# Reagent for Cellular Function Analysis

## Autophagy

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

## Senescence

- Cellular Senescence Detection Kit -SPiDER-βGal
- Cellular Senescence Plate Assay Kit -SPiDER-βGal

**Neurodegenerative Diseases**

**Cancer**

**Senescence**

## Mitochondria

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

## Cellular Metabolism

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

## Ferroptosis

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

# Neurodegenerative Diseases

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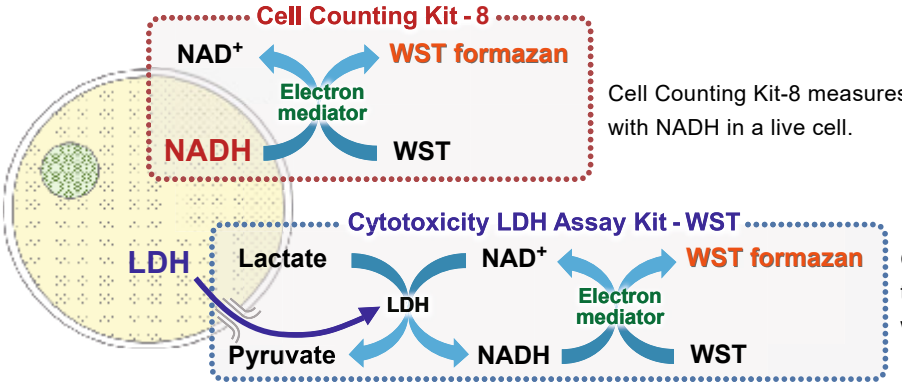
Cell Proliferation / Cytotoxicity Assay

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

Cell Counting Kit-8 Cytotoxicity LDH Assay Kit-WST

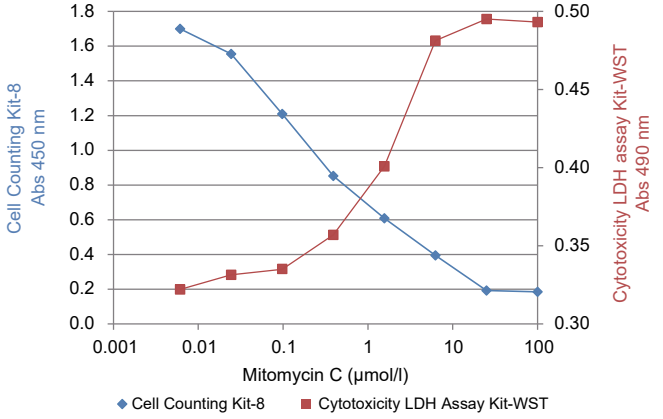
## Detection Principle




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

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

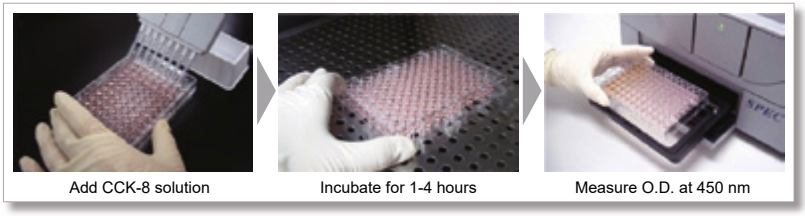
## Simultaneous Usage of CCK-8 and Cytotoxicity LDH Assay Kit-WST



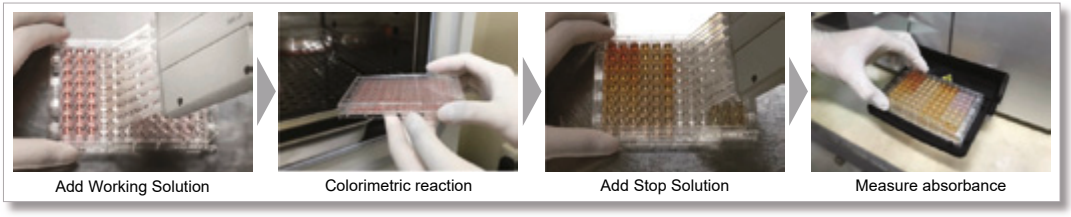

Drug: Mitomycin C
Cell Line: HeLa
Media: MEM, 10% FBS
Incubation: 37°C, 5% $CO_2$ for 48 hours
Measuring Condition: Cell Counting Kit-8 (450 nm)
Cytotoxicity LDH Assay Kit-WST (490 nm)

# Simple Procedure

・Cell Counting Kit-8

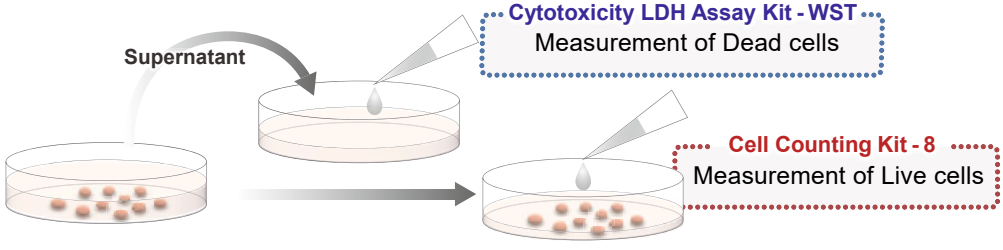



・Cytotoxicity LDH Assay Kit-WST




## Same Samples can be used

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



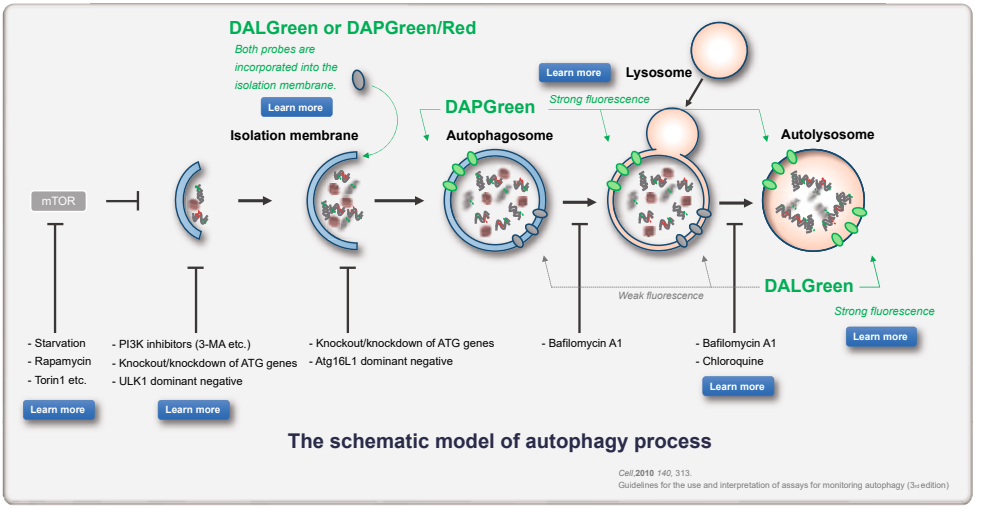

| Description | Unit | Code |
|---|---|---|
| Cell Counting Kit-8 | 500 tests | CK04-05 |
| | 1000 tests | CK04-11 |
| | 3000 tests | CK04-13 |
| | 10000 tests | CK04-20 |
| Cytotoxicity LDH Assay Kit-WST | 100 tests | CK12-01 |
| | 500 tests | CK12-05 |
| | 2000 tests | CK12-20 |

D675 D676 D677

## Autophagy

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

DAPGreen and DAPRed detect autophagosomes, while DALGreen detects autolysosomes. These dyes are permeable to cells and enables live cell imaging with fluorescence microscopy, and DAPGreen and DALGreen allow for quantitative assay by flow cytometry. Autophagy is an intracellular degradation system involving autophagosome formation, detected by DAPGreen and DAPRed, and lysosome fusion, detected by DALGreen, which fluoresces intensity increases in acidic conditions.

