DOJINDO

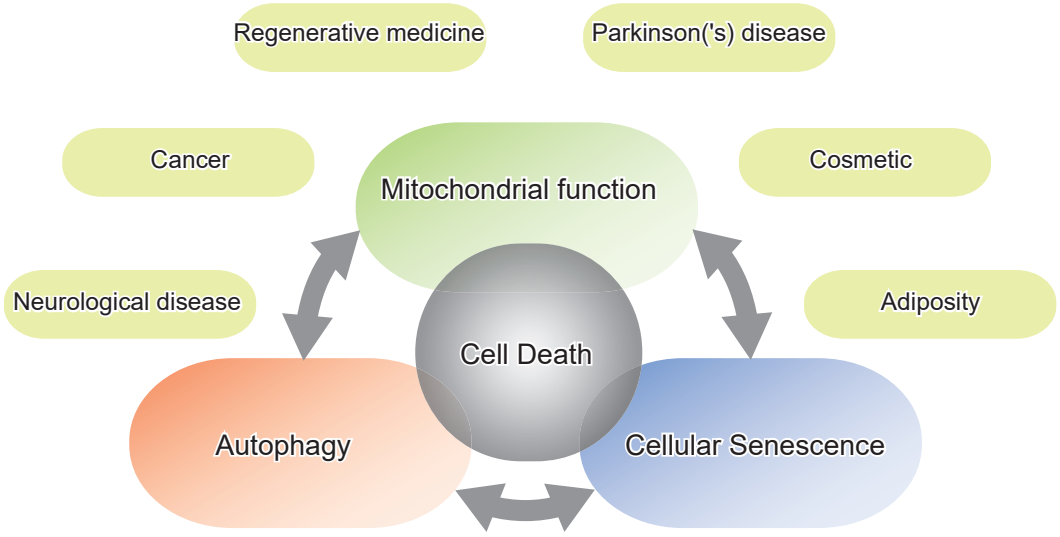
Analysis for Cellular Function

Mitocondrial Analysys
Autophagy
Cellular Senescence
Cell Proliferation Cytotoxicity

DOJINDO

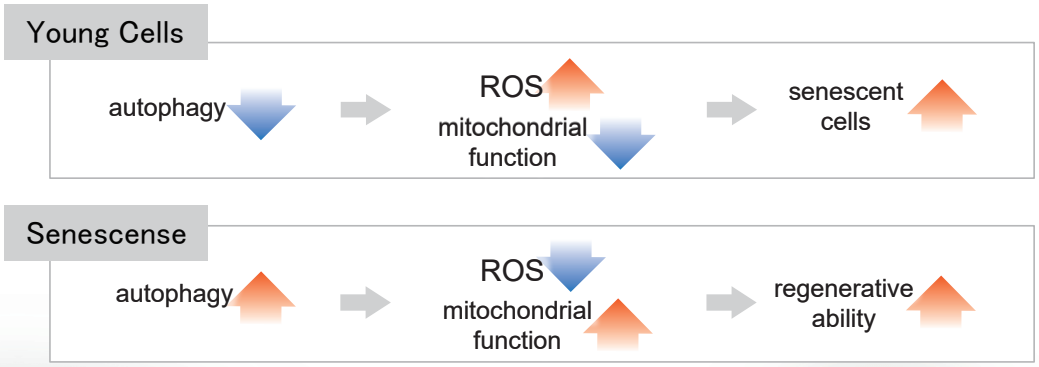
Analysis for Cellular Function

Understanding for cellular functions are important to reveal the mechanism of diseases and to develop a drug-discovery. Especially autophagy, cellular senescence and mitochondrial function have been found to be closely related each other, and have been attracting attention in the medical research and cosmetics industry.



Suppression of Cellular Senescence by Autophagy

As an example where autophagy, cellular senescence and mitochondrial function are closely related, there is an example using mouse stem cells. Muscle stem cells that remain resting stage of the cell cycle is dependent on autophagy. When the function of autophagy declines, the reactive oxygen species (ROS) increases due to mitochondrial dysfunction. As a result, it becomes cellular senescence. Even autophagy is inhibited in young cells, the cellular senescence is induced, conversely by inducing autophagy in senescent cells, the mitochondrial function is restored and recovery of regenerative ability has been confirmed.



L. García-Prat, M. Martínez-Vicente, E. Perdiguero, L. Ortet, J. Rodríguez-Ubreva, E. Rebollo, V. Ruiz-Bonilla, S. Gutarra, E. Ballestar, A. L. Serrano, M. Sandri & P. Muñoz-Cánoves, “Autophagy maintains stemness by preventing senescence.” *Nature*, **2016**, *529* (7584), 37

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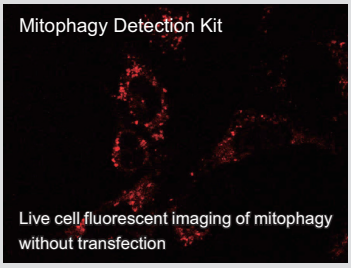
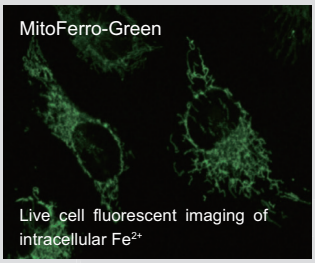
Reagents for Mitochondrial Research

Mitophagy Detection

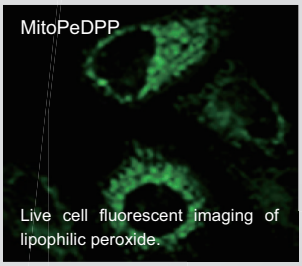


Current publication includes the principle of detection mechanism and experimental data of microscopy and flow cytometry using Mitophagy Detection dye. Visit our website by web searching with “MD01 Dojindo”.

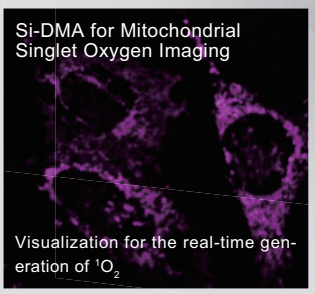
Mitochondrial Ferrous Ion Detection

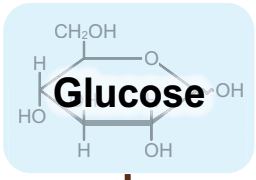


Mitochondrial Lipophilic Peroxide Detection

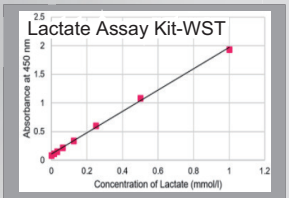


Mitochondrial Singlet Oxygen Detection





Lactate Quantification



As metabolite of glycolysis, lactate can be quantified by absorbance measurements using 96-well microplates.

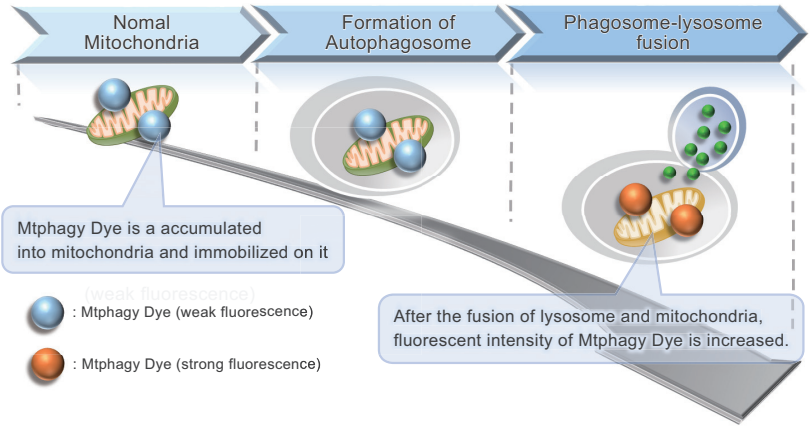
Mitochondrial Staining

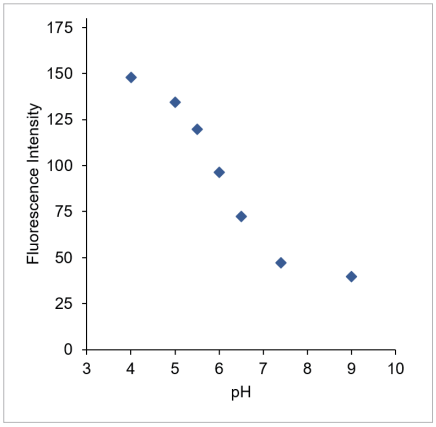
MitoBright Series (Green, Red, DeepRed)

Selectively staining of mitochondria in living cells

Mitophagy Detection Kit

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





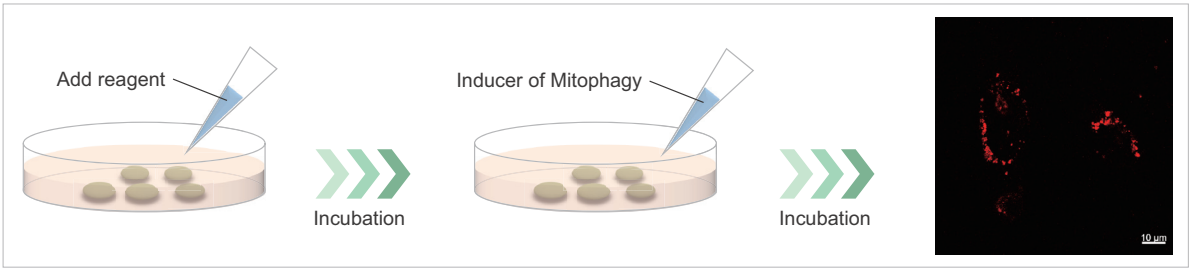
The fluorescent intensity of Mtphagy Dye is increased at pH4-5.

