Reagent for Cellular Function Analysis

DOJINDO

5th Edition

Reagent for Cellular Function Analysis

Cell Proliferation/Cytotoxicity Assay

Cell Counting Kit-8
Cytotoxicity LDH Assay Kit-WST
Viability/Cytotoxicity Multiplex Assay
Cell Count Normalization Kit

Cellular Senescence

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

Autophagy

Autophagosome Detection (DAPGreen / DAPRed)
Autolysosome Detection (DALGreen)

Oxidative Stress

ROS
- ROS Assay Kit -Highly Sensitive DCFH-DA-
- ROS Assay Kit -Photo-oxidation Resistant DCFH-DA-
- mtSOX Deep Red - Mitochondrial Superoxide
- Si-DMA for Mitochondrial Singlet Oxygen Imaging

Lipid Peroxide
- Liperfluo
- Lipid Peroxidation Probe -BDP 581/591 C11-
- MitoPeDPP

Intracellular Iron Ion
- FerroOrange
- Mito-FerroGreen

Glutathione
- GSSG/GSH Quantification Kit

Intracellular Metabolism

Starter Kit
- Glycolysis/JC-1 MitoMP Assay Kit
- Glycolysis/OXPHOS Assay Kit

Quantification for Intracellular Metabolism
- ATP Assay Kit-Luminescence
- ADP/ATP Ratio Assay Kit-Luminescence
- Glucose Assay Kit-WST
- Glutamine Assay Kit-WST
- Glutamate Assay Kit-WST
- α-Ketoglutarate Assay Kit-Fluorometric
- Lactate Assay Kit-WST
- NAD/NADH Assay Kit-WST
- NADP/NADPH Assay Kit-WST

Uptake Assay
- Glucose Uptake Assay Kit-Blue, Green, Red
- Amino Acid Uptake Assay
- Cystine Uptake Assay Kit
- Fatty Acid Uptake Assay Kit

Mitochondria

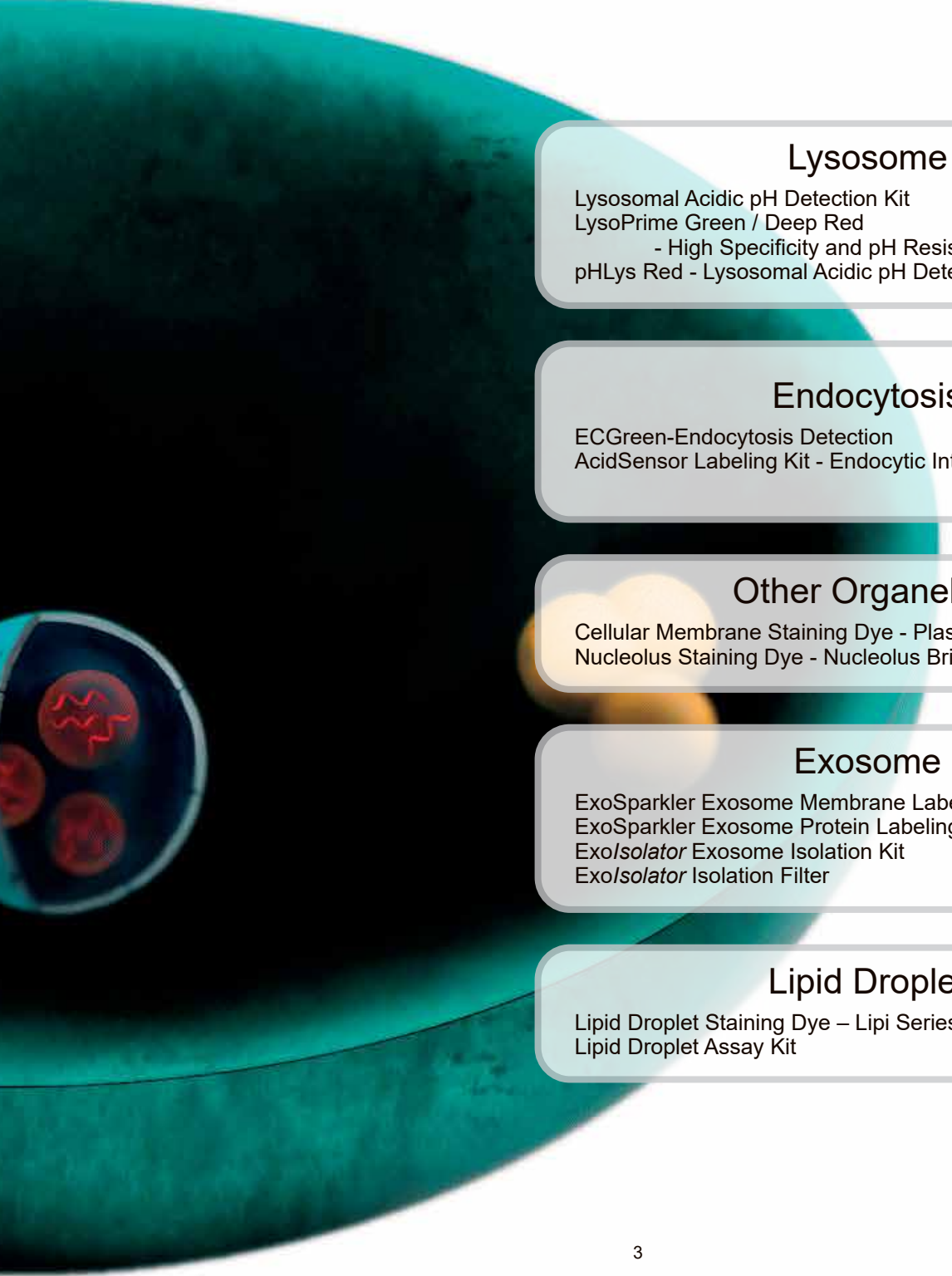
Metabolism
- Extracellular OCR Plate Assay Kit
- Glucose Assay Kit-WST
- Lactate Assay Kit-WST