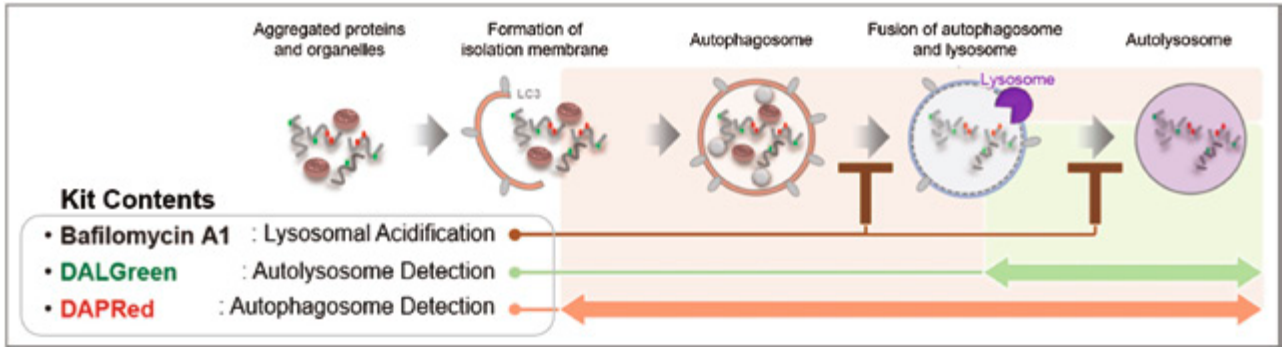



## Feature of Each Dye

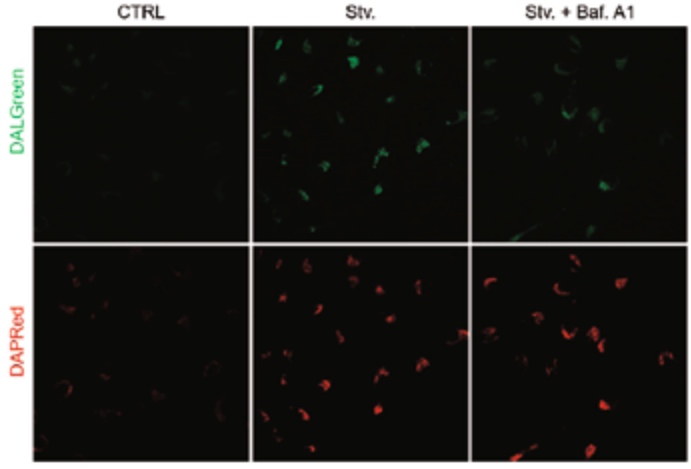
| | Applicable instruments | | | Fluorescent properties | Volume / the number of usable assays | Existing methods |
|---|---|---|---|---|---|---|
| | Fluorescent Microscope | Flow cytometer | Microplate reader | | | |
| DAPGreen | ✓ | ✓ | ✓ | Ex = 425-475 nm<br>Em = 500-560 nm<br>* For confocal microscope,the sample can be excited at 488 nm | 5 nmol x 1 /<br>35 mm dish: 25<br>(when used in 1.0 μmol/l) | LC3-GFP<br>MDC<br>Cyto-ID etc. |
| DAPRed | ✓ | | | Ex = 500-560 nm<br>Em = 690-750 nm | 5 nmol x 1 /<br>35 mm dish: 25<br>(when used in 1.0 μmol/l) | |
| DALGreen | ✓ | ✓ | | Ex = 350-450 nm<br>Em = 500-560 nm<br>* For confocal microscope,the sample can be excited at 488 nm | 20 nmol x 1 /<br>35 mm dish: 10<br>(when used in 1.0 μmol/l) | LC3-GFP-RFP<br>etc. |

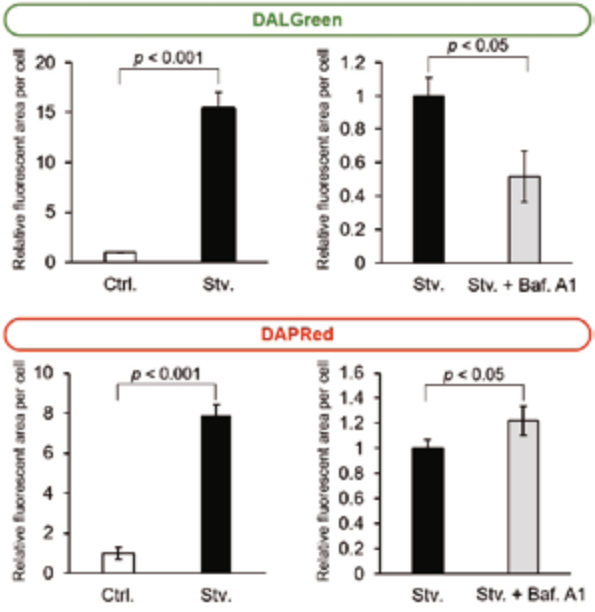
*Double staining imaging by DAPGreen and DALGreen is not possible

# Autophagic Flux Assay Kit




## Experimental Example: Autophagy Flux Analysis




By culturing HeLa cells in HBSS with starvation, autophagy was induced and DAPRed and DALGreen fluorescence increased. Addition of Baf. A1 decreased DALGreen fluorescence, indicating that autolysosomes were reduced and Autophagy Flux was inhibited.

Quantification method: Fluorescence values (area) were obtained in Image J and normalized by the number of cells per field of view*. Number of samples: n=3
*Please obtain images with the same number of cells per field of view as possible.

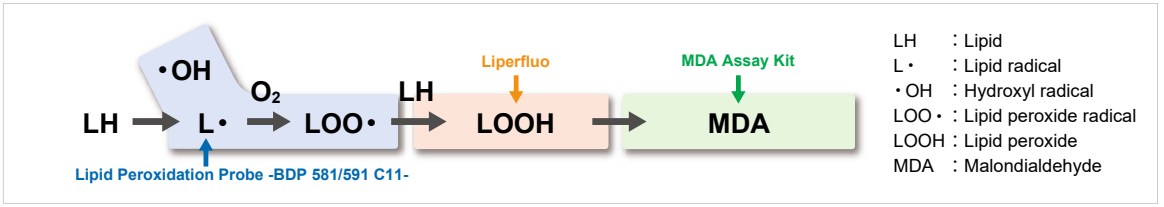
| Description | Unit | Code |
|---|---|---|
| Autophagic Flux Assay Kit | 1 set* | A562-10 |
| DALGreen - Autophagy Detection | 20 nmol | D675-10 |
| DAPGreen - Autophagy Detection | 5 nmol | D676-10 |
| DAPRed - Autophagy Detection | 5 nmol | D677-10 |

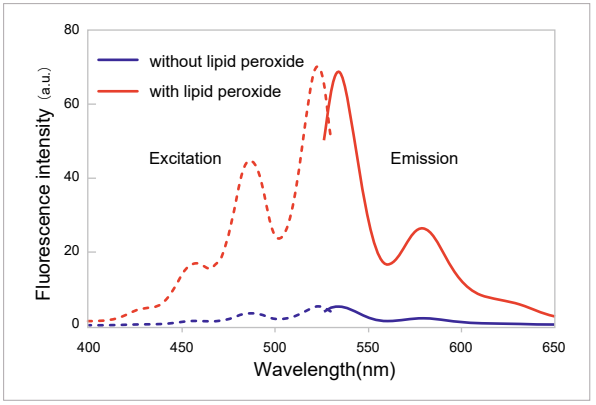
*Equivalent to 5 dishes (35 mm dish)

## Lipid Peroxide Detection

# Liperfluo

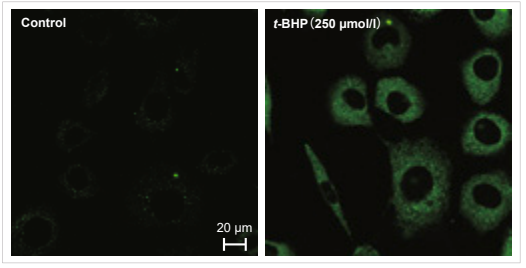
Liperfluo is a Dojindo-developed fluorescence probe to specifically detect lipid peroxides with minimal photo-damage or auto-fluorescence. It emits intense fluorescence in organic solvents and is nearly non-fluorescent in aqueous media. Liperfluo's tetraethyleneglycol group increases its solubility and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




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

## Lipid Peroxide Detection in Living Cells



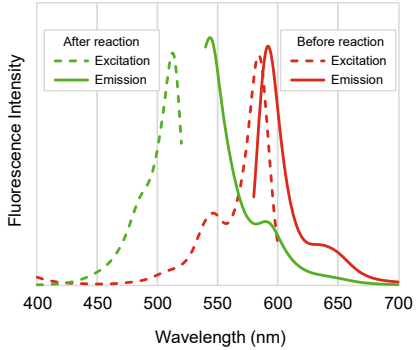

Liperfluo added to cells, t-BHP induced lipid peroxidation and cells were observed under confocal microscope to study ferroptosis.