The publication of Mitophagy Detection Kit includes the detection principle and experimental data.

H. Iwashita, S. Torii, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, S. Shimizu and K. Okuma, "Live Cell Imaging of Mitochondrial Autophagy with a Novel Fluorescent Small Molecule", *ACS Chem. Biol.*, **2017**, *12*. 2546.
※ Other publications are listed online. Please visit at www.dojindo.com.

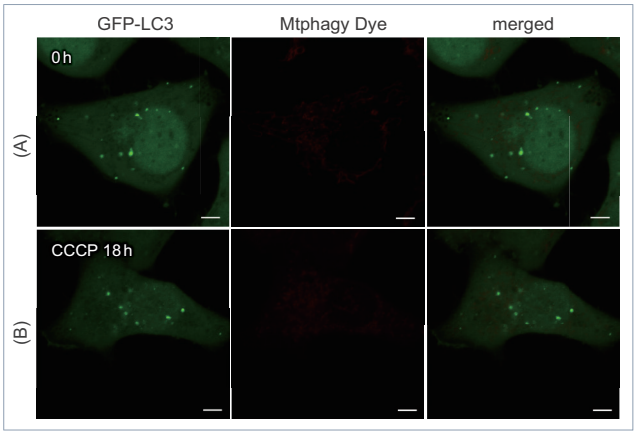
Simple procedure

Transfection procedure is not need for mitophagy imaging.

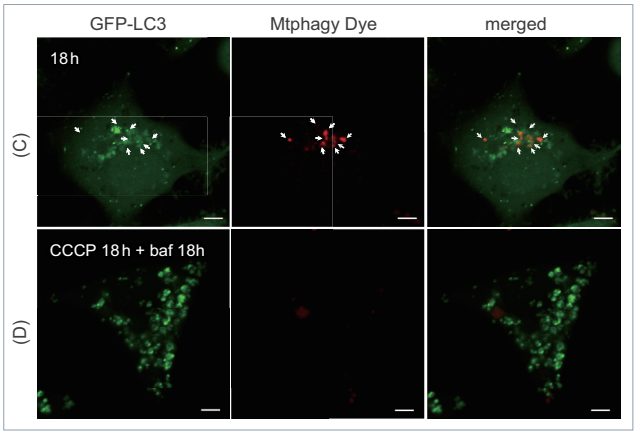


Comparison with autophagy marker

Normal HeLa cells



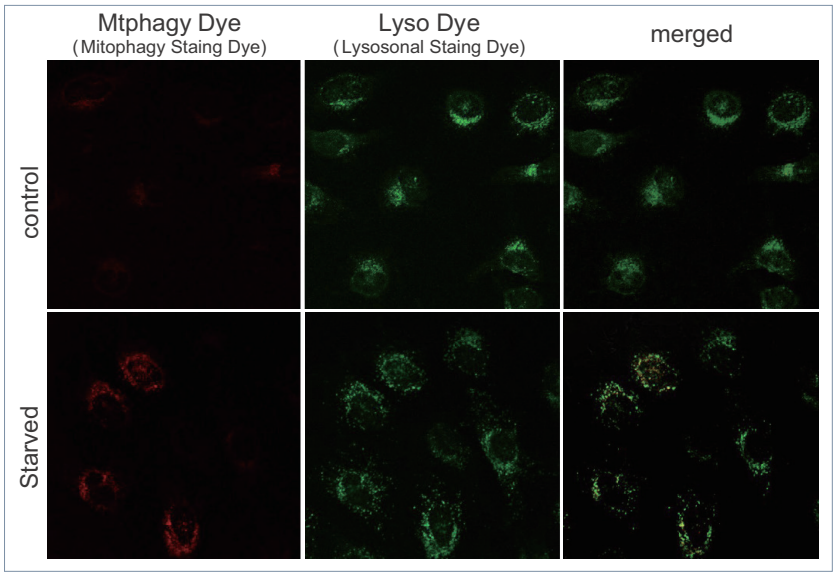
Parkin expressed HeLa cells



CCCP（carbonyl cyanide m-chlorophenyl hydrazone）has been added to normal and Parkin expressed cells.
The strong fluorescence was not observed in normal HeLa cells(A)(B). On the other hand, the strong fluorescence was shown in Parkin expressed cells in 18 hours after additon of CCCP(C). Some of the puncta are co-localized with the autophagy marker(GFP-LC3). In addition, suppressed fluorescence of Mtphagy dye was observed when autophagy inhibitor, bafilomycin was added to Parking expressed cells(D). Because lysosomal pH was increased by the additon of bafilomycin.

Mitophagy detection in starved cells

Living HeLa cells are co-stained with Mtphagy Dye and Lyso Dye under mitophady induced condition.



The condition of autophagy induction:

DMEM medium (amino acid free, included Pepstatin A and E-64d)

Filter

Mtphagy Dye : Ex. 561 nm/ Em. 650LP nm
Lyso Dye : Ex. 488 nm/ Em. 502-554 nm

Mtphagy Dye was added to HeLa cells and the cells were incubated for 6 hours under starved condition. Before observation of the cells, lysosomal staining dye, Lyso Dye was added to HeLa cells. The fluorescent intensity of Mtphagy Dye was increased in the starved HeLa cells but not in normal cells. In addition, Mtphagy Dye was co-localized with Lyso Dye in the starved cells.

Discription	Unit	Code
Mitophagy Detection Kit	1 set	MD01

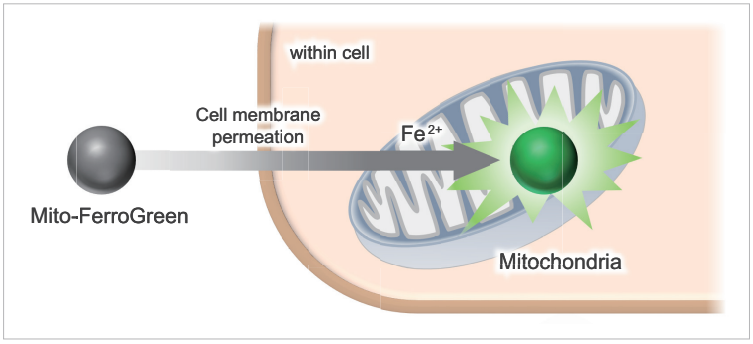
Mitochondrial Iron Detection Reagent

Mito-FerroGreen

Cell permeability and Fe^{2+} selectivity

It is reported that iron is the most abundant transition metal element within an organism and shows various physiological activities. Recently, free iron in living cells is getting attention because its high reactivity is suggested to be related to cellular damage or death. Free iron exists in its stable redox states, ferrous ion (Fe^{2+}) and ferric ion (Fe^{3+}). In living cells, it is considered that understanding the behavior of Fe^{2+} is more important than that of Fe^{3+} because of the intracellular reductive environment, metal transporters and water solubility of Fe^{2+}. Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion (Fe^{2+}) in mitochondria where Fe-S clusters and heme proteins are synthesized, and enables live cell fluorescent imaging of intracellular Fe^{2+}.

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



Double staining with mitochondrial staining probe

Co-localized Mito-FerroGreen and mitochondrial staining dye, MitoBright Deep Red (Dojindo, Code: MT08) were observed after addition of iron(II) in living HeLa cells.

