis) and zeta potential were measured to determine the changes in exosomes before and after staininge with Mem Dyes.
As a result, the Mem-Dye series (green, red, deep red) had little effect on exosome properties. Comparison data is available on our website.



NTA comparison of dye-stained (Mem-Dye) or unstained exosomes

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

* Protein amount : 1-10 μg / sample, Particle count : 10 to 100 x 10^8 / sample (As purified exosome using ultracentrifugation)

Antibody Labeling

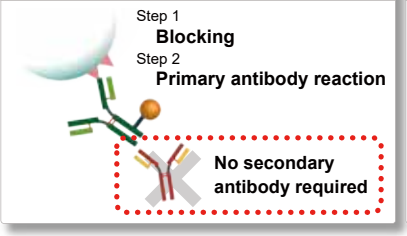
DOJINDO MOLECULAR TECHNOLOGIES, INC.

Dojindo Labeling Kit series

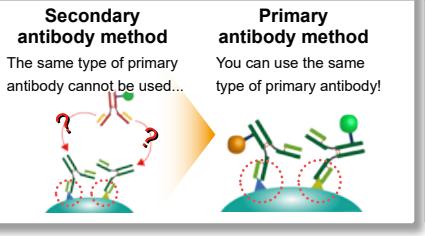
Dojindo Labeling Kit series is a kit that allows you to directly label your primary antibodies with fluorescent dyes, biotin, or enzymes.

Features

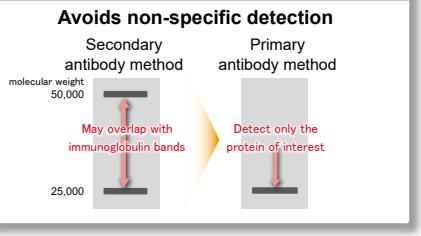
Fewer steps



No risk of Cross-reaction



Useful for immunopercipitation



Product Lineup

References using each product are available on our website.

Biotin	Sample amount / type	Target	Description	Unit	Code	Detection
Biotin	10 µg antibody	-NH_2	Ab-10 Rapid Biotin Labeling Kit *	3 samples	LK37	Micro scope
	50-200 µg antibody・protein	-NH_2	Biotin Labeling Kit -NH_2 *	3 samples	LK03	
		-SH	Biotin Labeling Kit -SH	3 samples	LK10	FCM
	1 mg antibody・protein	-NH_2	Biotin Labeling Kit - NH_2 (for 1mg) *	1 sample	LK55	Plate Reader
	1-5 mg antibody・protein	-NH_2	Biotinylation Kit (Sulfo-OSu)*	1 set	BK01	Western Blot

fluorescent dye	Sample amount / type	Target	Description	Unit	Code	Detection
Fluorescein	10 µg antibody	-NH_2	Ab-10 Rapid Fluorescein Labeling Kit *	3 samples	LK32	
	50-200 µg antibody・protein	-NH_2	Fluorescein Labeling Kit -NH_2 *	3 samples	LK01	
HiLyte Fluor	10 µg antibody	-NH_2	Ab-10 Rapid HiLyte Fluor™ 555 Labeling Kit -NH_2 *	3 samples	LK35	
	50-200 µg antibody・protein	-NH_2	HiLyte Fluor™ 555 Labeling Kit -NH_2 *	3 samples	LK14	Micro scope
	10 µg antibody	-NH_2	Ab-10 Rapid HiLyte Fluor™ 647 Labeling Kit-NH_2 *	3 samples	LK36	FCM
	50-200 µg antibody・protein	-NH_2	HiLyte Fluor™ 647 Labeling Kit-NH_2 *	3 samples	LK15	
ICG	50-200 µg antibody・protein	-NH_2	ICG Labeling Kit-NH_2 *	3 samples	LK31	

fluorescent protein	Sample amount / type	Target	Description	Unit	Code	Detection
R-Phycoerythrin	10 µg antibody	-NH_2	Ab-10 Rapid R-Phycoerythrin Labeling Kit *	3 samples	LK34	
	50-200 µg antibody・protein	-NH_2	R-Phycoerythrin Labeling Kit-NH_2 *	3 samples	LK23	Micro scope
		-SH	R-Phycoerythrin Labeling Kit-SH	3 samples	LK26	
Allopycocyanine	50-200 µg antibody・protein	-NH_2	Allophycocyanin Labeling Kit-NH_2 *	3 samples	LK21	FCM
		-SH	Allophycocyanin Labeling Kit-SH	3 samples	LK24	

Enzyme	Sample amount / type	Target	Description	Unit	Code	Detection
Peroxidase	10 µg antibody	-NH_2	Ab-10 Rapid Peroxidase Labeling Kit *	3 samples	LK33	
	50-200 µg antibody・protein	-NH_2	Peroxidase Labeling Kit-NH_2 *	3 samples	LK11	Micro scope
		-SH	Peroxidase Labeling Kit-SH	3 samples	LK09	
	1 mg antibody・protein	-NH_2	Peroxidase Labeling Kit - NH_2 (for 1mg) *	1 sample	LK51	Plate Reader
Alkaline Phosphatase	50-200 µg antibody・protein	-NH_2	Alkaline Phosphatase Labeling Kit-NH_2 *	3 samples	LK12	Western Blot
		-SH	Alkaline Phosphatase Labeling Kit-SH	3 samples	LK13	

* Labeling reaction may interfere the antigen recognition site of the antibody. If you have any questions, please contact our Customer Support or your sales representative.

Applicable Instruments

Search for products by your device

Plate Reader

Microscope

Flow Cytometry

Western Blot

1. Information on this leaflet is as of July 2024. It may change without prior notice.
2. For research use only.
3. The company names and product names on this leaflet are the trademarks or the registered trademarks of Dojindo or their respective owners.

Product Search
【Product Code】Dojindo Search

Manufacturer

DOJINDO LABORATORIES

Please feel free to ask any technical questions about our products.
Inquiry Dojindo Search

URL: https://www.dojindo.com/ASIA/

Distributor

240522-1 (S)