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

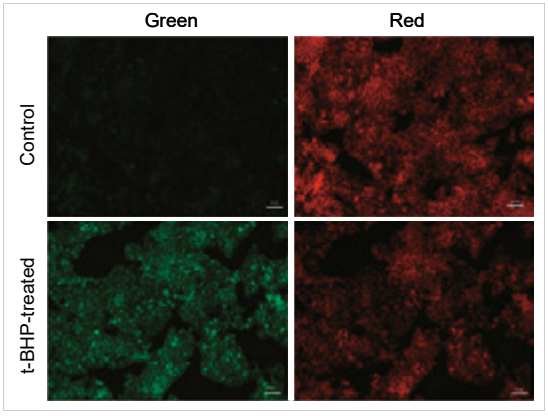
| Description | Unit | Code |
|---|---|---|
| Liperfluo | 1 set (50 μg × 5) | L248-10 |

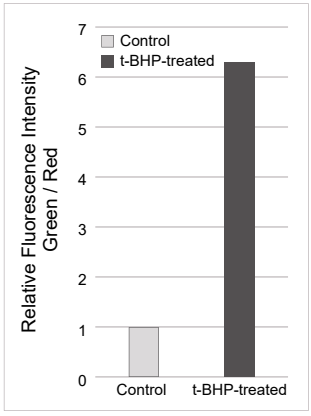
# Lipid Peroxidation Probe -BDP 581/591 C11-

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




## Lipid Peroxidation Assay




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

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

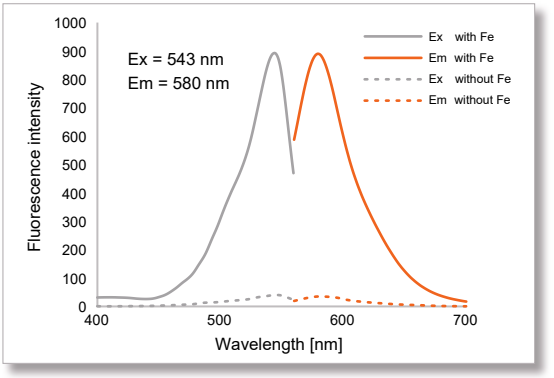
Fluorescent Plate Reader
Green: Ex = 490 nm, Em = 520-540 nm
Red: Ex = 570 nm, Em = 600-620 nm

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

## Intracellular Iron Ion Measurement

# FerroOrange

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




## Experimental Example


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

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

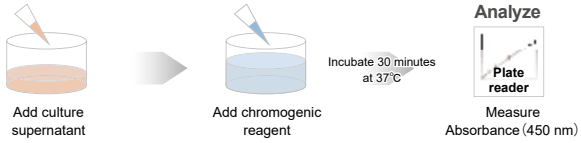
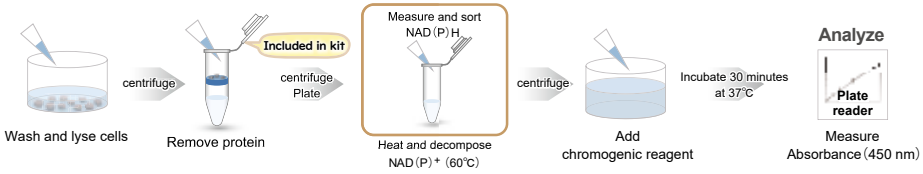
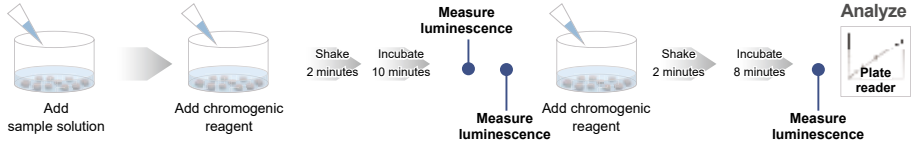
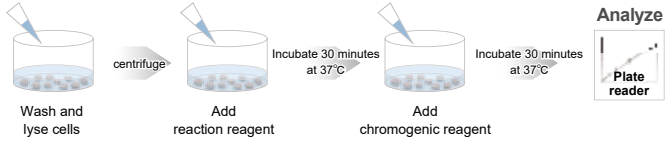
| Description | Unit | Code |
|---|---|---|
| FerroOrange | 1 tube | F374-10 |
| | 3 tube | F374-12 |

# Measurements of Intracellular Metabolism

| Description | Unit | Code |
|---|---|---|
| **Starter Kit** | | |
| Glycolysis/OXPHOS Assay Kit | 50 tests | G270-10 |
| Glycolysis/JC-1 MitoMP Assay Kit | 50 tests | G272-10 |
| **Quantification for Intracellular Metabolism** | | |
| ATP Assay Kit-Luminescence | 50 tests | A550-10 |
| | 200 tests | A550-12 |
| ADP/ATP Ratio Assay Kit-Luminescence | 100 tests | A552-10 |
| Glucose Assay Kit-WST | 50 tests | G264-05 |
| | 200 tests | G264-20 |
| Glutamine Assay Kit-WST | 100 tests | G268-10 |
| Glutamate Assay Kit-WST | 100 tests | G269-10 |
| α-Ketoglutarate Assay Kit-Fluorometric | 100 tests | K261-10 |
| Lactate Assay Kit-WST | 50 tests | L256-10 |
| | 200 tests | L256-20 |
| NAD/NADH Assay Kit-WST | 100 tests | N509-10 |
| NADP/NADPH Assay Kit-WST | 100 tests | N510-10 |
| **Uptake Assay Kit** | | |
| Glucose Uptake Assay Kit-Blue | 1 set | UP01-10 |
| Glucose Uptake Assay Kit-Green | 1 set | UP02-10 |
| Glucose Uptake Assay Kit-Red | 1 set | UP03-10 |
| Amino Acid Uptake Assay | 20 tests | UP04-10 |
| | 100 tests | UP04-12 |
| Cystine Uptake Assay Kit | 20 tests | UP05-10 |
| | 100 tests | UP05-12 |
| Fatty Acid Uptake Assay Kit | 100 tests | UP07-10 |

# Simple Procedure for First Time User

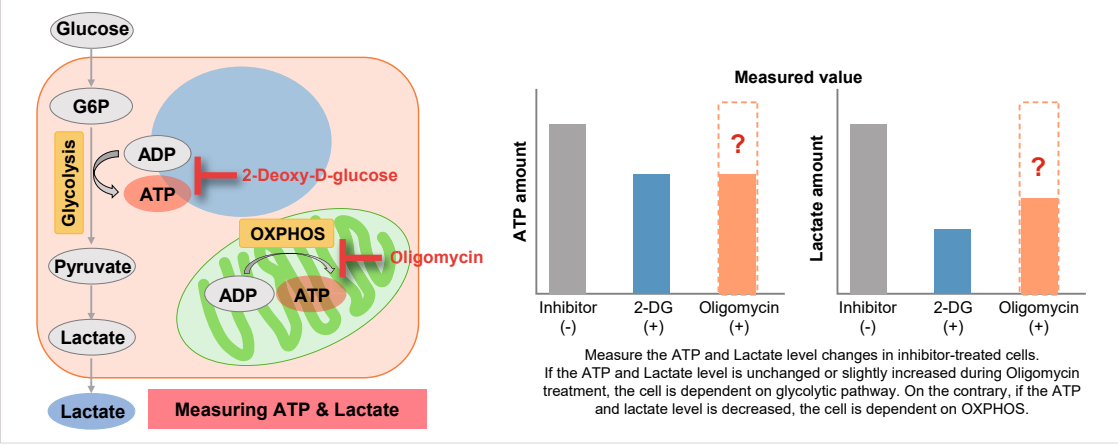
For a first-time user, the kit includes the reagents and components necessary for measuring samples. You'll soon realize how easy it is to use.