■ Mito-FerroGreen
Excitation : 488 nm
Fluorescence : 500-565 nm

■ MitoBright Deep Red
Excitation : 640 nm
Fluorescence : 656-700 nm

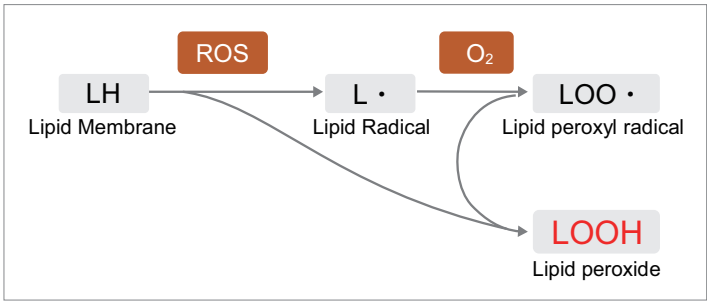
The final concentration of 5 µmol/l Mito-FerroGreen and 200 nmol/l MitoBright Deep Red were added to HeLa cells and the cells were incubated in a 5% CO_2 incubator for 30 minutes. Then, the final concentration of 100 µmol/l ammonium iron(II) sulfate was added to the cells and the cells were incubated in a 5% CO_2 incubator for 1 hour. After incubation, the cells were observed by a confocal fluorescent microscopy.

Discription	Unit	Code
Mito-FerroGreen	50 µg × 2	M489

Mitochondrial Lipophilic Peroxide Detection Reagent

MitoPeDPP

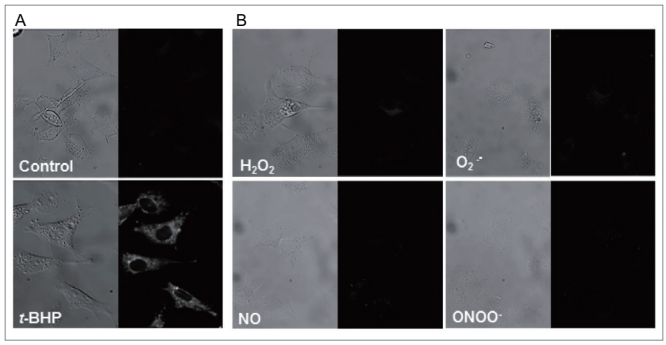
MitoPeDPP is a newly developed fluorescent dye which can penetrate cell membranes and is accumulated in the mitochondria. MitoPeDPP accumulated in mitochondrial inner membranes is oxidized by lipophilic peroxide and then emits strong fluorescence. Since the excitation and emission wavelengths of the oxidized MitoPeDPP (Ox-MitoPeDPP) are 452 nm and 470 nm, respectively, photodamage and autofluorescence of the samples can be minimized. Therefore, MitoPeDPP can be applicable for imaging of the lipophilic peroxide in living cells under a fluorescence microscope.
This product was developed by Dr. Shioji, Department of Chemistry, Fukuoka University.



Lipid peroxidation is the metabolic process under the oxidative stress such as the presence of reactive oxygen species (ROS). Lipid peroxide is an important compound which formed by the chain reaction of lipid radicals and is a marker of oxidative damage in organelle membranes.

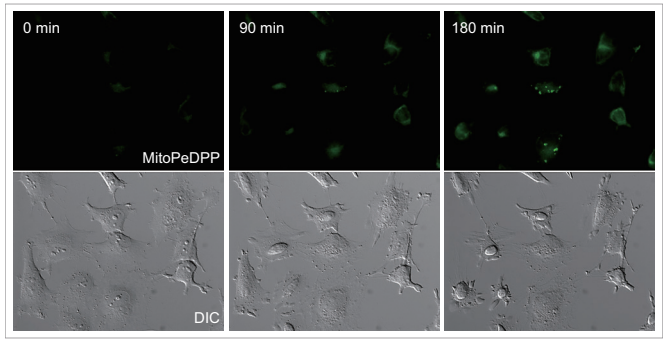
Lpophilic peroxide Selectivity

Even though MitoPeDPP reacts with various peroxides (H_2O_2, *t*-BHP, $ONOO^-$) in homogeneous systems (data is not shown), the MitoPeDPP is specifically-oxidized by *t*-BHP in mitochondria (A) but not with ROS and RNS (B).



A: MitoPeDPP stained cells with *t*-BHP treatment (*t*-BHP) and without (control).
B: MitoPeDPP stained cells with ROS or RNS exposure.
*As ROS generators, PMA ($O_2^{\cdot-}$), NOC7(NO), and SIN-1($ONOO^-$) were used in the experiments.
*Abbreviations
t-BHP : *tert*-butylhydroperoxide
PMA : Phorbol Myristate Acetate
NOC7 : 1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazine
SIN-1 : 3-(Morpholinyl)sydnonimine, hydrochloride
Emission : 470/40 nm, Excitation: 525/50 nm

Detection of lipophilic peroxide generated with rotenone



The final concentration of 0.1 µmol/l MitoPeDPP was added to HeLa cells. The cells were then incubated in a 5% CO_2 incubator for 15 minutes. Then, the final concentration of 1 µmol/l rotenone was added to the cells and time-dependent fluorescence change was observed under a fluorescence microscope for 3 hours.

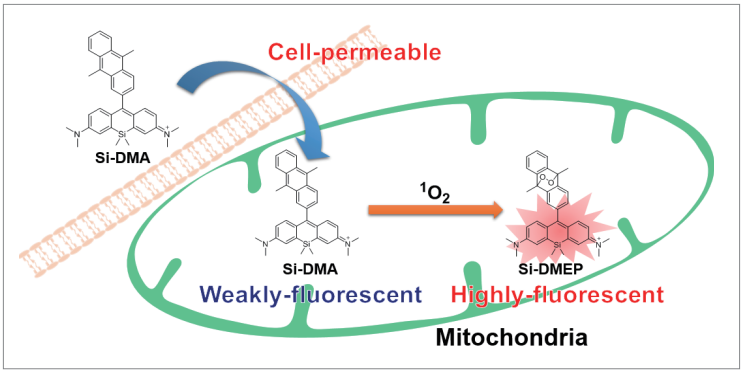
Emission : 470/40 nm
Excitation : 525/50 nm

Discription	Unit	Code
MitoPeDPP	1 set (5 µg×3)	M466

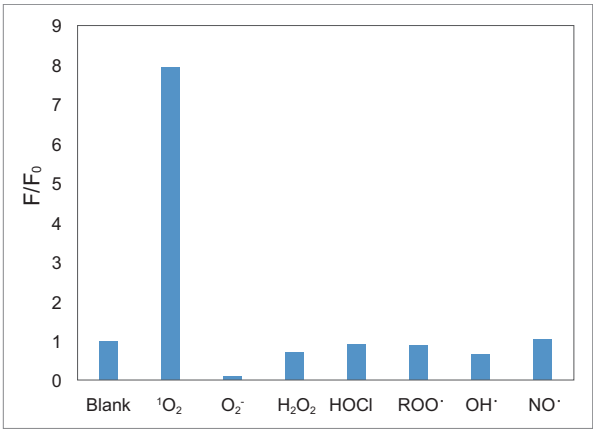
Mitochondrial Singlet Oxygen Detection Reagent

Si-DMA for Mitochondrial Singlet Oxygen Imaging

Majima *et al.* synthesized a new far-red fluorescence probe composed of silicon-containing rhodamine and anthracene moieties, namely Si-DMA, as a chromophore and a $^1O_2$ reactive site, respectively. In 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 is able to selectively detect the $^1O_2$. In addition, Si-DMA is able to visualize the real-time generation of $^1O_2$ from protoporphyrin IX in mitochondria with 5-aminolevulinic acid (5-ALA), a precursor of heme.



Singlet oxygen selectivity

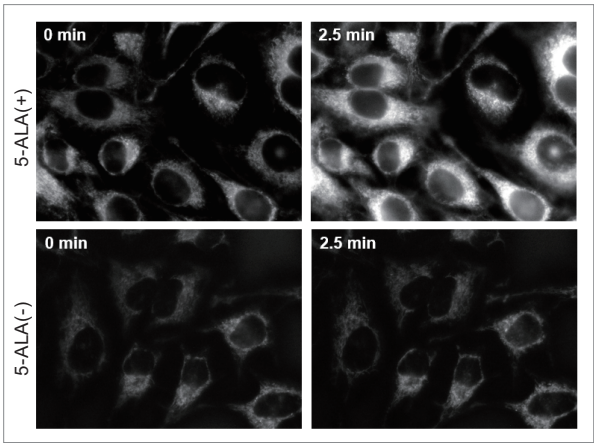


Si-DMA showed significant signal to singlet oxygen but not to other ROS and RNS.

Experimental condition

PBS(pH7.4) MeOH = 1 : 1
Si-DMA 1 μmol/l
ROS 10 mmol/l
RNS 10 mmol/l

Singlet oxygen detection after the addition of 5-ALA



By the addition of 5-ALA(5-aminolevulinic acid) to HeLa cells, protoporphyrin IX was produced in mitochondria. Once the cells were irradiated under the excitation laser, singlet oxygen was generated. In the experiment, the generated singlet oxygen was detected by Si-DMA under a fluorescence microscope.

Emission : 575-625 nm
Excitation : 660-710 nm

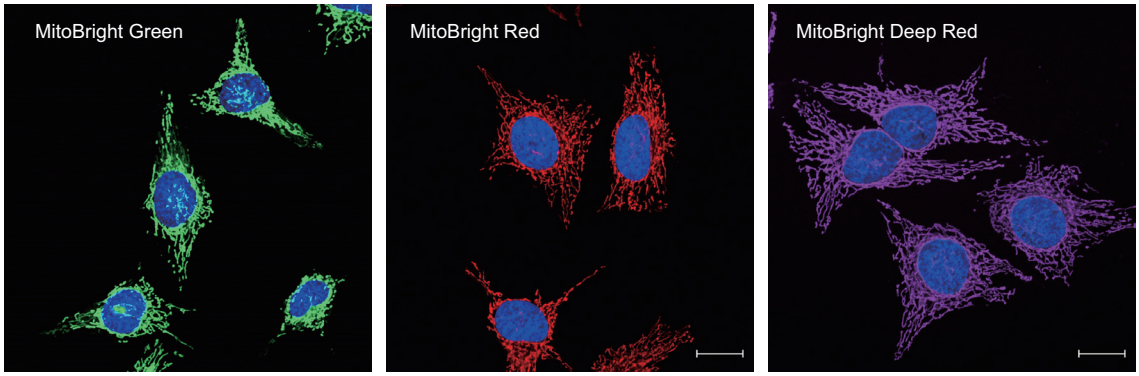
Discription	Unit	Code
Si-DMA for Mitochondrial Singlet Oxygen Imaging	2 μg	MT05

Mitochondrial Staining Reagent

MitoBright Series

Green / Red / DeepRed

There are various organelles playing an important role in cells. Mitochondria is not only a principal site of oxidative phosphorylation to produce ATP, but also an important organelle whose activity and dysfunction are relevant to cancer, cell senescent and neurodegenerative diseases such as Alzheimer's and Parkinson's. “MitoBright” is a fluorescent probe for selectively staining of mitochondria in living cells. It accumulates in healthy mitochondria due to the membrane potential dependency. In addition, MitoBright is well retained in mitochondria because of its covalent bond ability to proteins.



Co-staining of each mitochondrial staining dye and Hochst 33342 (blue) in HeLa cell

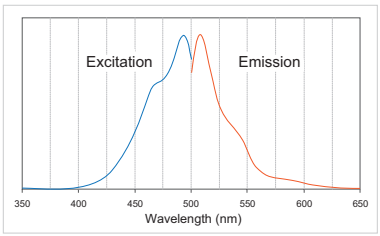
MitoBright series line-up

Description	λex	λem	live imaging	staining before fixation*	staining after fixation
MitoBright Green	493 nm	508 nm	○	○	×
MitoBright Red	547 nm	563 nm	○	○	×
MitoBright Deep Red	643 nm	663 nm	○	○	×

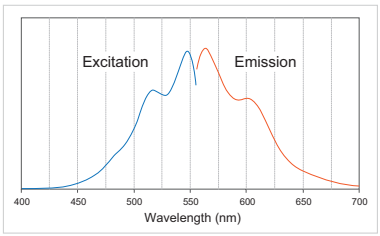
※ PFA (paraformaldehyde) fixation

Fluorescent Property

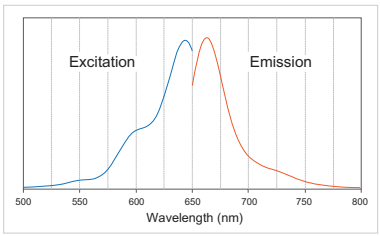
MitoBright Green



MitoBright Red



MitoBright Deep Red



Suitable unit size

The one tube (50 μg) of each MitoBright series is corresponded more than 300 staining experiments when 35 mm dish is used.

Discription	Unit	Code
MitoBright Green	50 μg ×3	MT06
MitoBright Red	50 μg ×3	MT07
MitoBright Deep Red	50 μg ×3	MT08

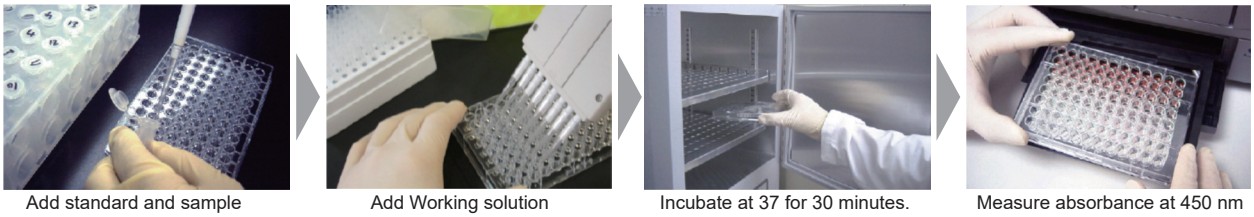
Lactate Quantification Kit

Lactate Assay Kit-WST

Lactate is a metabolite of glycolysis that is one of the main metabolic pathways in cells, and is known to be a biomarker for muscular fatigue and hyperlactacidemia. It also serves as a marker for monitoring the changes of intracellular metabolic pathways. In addition, recent metabolomic study suggests that lactate contributes as a major carbon source in the TCA cycle of tissues and cancer cells. Lactate Assay Kit-WST enables quantitation of lactate produced by glycolysis. This kit is formated for 96-well microplate assays with a detection sensitivity limit of 0.02 mmol/l lactate.

Simple procedure

After transferring the supernatant to a microplate, only the mixing with reagent and incubation procedures are needed.



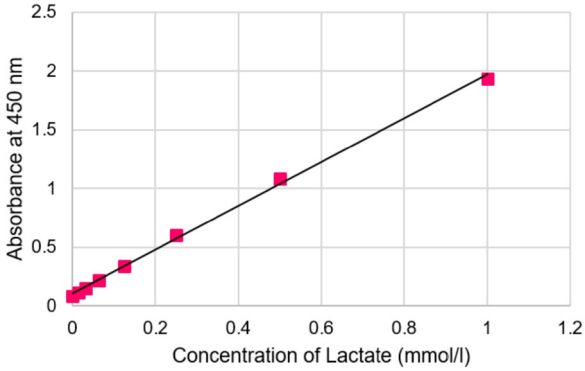
Add standard and sample in each well

Add Working solution

Incubate at 37 for 30 minutes.

Measure absorbance at 450 nm

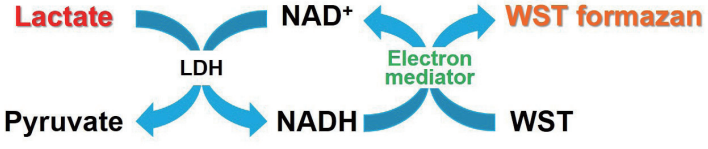
Quantify through standard curve



The concentration of lactate in a sample is quantified with a standard curve which is prepared from the lactate standard in a kit. In case the concentration of lactate is over 1 mmol/l, the sample can be measured after a dilution procedure.

Assay Principle

The kit has been optimized to quantitate lactate in cell culture supernatant by measuring the absorption derived from a colorimetric reaction of WST.