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

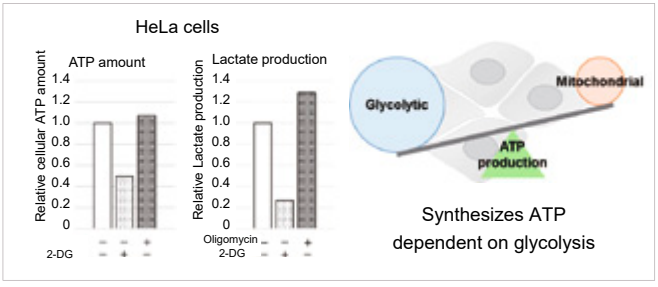
# Glycolysis/OXPHOS Assay Kit

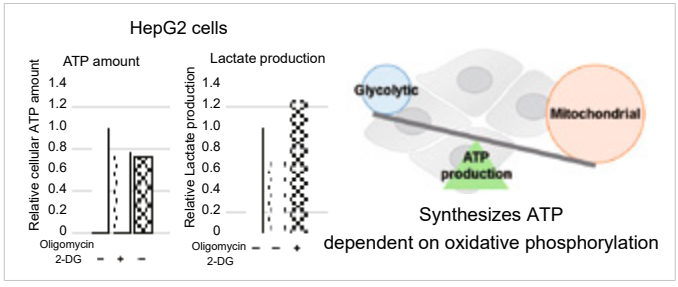
- Easy test via plate reader, no need for expensive equipment
- All reagent acquired is included, ready to use kit
- Easy-to-understand detailed protocol

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




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



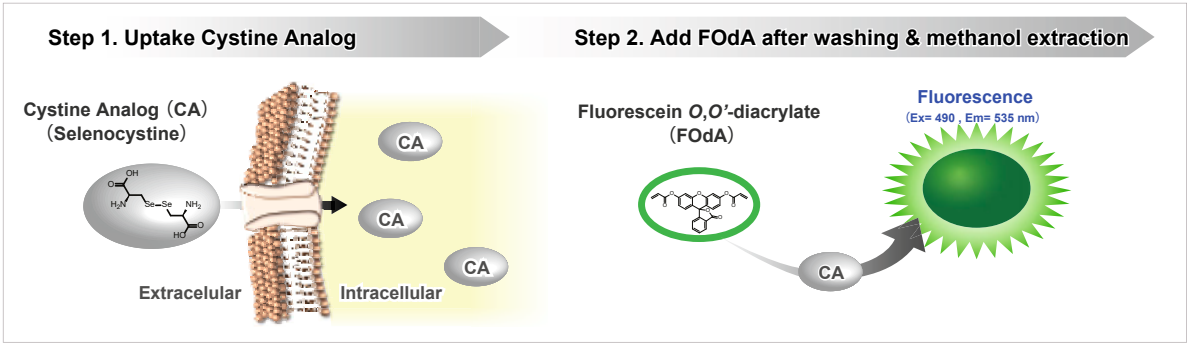




| Description | Unit | Code |
|---|---|---|
| Glycolysis/OXPHOS Assay Kit | 50 tests | G270-10 |

# Cystine Uptake Assay Kit

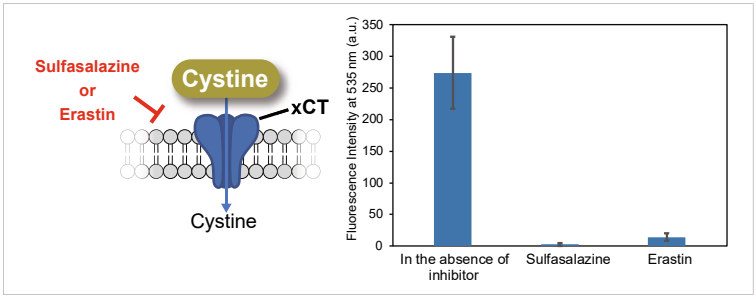
- Easier way to cystine uptake assay
- Applied for plate assay

The Cystine Analog (CA) in this kit can be taken up into cells via xCT, and the incorporated CA can be specifically detected using the Fluorescent Probe and Reducing Agent. Thus, the xCT activity can be measured easily.[Patent applied]




## Evaluation of xCT inhibitor Sulfasalazine or Erastin

Using this kit, we measured the inhibitory effect of sulfasalazine and erastin on cystine uptake by HeLa cells. The fluorescence intensity of the sulfasalazine and elastin groups decreased significantly, indicating that both reagents inhibit cystine uptake.




Experiment Condiitons
Cell Line: HeLa cells
Pretreatment: DMEM (cystine-free, serum-free), 37°C, 5 min
Uptake conditions: 0.5 mmol/l sulfasalazine or 2 µmol/l erastin / Cystine Analog / DMEM (cystine-free, serum-free), 37°C, 30 min
Instrument: Fluorescent Plate Reader
Filter: Ex=485 nm, Em=535 nm

| Description | Unit | Code |
|---|---|---|
| Cystine Uptake Assay Kit | 20 tests | UP05-10 |
| | 100 tests | UP05-12 |

# Mitochondrial Research

## Mitochondrial Superoxide Detection

MitoBright ROS Deep Red - Mitochondrial Superoxide Detection

Allow to detecting mitochondrial superoxide with a long wavelength (Deep Red)

## Ferrous Ion Detection

Mito-FerroGreen

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

## Mitophagy Detection

Mitophagy Detection Kit

Live-cell fluorescent imaging of mitophagy without transfection

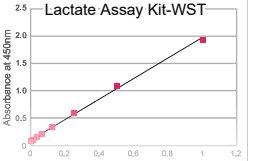
## Measurement of Glucose

Glucose Assay Kit-WST

Measurement of intracellular glucose concentrations via fluorescence

## Measurement of Lactate

Lactate Assay Kit-WST

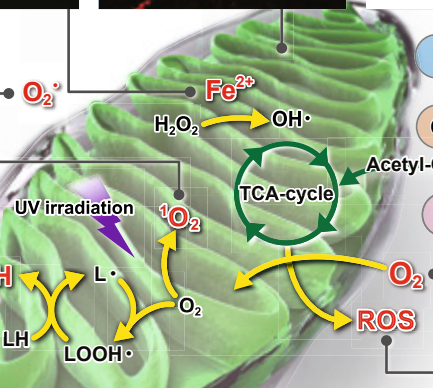



Measuring lactate to infer glycolytic activity

## Singlet Oxygen Detection

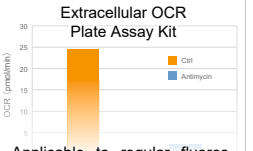
Si-DMA for Mitochondrial Singlet Oxygen Imaging

Real-time visualization of $^1O_2$ generation




## Oxygen consumption rate (OCR) Detection

Extracellular OCR Plate Assay Kit




Applicable to regular fluorescent plate reader with temperature-controlled incubation

## Lipophilic Peroxide Detection

MitoPeDPP

Live-cell fluorescent imaging of lipophilic peroxide

## Mitochondria Fluorescent Probe for Immunostaining

MitoBright IM Red for Immunostaining

Capable of co-stained with immunostaining.
Higher retention in mitochondria after fixation & membrane permeabilization

## Membrane Potential Detection

MT-1 MitoMP Detection Kit

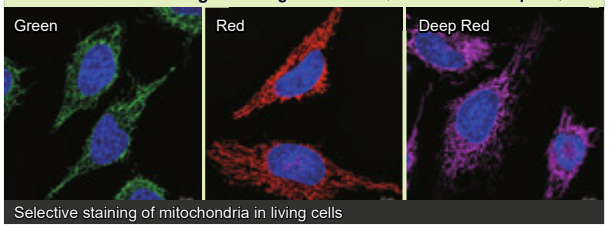
Monitoring and observation even after fixation, with more sensitive detection than JC-1