Discription	Unit	Code
Lactate Assay Kit-WST	50 tests 200 tests	L256

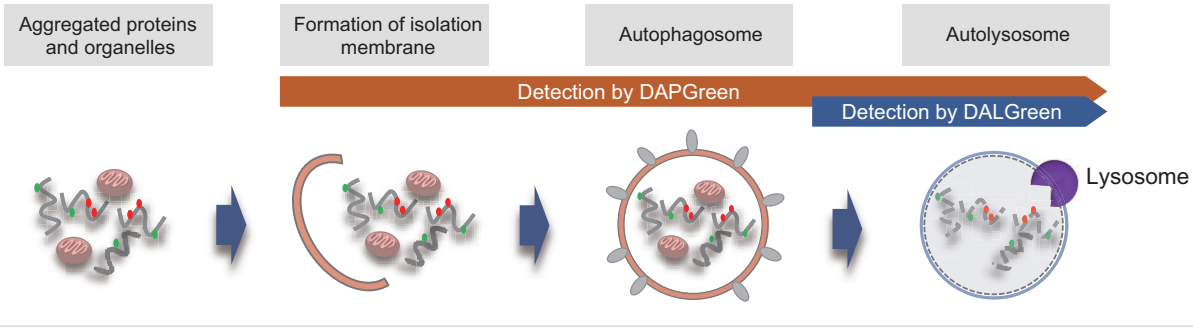
Autophagy Detection Reagent

Detection of Autophagosome
DAPGreen - Autophagy Detection

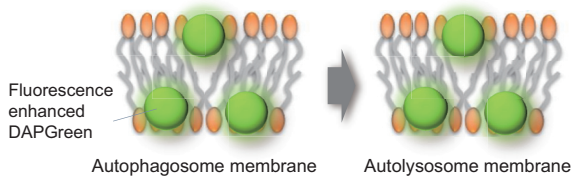
Detection of Autolysosome
DALGreen - Autophagy Detection

How they work

DAPGreen has been developed to monitor early-phase to late-phase autophagy, autophagosome and autolysosome. DALGreen is favorable for monitoring late-phase autophagy, autolysosome. With these probes that stain autophagosomes as they are being formed, the real-time change of autophagic phenomena of live cells may be traced.

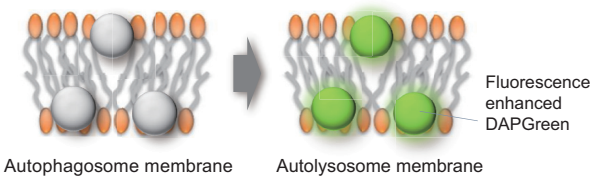


Autophagosome Detection Reagent DAPGreen



When an autophagosome membrane is formed, DAPGreen is incorporated inside of the membrane. The fluorescence of incorporated DAPGreen is enhanced under lipophilic condition. The analysis of DAPGreen also has a high correlation with that of LC3 which is a well-known autophagy marker. For details, please refer to the experimental data of DAPGreen.

Autolysosome Detection Reagent DALGreen



In the same way as DAPGreen, DALGreen is incorporated inside of the autophagosome membrane when the membrane is formed. The fluorescence of DALGreen is enhanced under acidic condition after the autophagosome is fused with the lysosome.

References

The following paper includes the detail principle of DAPGreen and DALGreen. The co-staining results with a lysosomal staining dye and the staining data in ULK1/2* double-knockout cells are also discribed in this article.
*ULK1/2: Uncoordinated 51-like kinases 1 and 2 which plays for homologous function of yeast Atg1.

H. Iwashita, H. T. Sakurai, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, K. Okuma, S. Shimizu, and Y. Ueno, "Small fluorescent molecules for monitoring autophagic flux", *FEBS Lett.*, **2018**, *592*(4), 559.

General information

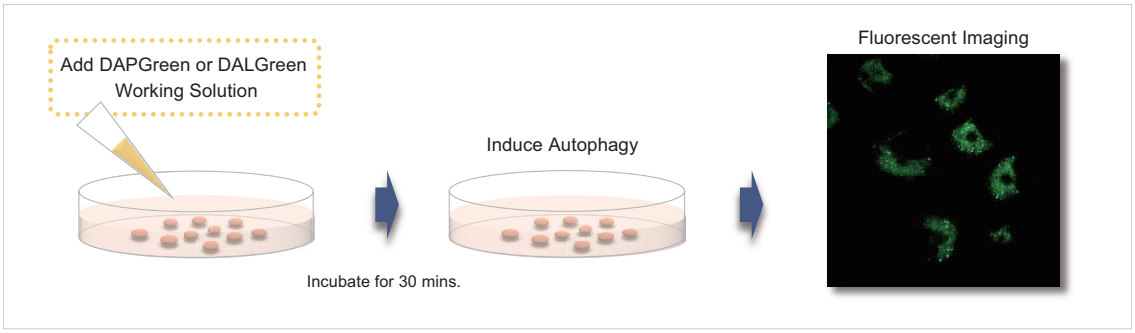
Below table guides you to choose the most suitable application for you.

	Applicable instruments			Fluorescent property	Volume / the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	○	○	○	Ex. 425-475 Em. 500-560 *For confocal microscope, the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish x 25 (when used with max. concentration)	LC3-GFP MDC Cyto-ID etc.
DALGreen	○	○	×	Ex. 350-450 Em. 500-560 *For confocal microscope, the sample can be excited at 488 nm	20 nmol x 1 / 35 mm dish x 10 (when used with max. concentration)	LC3-GFP-RFP etc.

* Fluorescent property is available on Dojindo HP.
* Double staining with DAPGreen and DALGreen is not possible, unfortunately. If you like to double stain autophagosome and autolysosome in different color, please contact us.

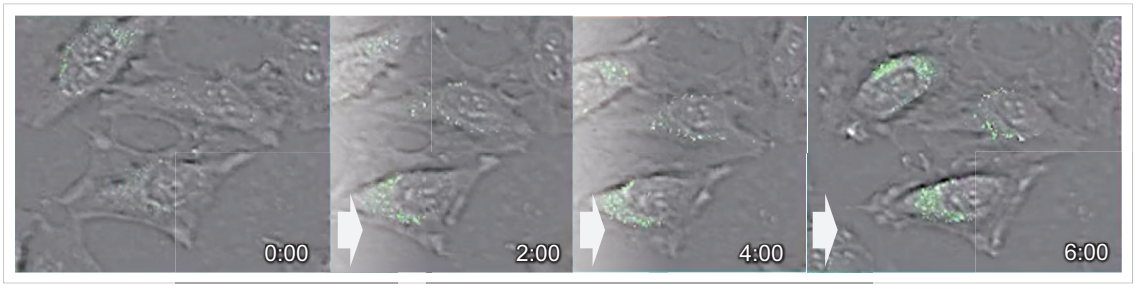
Without genetic engineering

DAPGreen and DALGreen do not require any transfection. You only need to add the reagent to cultured cells.



Time-lapse imaging of autophagy

Six hours of time-lapse imaging was taken using DALGreen and starved HeLa cells.



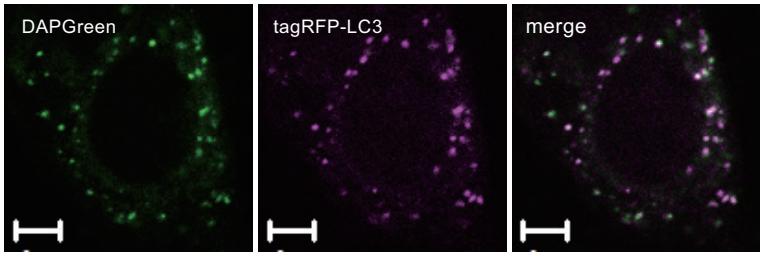
Imaging condition

Medium : Amino acids-free culture medium
Machine : Confocal Quantitative Image Cytometer (YOKOGAWA Electric Corporation : CQ1)
Fluorescence Filters : Ex. 405 / Em. 525/50,
Magnification : 20X

The imaging movie has been uploaded on Dojindo HP.

Good correlation with LC3

The HeLa cells were double stained by DAPGreen and tagRFP-LC3 to determine their colocalization.



Result

Almost all of DALGreen signals were colocalized with LC3 reasonably.

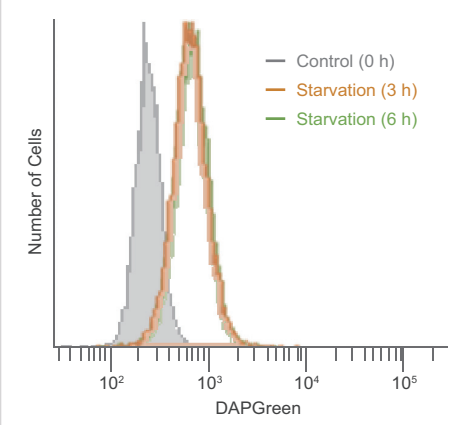
Imaging condition

DAPGreen : Ex. 488 nm / Em. 500-563 nm
Scale bar : 10 μm

Autophagy induced condition

After adding DAPGreen, RFP-LC3 expressed HeLa cells were treated with rapamycin. The images were obtained after 4 hours of incubation.

Quantitative analysis using flow cytometer



Result

Starved HeLa cells showed strong fluorescent signal after 3 hours of incubation.