## Total ROS Detection

ROS Assay Kit -Highly Sensitive DCFH-DA-

Detection with higher sensitivity than the original DCFH-DA

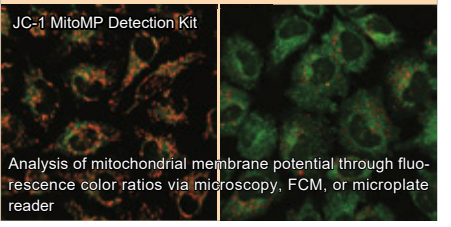
## Mitochondrial Staining MitoBright LT Series (Green / Red / DeepRed)




Selective staining of mitochondria in living cells

## Membrane Potential Detection

JC-1 MitoMP Detection Kit



Analysis of mitochondrial membrane potential through fluorescence color ratios via microscopy, FCM, or microplate reader

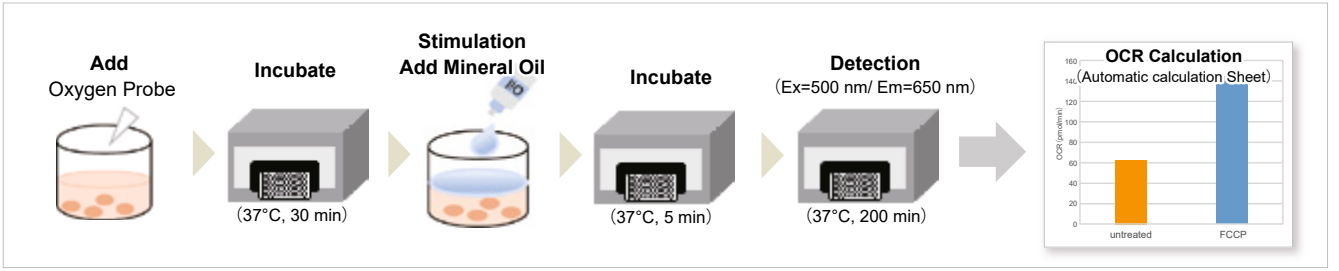
| Description | Unit | Code |
|---|---|---|
| Metabolism | | |
| Extracellular OCR Plate Assay Kit | 100 tests | E297-10 |
| Glucose Assay Kit-WST | 50 tests | G264-05 |
| | 200 tests | G264-20 |
| Lactate Assay Kit-WST | 50 tests | L256-10 |
| | 200 tests | L256-20 |
| Mitochondrial Membrane Potential | | |
| MT-1 MitoMP Detection Kit | 1 set | MT13-10 |
| JC-1 MitoMP Detection Kit | 1 set | MT09-10 |
| Mitophagy | | |
| Mitophagy Detection Kit | 1 set | MD01-10 |
| Mtphagy Dye | 5 µg × 3 | MT02-10 |
| Mitochondrial Staining | | |
| MitoBright LT Green | 400 µl | MT10-12 |
| MitoBright LT Red | 400 µl | MT11-12 |
| MitoBright LT Deep Red | 400 µl | MT12-12 |
| MitoBright IM Red for Immunostaining | 20 µl×1 | MT15-10 |
| | 20 µl×3 | MT15-12 |
| Oxidative Stress | | |
| MitoBright ROS Deep Red - Mitochondrial Superoxide Detection | 100 nmol × 1 | MT16-10 |
| | 100 nmol × 3 | MT16-12 |
| Mito-FerroGreen | 1 set (50 µg × 2) | M489-10 |
| Si-DMA for Mitochondrial Singlet Oxygen Imaging | 2 µg | MT05-10 |
| MitoPeDPP | 5 µg × 3 | M466-10 |

Mitochondrial Research

# Extracellular OCR Plate Assay Kit

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

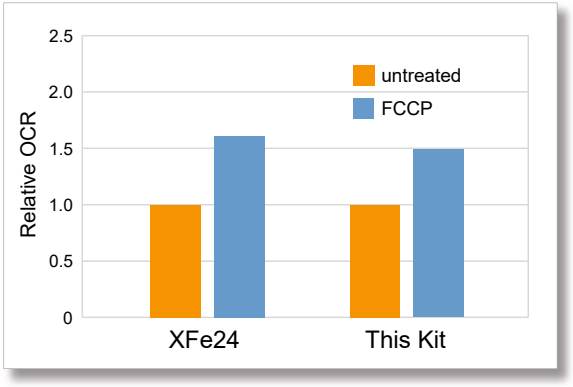

**Procedure**




## Comparison with Flux Analyzer

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

Cells: HepG2
Cell Number: $5 \times 10^4$ cells/well
Stimulation: FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone)
FCCP Concentration: 2 µmol/l



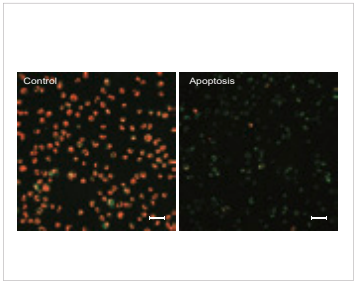

| Description | Unit | Code |
|---|---|---|
| Extracellular OCR Plate Assay Kit | 100 tests | E297-10 |

## Mitochondrial Membrane Potential Detection

# JC-1 MitoMP Detection Kit

JC-1 forms aggregate (in healthy mitochondria) with red fluorescence. As membrane potential decreases, JC-1 becomes monomers, which shows in green fluorescence. The change in ratio of red to green fluorescence is used as a indicator of mitochondrial condition.

Fluorescence Microscope




Flow Cytometry

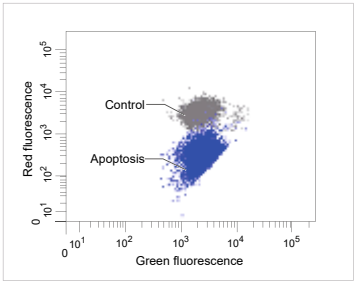
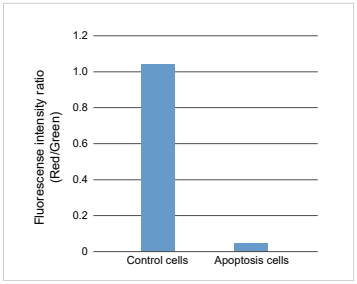



Plate Reader



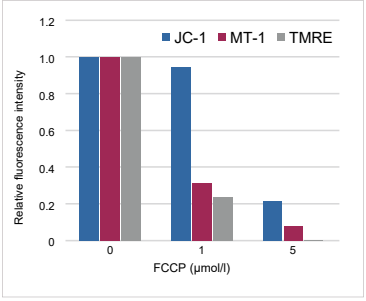

| Description | Unit | Code |
|---|---|---|
| JC-1 MitoMP Detection Kit | 1 set | MT09-10 |

## Mitochondrial Membrane Potential Detection

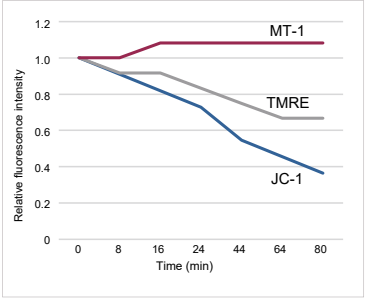
# MT-1 MitoMP Detection Kit

JC-1 dye, TMRE, and TMRM are widely used to monitor MMP, however, these dyes have some limitations, such as low photostability and poor retention after aldehyde fixation. These limitations result in poor reproducibility of experiments. Dojindo's MT-1 MitoMP Detection Kit overcomes these limitations. In addition, the Imaging Buffer included in this kit minimizes background fluorescence and maintains cell vitality while the assay is being performed.