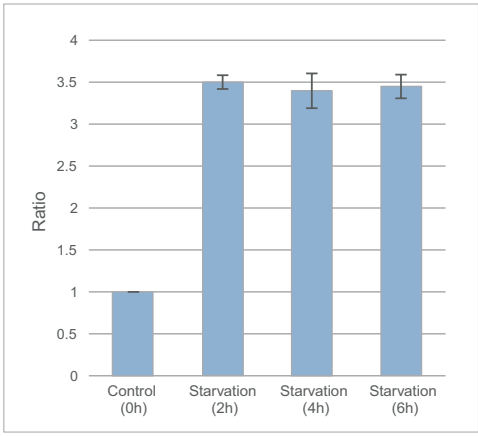
Detection

Wavelengths: Ex. 488 nm / Em. 500-560 nm

The condition of autophagy induction

After staining with DAPGreen, HeLa cells were incubated for 0, 3, 6 hours with amino acid-free medium.

Quantitative analysis using microplate reader



Result

After 2 hours of incubation under starved condition, enhanced fluorescence was observed. (3.5 times stronger than “Control”)

Detection

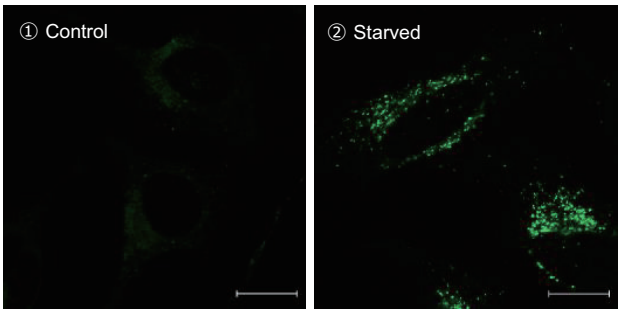
Wavelengths: Ex. 450 nm / Em. 535 nm

The condition of autophagy induction

After staining with DAPGreen, HeLa cells were incubated for 0, 2, 4, 6 hours with amino acid-free medium.

Autolysosome analysis by microscopy

Once DALGreen is added, HeLa cells were incubated either with a growth medium(1) or an amino acid-free medium(2).



Result

The strong fluorescence of DALGreen was observed as puncta in starved HeLa cells.

Imaging condition

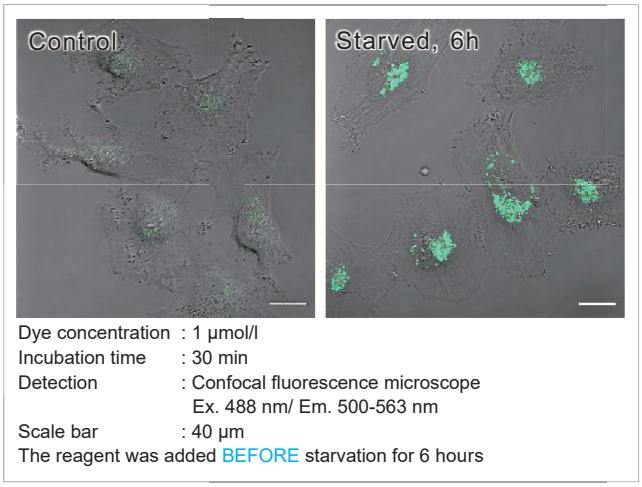
Detection : Ex.488 nm / Em.500-563 nm
Scale bar : 20 μm

The condition of autophagy induction

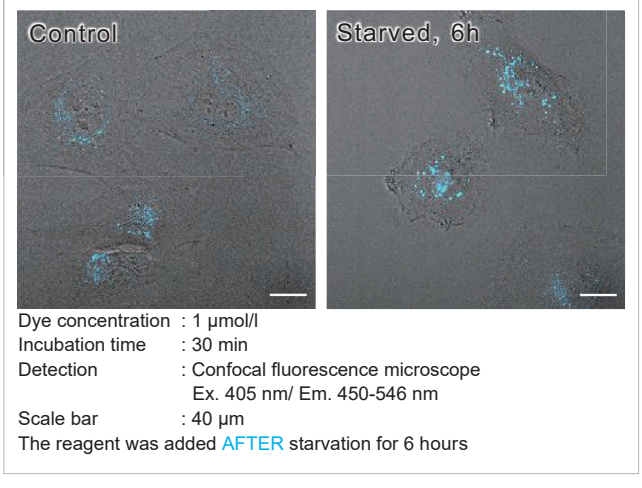
① Control: 6 hours incubation in growth medium
② Starved: 6 hours incubation in amino acid-free growth medium

Comparison with MDC

Autophagy in starved HeLa cells was observed with DALGreen and MDC(Monodansylcadaverine).



Dye concentration : 1 μmol/l
Incubation time : 30 min
Detection : Confocal fluorescence microscope
Ex. 488 nm/ Em. 500-563 nm
Scale bar : 40 μm
The reagent was added BEFORE starvation for 6 hours



Dye concentration : 1 μmol/l
Incubation time : 30 min
Detection : Confocal fluorescence microscope
Ex. 405 nm/ Em. 450-546 nm
Scale bar : 40 μm
The reagent was added AFTER starvation for 6 hours

Results

The fluorescent signal of DALGreen was increased in the starved HeLa cells, whereas MDC showed low S/N ratio.

Advantage

MDC requires excitation by ultraviolet which can damage living cells. On the other hand, DALGreen can be excited at a longer wavelength.

The condition of autophagy induction

DALGreen : Addition of reagent ⇒ Induction of starvation
MDC : Induction of starvation ⇒ Addition of reagent

DALGreen can be used for monitoring autophagy because the reagent is added prior to induction of autophagy unlike MDC.

Discription	Unit	Code
DALGreen - Autophagy Detection	20 nmol	D675
DAPGreen - Autophagy Detection	5 nmol	D676

Cellular Senescence Assay Kit

Cellular Senescence Detection Kit - SPiDER-βGal

Comparison with conventional method, X-Gal

Since X-Gal requires a counting by colorimetric visualization under a microscope, it is not suitable as a quantitative detection. Dojindo's β-galactosidase substrate, SPiDER-βGal allows fluorescent co-staining and quantitative analysis through flow cytometer, image cytometer and so on.

X-Gal

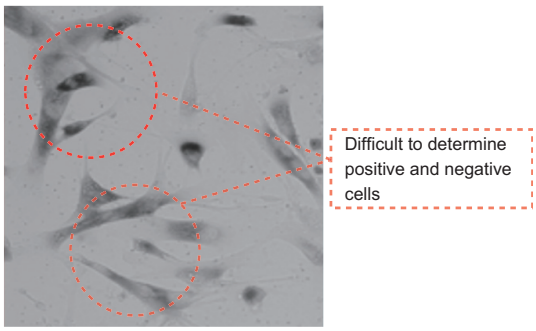
Visual counting required

Disadvantages : - Personal errorv
- Time consuming procedure

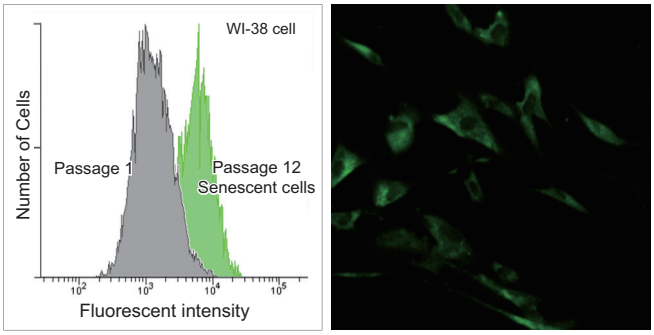
SPiDER-βGal

Reliable analysis by flowcytometry

Advantages : - Quantitative method
- Simple procedure



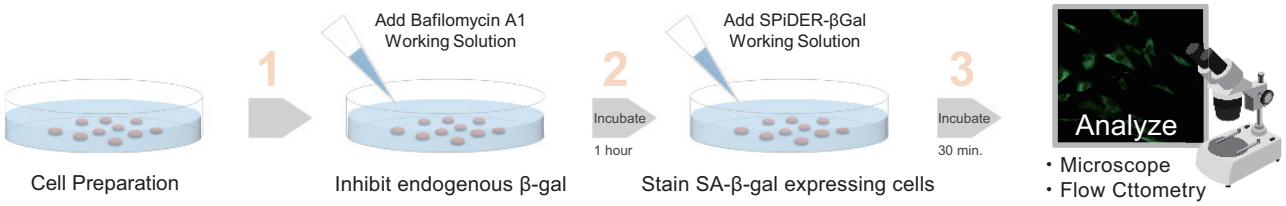
Senescent cells stained with X-gal



Senescent cells stained with this kit

Simple Procedure

The kit is applicable for both living and fixed cells. The SA-β-gal staining can be done in only 30 minutes. For living cells, just need to add 2 types of reagent included in the kit to cultured cells. If fixation is needed, it can be performed before staining.