High Sensitivity Detection




Allow to monitor mitochondrial membrane potential

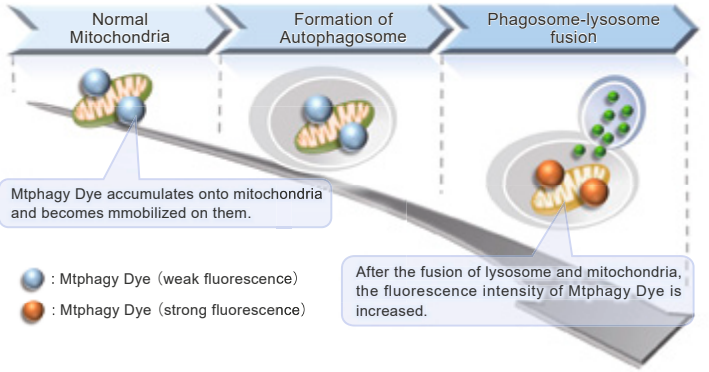


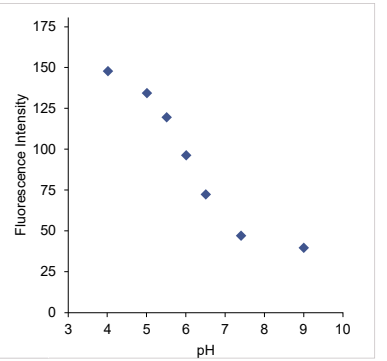

| Description | Unit | Code |
|---|---|---|
| MT-1 MitoMP Detection Kit | 1 set | MT13-10 |

Mitochondrial Research

# Mitophagy Detection Kit

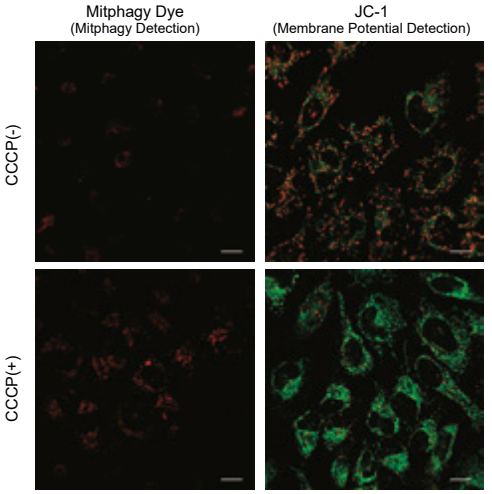
This kit is composed of Mtphagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtphagy Dye accumulates in intact mitochondria, is immobilized on it with chemical bond and exhibits a weak fluorescence from the influence of surrounding condition. When Mitophagy is induced, the damaged mitochondria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




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

## Mitophagy Induction and Mitochondrial Membrane Potential Changes




Mitochondrial condition in the carbonyl cyanide m-chlorophenyl hydrazine (CCCP) treated Parkin-expressing HeLa cells was compared with untreated cells using Mitophagy Detection Kit (MD01, MT02) and JC-1 MitoMP Detection Kit (MT09).
Result:
As a result, mitophagy was hardly detected in the CCCP-untreated cells, and mitochondrial membrane potential was maintained normally. On the other hand, in CCCP-treated cells, we observed a decrease in mitochondrial membrane potential (decrease in red fluorescence of JC-1)
and induction of mitophagy (increase in fluorescence of Mtphagy Dye).

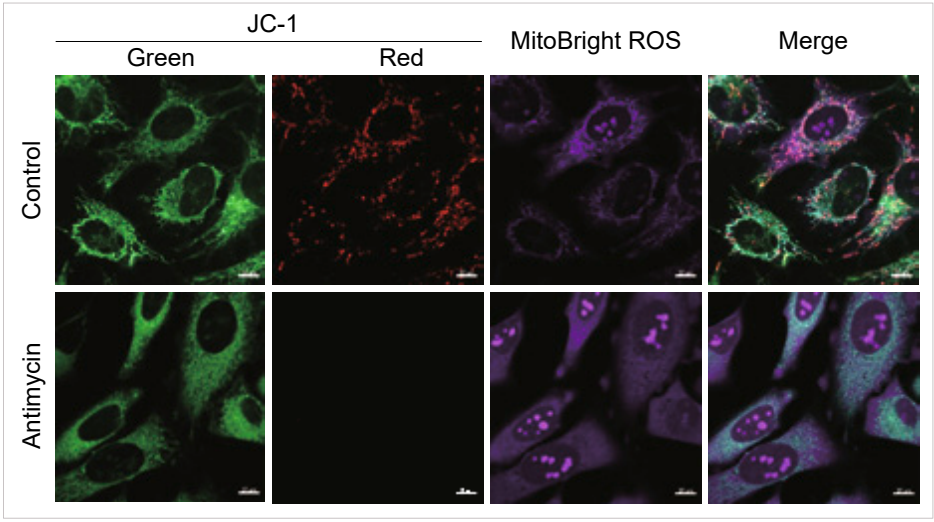
| Description | Unit | Code |
|---|---|---|
| Mitophagy Detection Kit | 1 set | MD01-10 |
| Mtphagy Dye | 5 µg × 3 | MT02-10 |

# MitoBright ROS Deep Red - Mitochondrial Superoxide Detection

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

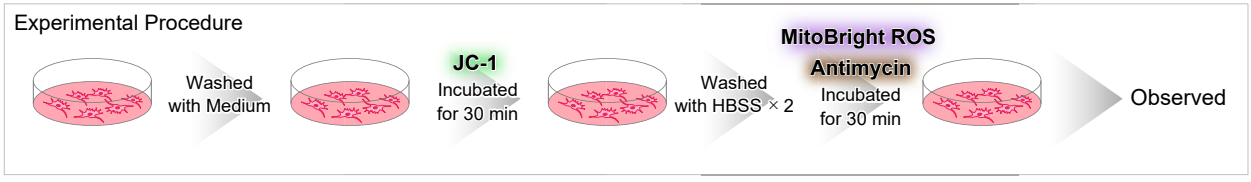
## Experimental Example

### Simultaneously Evaluation of Mitochondrial Superoxide and Membrane Potential




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

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




| Description | Unit | Code |
|---|---|---|
| MitoBright ROS Deep Red - Mitochondrial Superoxide Detection | 100 nmol × 1 | MT16-10 |
| | 100 nmol × 3 | MT16-12 |

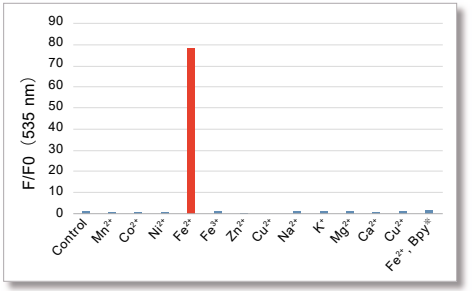
M489 F374

## Mitochondrial Superoxide Detection

# Mito-FerroGreen

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

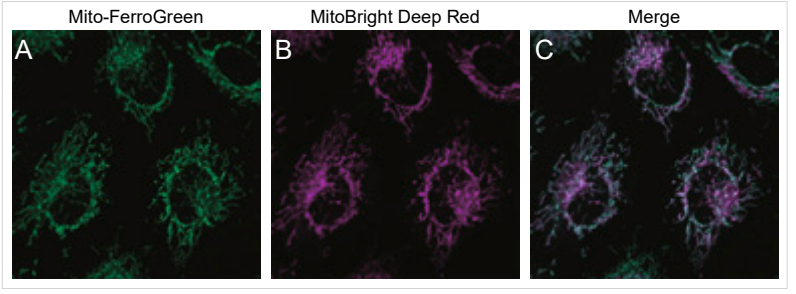
Metal ion Selectivity




※ Bpy : 2,2′ -Bipyridyl

## Double staining with mitochondrial staining probe

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




Double staining with mitocondrial staining probe

Mito-FerroGreen (5 μmol/l) Ex/Em = 488 nm/ 500-550 nm

MitoBright Deep Red (200 nmol/l) Ex/Em = 640 nm/ 656-700 nm

A Mito-FerroGreen

B MitoBright Deep Red

C Merge

## Iron Detection Dyes

| | Mito-FerroGreen (M489) | FerroOrange (F374) |
|---|---|---|
| Localization | Mitochondria | Intracellular |
| Fluorescent Property | λex 505 nm, λem 535 nm | λex 543 nm, λem 580 nm |
| Instrument (filter) | Fluorescence microscope (FITC, GFP) | Fluorescence microscope, plate reader (Cy3) |
| Sample | Live Cell | Live cell |
| The number of assays | 1 set (50 μg x 2)<br>10 assays at 35 mm dish<br>(final concentration 5 μmol/l) | 1 tube (24 μg)<br>17 assays at 35 mm dish<br>(final concentration 1 μmol/l) |

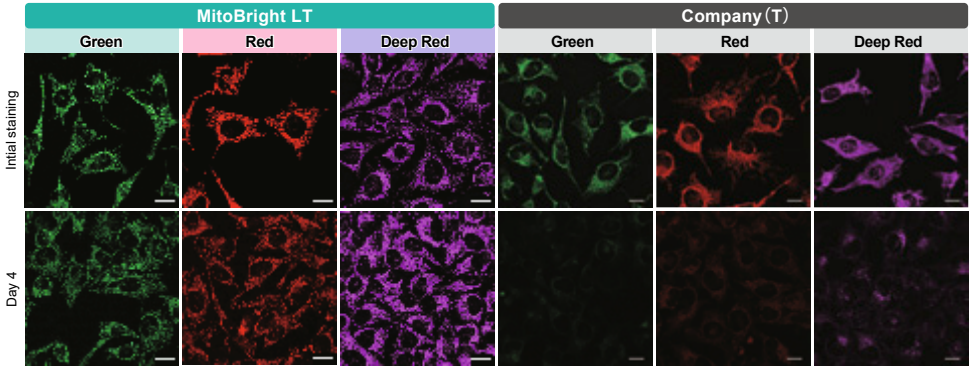
| Description | Unit | Code |
|---|---|---|
| Mito-FerroGreen | 1 set (50 μg × 2) | M489-10 |
| FerroOrange | 1 tube | F374-10 |
| | 3 tube | F374-12 |

# MitoBright LT Series

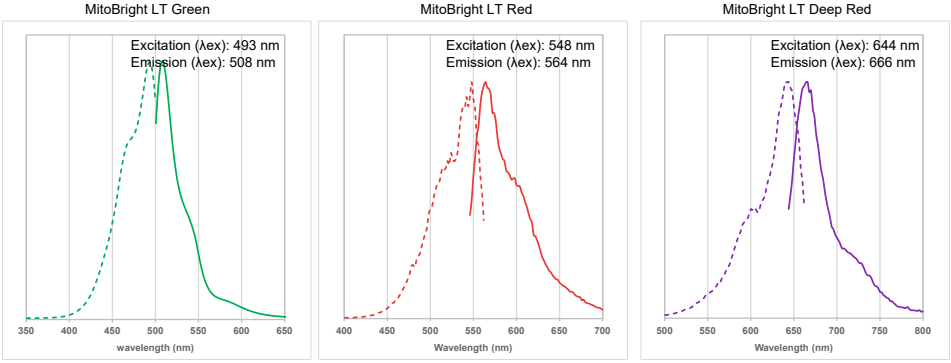
MitoBright LT dyes are designed to exhibit mitochondria retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color options (Green, Red and Deep Red), and are provided as a ready-to-use DMSO solution. A working solution can easily be prepared in a single dilution step with growth medium or HBSS.

## Stained in serum-contained media

HeLa cells were stained with MitoBright LTs or an existing reagent and observed after 4 days. MitoBright LT remained unchanged and observable even after 7 days, while the existing reagent's intensity decreased.




## Fluorescence Properties

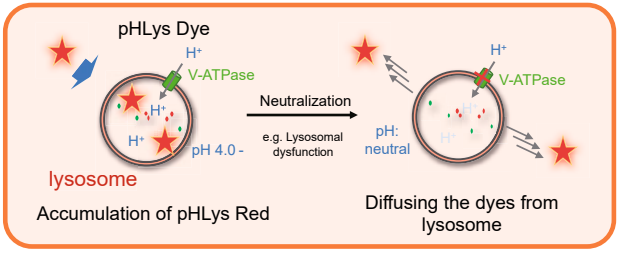



| Description | Unit | Code |
|---|---|---|
| MitoBright LT Green | 400 μl | MT10-12 |
| MitoBright LT Red | 400 μl | MT11-12 |
| MitoBright LT Deep Red | 400 μl | MT12-12 |

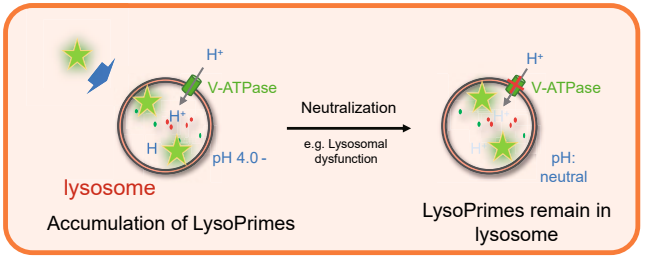
Lysosomal Analysis

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

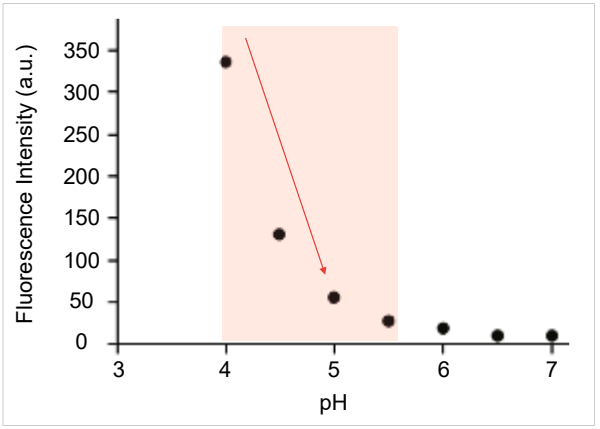
## Lysosomal pH-dependent Fluorescent Probe




## Lysosomal pH-independent Fluorescent Probe

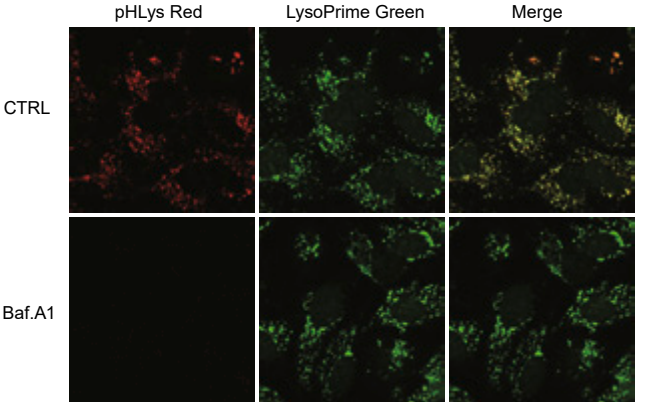



## pH dependence of pHLys Red




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

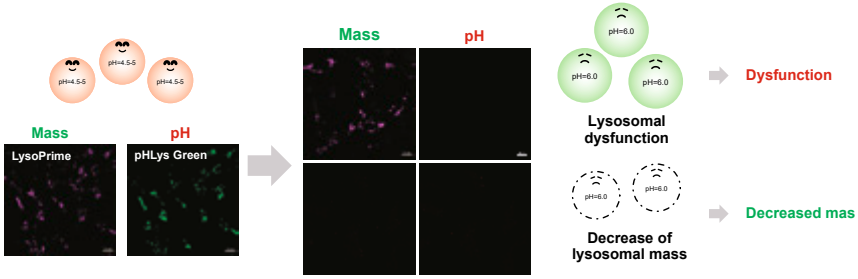
## Resistance to pH changes in lysosomes



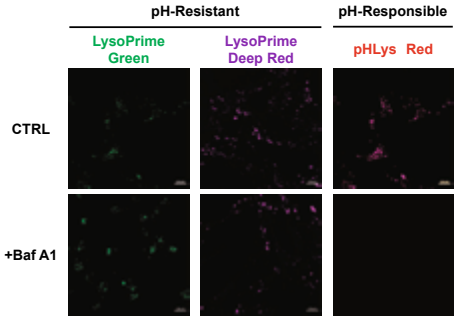

LysoPrime Green and existing dyes accumulate in acidic lysosomes, but when treated with Bafilomycin A1, a lysosomal acidity inhibitor, the existing dyes leave the lysosomes when the lysosomes are changed from acidic to neutral, resulting in a significant decrease in the fluorescence signal. On the other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

# Lysosomal Acidic pH Detection Kit

The kit includes lysosome staining dyes, pHLys Red/Green (pH dependent), and LysoPrime Green/Deep Red (pH-independent). The pHLys and LysoPrime dyes accumulate in the intact lysosomes. The fluorescence intensity of pHLys dyes are enhanced as the acidity increases, and weak fluorescence is observed when lysosomes are neutralized due to the lysosomal dysfunction. On the other hand, LysoPrime dyes gives stable emissions even lysosomes are neutralized. Lysosomal pH and lysosomal mass can be measured by combining these pHLys and LysoPrime dyes.