Cell Preparation

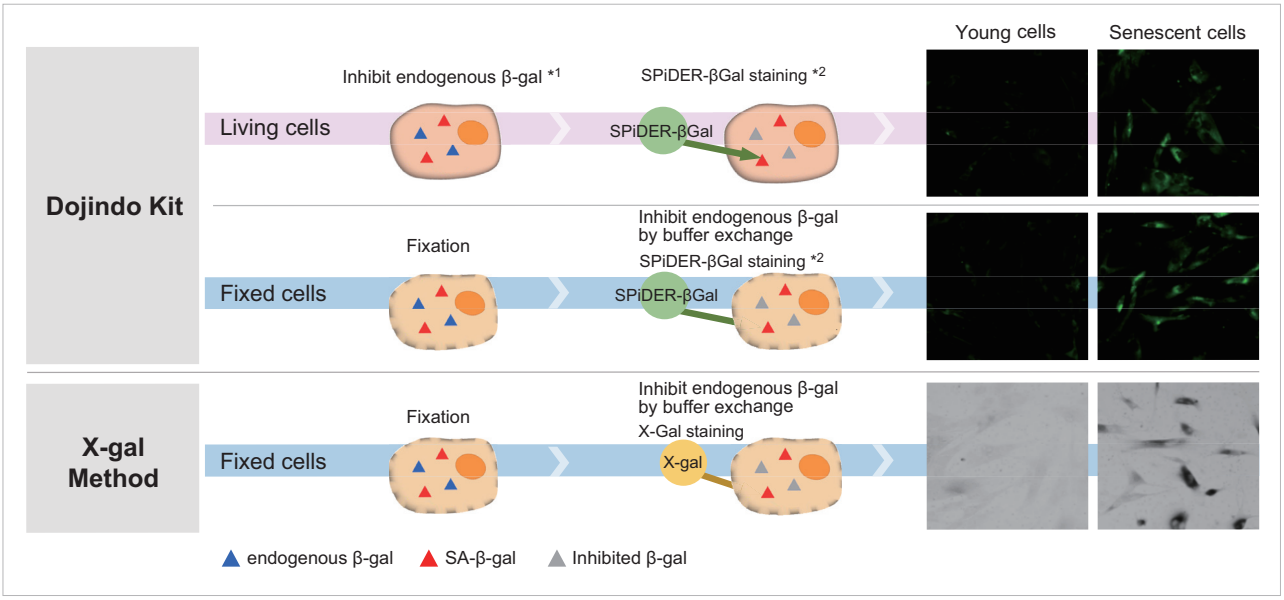
Inhibit endogenous β-gal

Stain SA-β-gal expressing cells

- Microscope
- Flow Cttometry

This kit is applicable for both living and fixed cells.

The staining principles are shown in below.



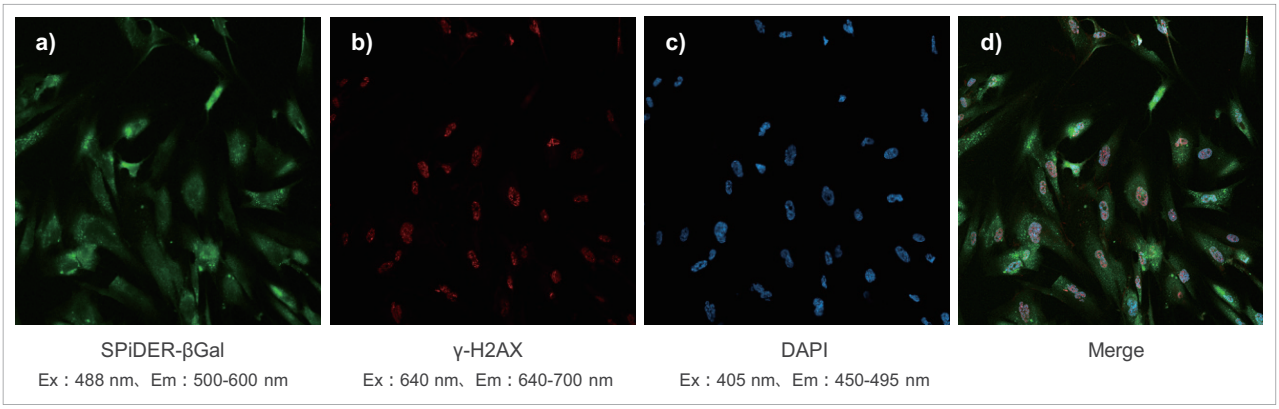
∗1 Why endogenous β-galactosidase suppressed?

Normally endogenous β-galactosidase exist in lysosome where acid pH envilopment. To detect acculate SA-β-gal selectively, Bafilomycin A1(included in the kit) works to supress such endogenous β-galactosidase by pH neutralization.

∗2 How SPiDER-βGal beneficial compared with other small molecules?

SPiDER-βGal has better cell permeability and retention ability than other commercially available dyes such as FDG and C12FDG. Therefore, it enables high sensitive fluorescence imaging.

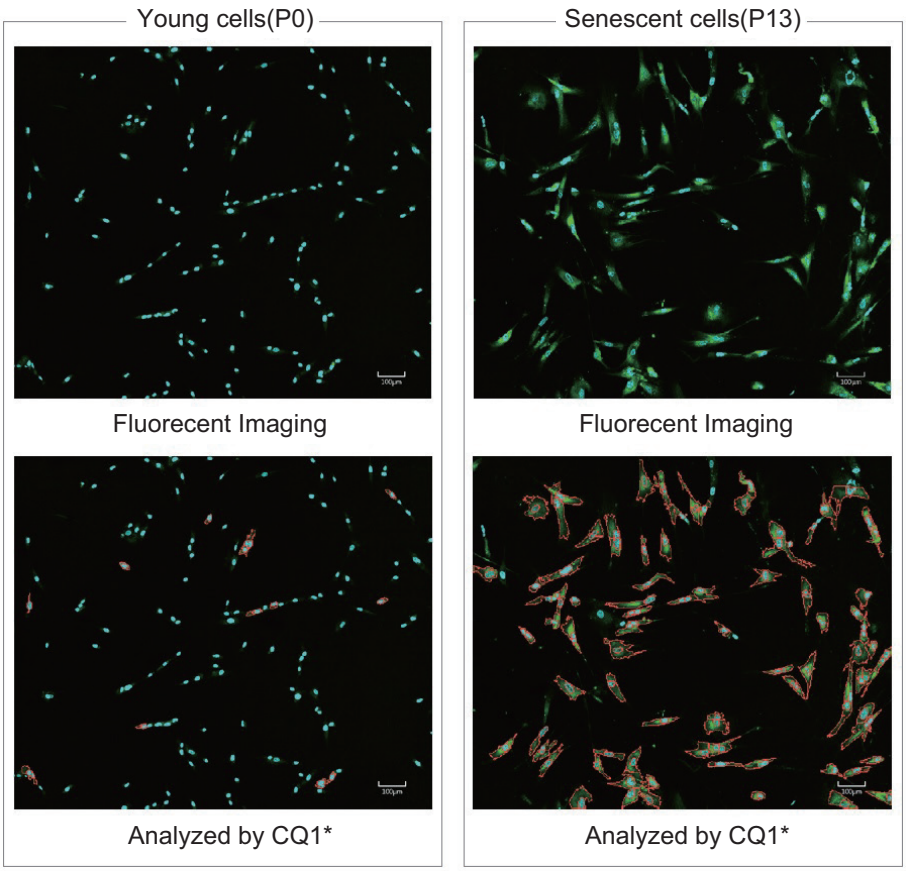
Co-staining with other cellular senescence markers



In this experiment, WI-38 cells were used after ten times of subculture (Passage 10) as the model of senescent cells. The cells were stained with a) this kit for SA-β-gal detection, b) anti γ-H2AX antibody for DNA damage detection, and DAPI for nuclear staining. In the results, both senescent markers, SA-β-gal and γ-H2AX staining were observed. The experimental procedure is informed at the product HP.

Quantification with confocal quantitative image cytometer

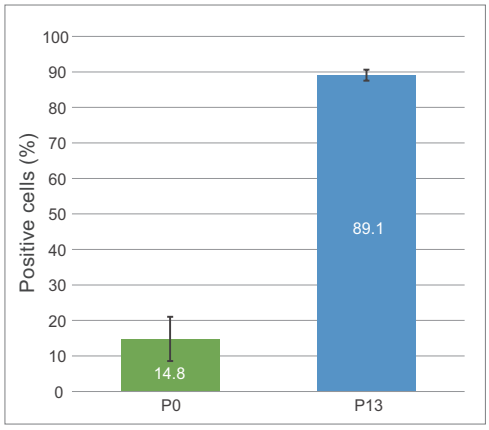
In the conventional method of X-gal, SA-β-gal-positive cells are counted under microscope and calculate the percent of the senescent cells by compared with toal cells. The SA-β-gal-positive cells were stained with this kit and analyzed using confocal quantitative image cytometer CQ1(Yokogawa Electric Corporation).