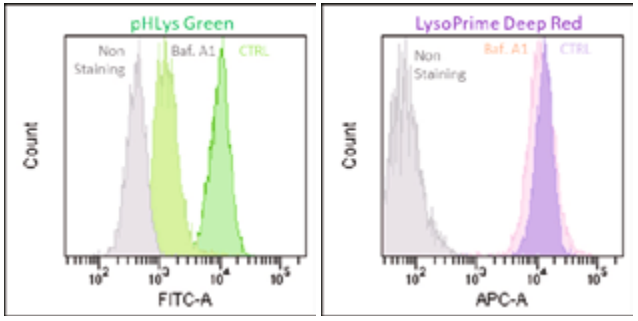



## Imaging Analysis: Green/Red (#L266-10)




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

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



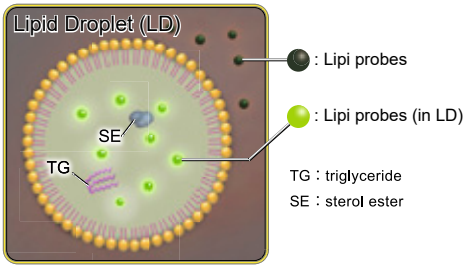

<Experimental Conditions>
pHLys Green: FITC Filter (Ex = 488 nm, Em = 515 – 545 nm)
LysoPrime Deep Red: APC Filter (Ex = 640 nm, Em = 650 – 670 nm)

| Description | Unit | Code |
|---|---|---|
| Lysosomal Acidic pH Detection Kit – Green/Red *1 | 1 set | L266-10 |
| Lysosomal Acidic pH Detection Kit – Green/Deep Red *2 | 1 set | L268-10 |
| LysoPrime Green – High Specificity and pH Resistance | 10 µl × 1 | L261-10 |
| | 10 µl × 3 | L261-12 |
| LysoPrime Deep Red - High Specificity and pH Resistance | 1 tube | L264-10 |
| | 3 tube | L264-12 |
| pHLys Red - Lysosomal Acidic pH Detection | 1 tube | L265-10 |
| | 3 tube | L265-12 |

*1 Green/Red: combination of LysoPrime Green and pHLys Red, *2 Green/Deep Red: combination of pHLys Green and LysoPrime Deep Red

## Lipid Droplet Staining

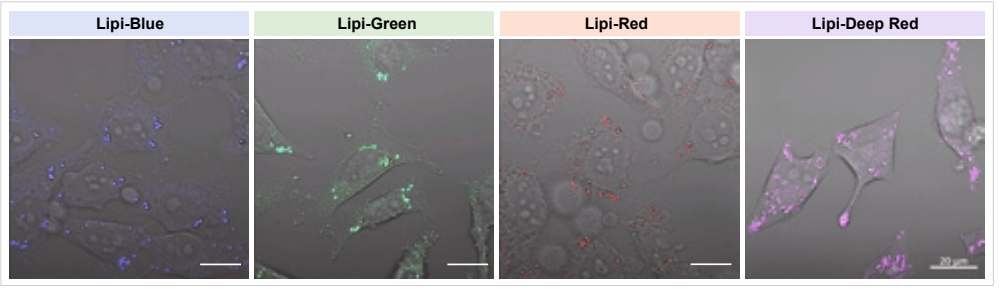
# Lipi-Blue / Green / Red / Deep Red




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

A medium that contained oleic acid (200 µmol/l) was added and incubated overnight. Then, the supernatant was removed and the cells were washed with PBS. Each Lipi product series (1 µmol/l) was added and the cells were incubated for 15 minutes.

Lipi-Blue: Ex. 405 nm / Em. 450 – 500 nm, Lipi-Green: Ex. 488 nm / Em. 500 – 550 nm,
Lipi-Red: Ex. 561 nm / Em. 565 – 650 nm, Lipi-Deep Red: Ex.640 nm / Em.650-700 nm




## Comparison of Reagents

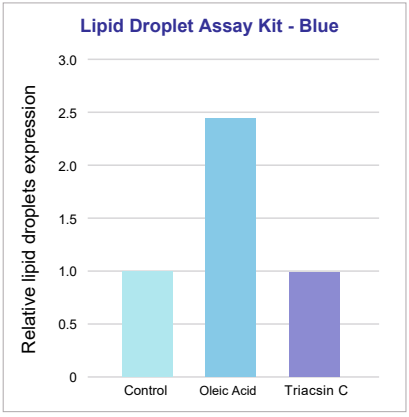
| | Dojindo | | | Other Products | | |
|---|---|---|---|---|---|---|
| | Lipi-Blue | Lipi-Green | Lipi-Red | Oil Red O | Nile Red | Reagent B |
| Live Cells | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Fixed Cells | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Selectivity towards Lipid Droplet (Level of Background) | ✓ | ✓ | ✓ | | | |
|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

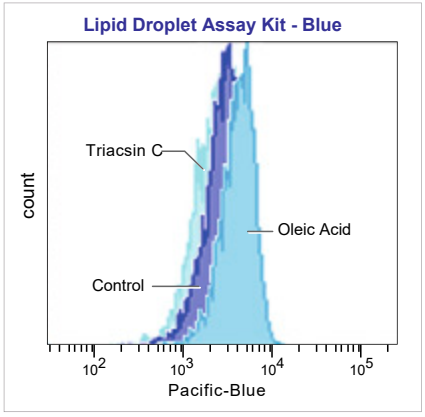
*1 Please refer to our website for the co-staining filter.
*2 When co-staining with a green fluorescent dye, a green fluorescent emission filter less than 550 nm is recommended.
*3 Leaks in GFP filter (500 ～ 540 nm)

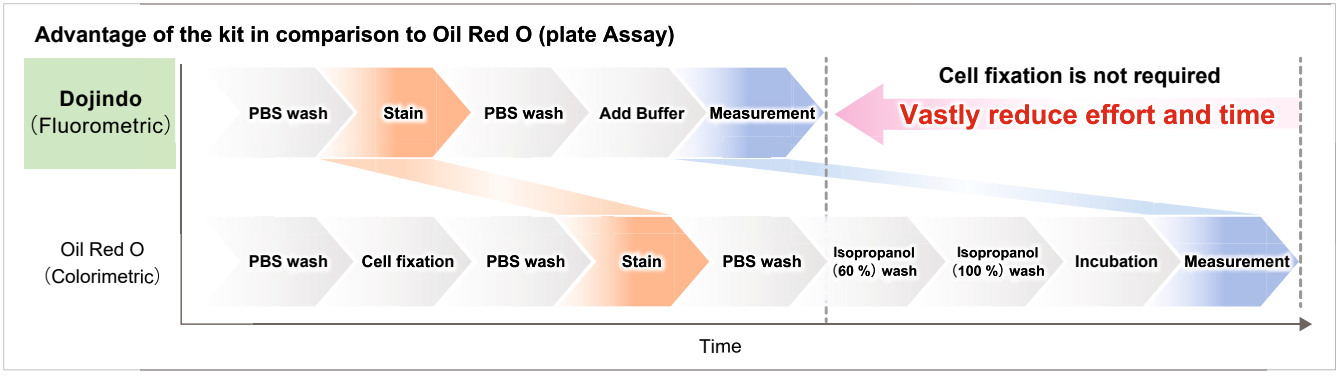
| Description | Unit | Code |
|---|---|---|
| Lipi-Blue | 10 nmol × 1 | LD01-10 |
| Lipi-Green | 10 nmol × 1 | LD02-10 |
| Lipi-Red | 100 nmol × 1 | LD03-10 |
| Lipi-Deep Red | 10 nmol × 1 | LD04-10 |

# Lipid Droplet Assay Kit - Blue / Deep Red

The Lipid Droplet Assay Kit simplifies the quantification of fat droplets with provided protocols and buffers. It works for live cells, and its fluorescent dye is suitable for both live and fixed cells. Compared to colorimetric reagents, it reduces measuring time and increases experiment repeatability by avoiding dye deposition in the plate.




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

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