* SA-β-gal positive cells are marked with red circles.

Imaging condition

Culture plate : 96 well plate
Objective lens : 10 times
Emission Detector
405 nm (Hoechst 33342) : cyan
488 nm (SPiDER-βGal) : green



Quantification for SA-β-gal-positive cells

The total cells were stained with DNA staining dye, Hoechst 33342. The SA-β-gal-positive cells and total were counted and calculate the ratio of senescent cells.

The difference of SA-β-gal-positive cells ratio were shown in WI-38 cells depending on the number of passage. The data was quickly analysed with the confocal quantitative image cytometer compared with the manualy counting procedure with X-gal staining method.

Discription	Unit	Code
Cellular Senescence Detection Kit - SPiDER-βGal	-	SG03 or SG04

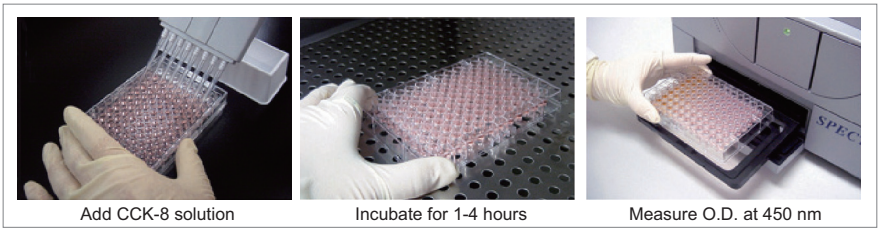
Cell Proliferation / Cytotoxicity Assay Kit

Cell Counting Kit - 8

Cell Counting Kit-8 (CCK-8) allows sensitive colorimetric assays for the determination of cell viability in cell proliferation and cytotoxicity assays. Dojindo's highly water-soluble tetrazolium salt, WST-8, is reduced by dehydrogenase activities in cells to give a yellow-color formazan dye, which is soluble in the tissue culture media. The amount of the formazan dye, generated by the activities of dehydrogenases in cells, is directly proportional to the number of living cells. The detection sensitivity of CCK-8 is higher than the other tetrazolium salts such as MTT, XTT, MTS or WST-1.

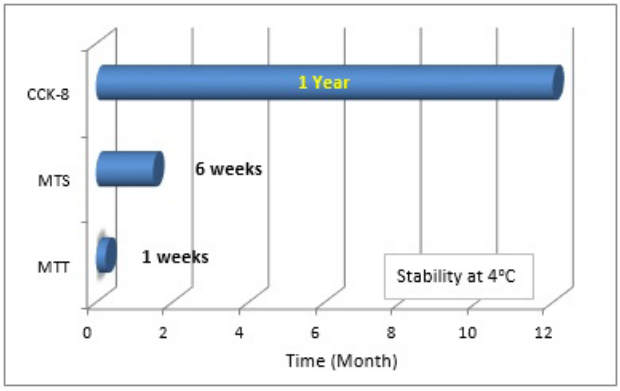
Simple procedure

Cell Counting Kit-8 requires 3 simple steps.



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

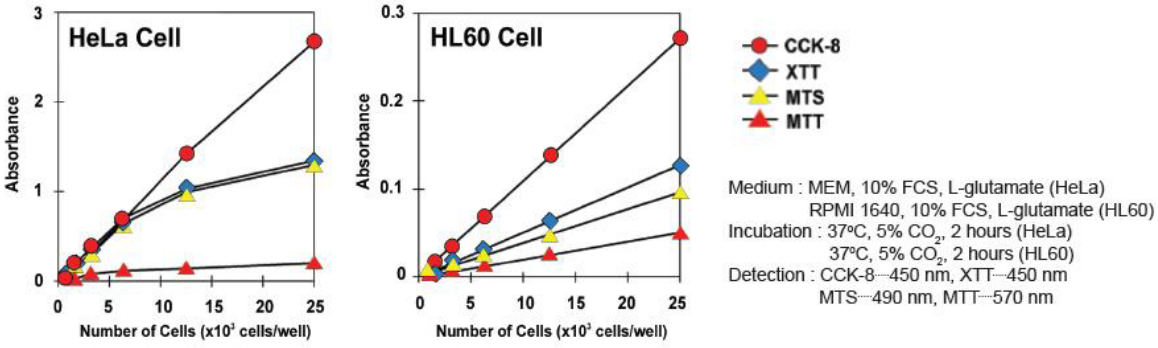
Longer stability



Cell Counting Kit-8 is a ready-to-use solution. It is also stable at 4ºC for 1 year. Your assay can be done anytime without thaw and freeze.

Higher sensitivity

CCK-8 (WST-8) is the highest sensitive kit for cell based assay.



Medium : MEM, 10% FCS, L-glutamate (HeLa)
RPMI 1640, 10% FCS, L-glutamate (HL60)
Incubation : 37ºC, 5% CO_2, 2 hours (HeLa)
37ºC, 5% CO_2, 2 hours (HL60)
Detection : CCK-8---450 nm, XTT---450 nm
MTS---490 nm, MTT---570 nm

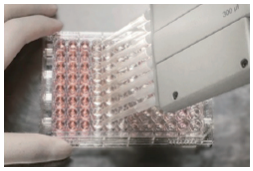
Discription	Unit	Code
Cell Counting Kit-8	100 - 10,000 tests	CK04

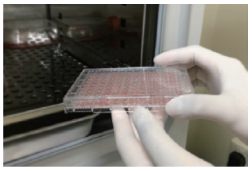
Cytotoxicity Assay Kit

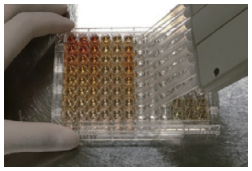
Cytotoxicity LDH Assay Kit-WST

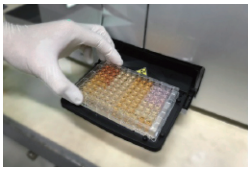
Lactate dehydrogenase(LDH) is an enzyme that presents in almost cell types and it catalyzes the oxidation of lactate to pyruvate in the presence of co-enzyme NAD+. Once Cells are impaired by stress, injuries, chemicals, or intercellular signals, LDH is rapidly released from the cell membrane. Thus, the measurement of the amount of released LDH from cells is one of the major methods to assess the cell death. Cytotoxicity LDH Assay Kit-WST is designed for use with 96-well micro plates formats for high-throughput screening.

Simple procedure

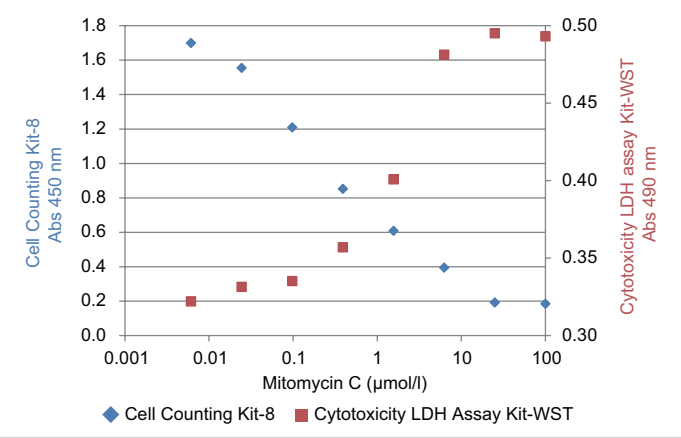

Add Working Solution


Colorimetric reaction


Add Stop Solution


Measure absorbance

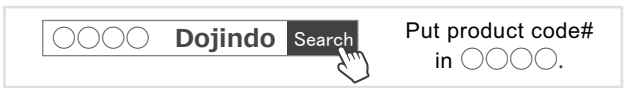
Combination use



To obtain the accurate result in cytotoxicity assay, the samples are measured by different principles. CCK-8 and LDH assay measure NADH as living cells and released LDH as dead cells, respectively. According to these results, the living cells were reduced and dead cells were increased with the high concentration of toxicant.

Discription	Unit	Code
Cytotoxicity LDH Assay Kit-WST	100 tests 500 tests 2,500 tests	CK12

For the detail information of each product, put the product code with "dojindo" and visit our web site.



If you have any questions or need any assistances, please feel free to contact us.

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