Mitochondrial Membrane Potential
- MT-1 MitoMP Detection Kit
- JC-1 MitoMP Detection Kit

Mitophagy
- Mitophagy Detection Kit
- Mtphagy Dye

Mitochondrial Staining
- MitoBright LT Green
- MitoBright LT Red
- MitoBright LT Deep Red
- MitoBright IM Red for Immunostaining

Oxidative Stress
- mtSOX Deep Red
  - Mitochondrial Superoxide Detection
- Mito-FerroGreen
- Si-DMA for Mitochondrial Singlet Oxygen Imaging
- MitoPeDPP



Lysosome

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

Endocytosis

ECGreen-Endocytosis Detection
AcidSensor Labeling Kit - Endocytic Internalization Assay

Other Organelles

Cellular Membrane Staining Dye - PlasMem Bright
Nucleolus Staining Dye - Nucleolus Bright

Exosome

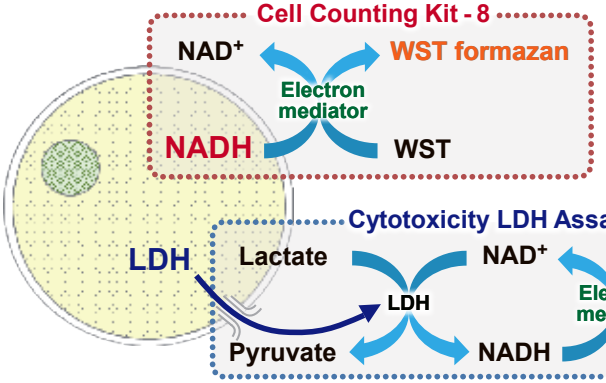
ExoSparkler Exosome Membrane Labeling Kit
ExoSparkler Exosome Protein Labeling Dye
Exo*Isolator* Exosome Isolation Kit
Exo*Isolator* Isolation Filter

Lipid Droplet

Lipid Droplet Staining Dye – Lipi Series
Lipid Droplet Assay Kit

Cell Counting Kit-8
Cytotoxicity LDH Assay Kit-WST

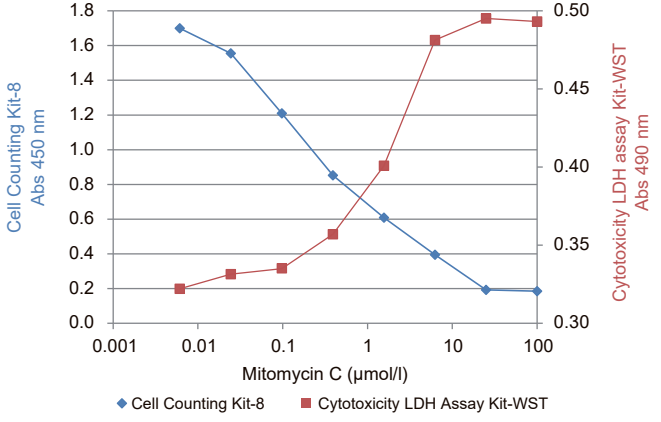
Detection Principle



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.

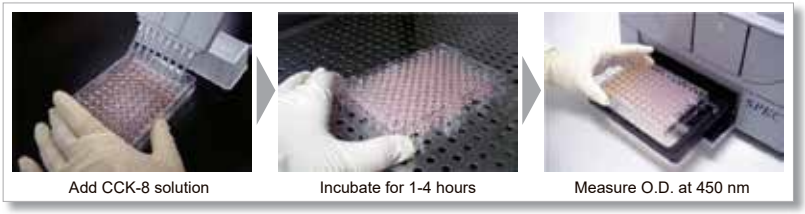
Simultaneous Usage of CCK-8 and Cytotoxicity LDH Assay Kit-WST



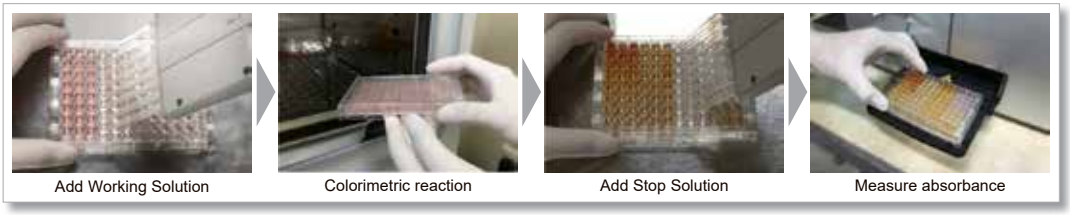
Drug: Mitomycin C
Cell Line: HeLa
Media: MEM, 10% FBS
Incubation: 37°C, 5% CO_2 for 48 hours
Measuring Condition: Cell Counting Kit-8 (450 nm)
Cytotoxicity LDH Assay Kit-WST (490 nm)

Simple Procedure

・Cell Counting Kit-8

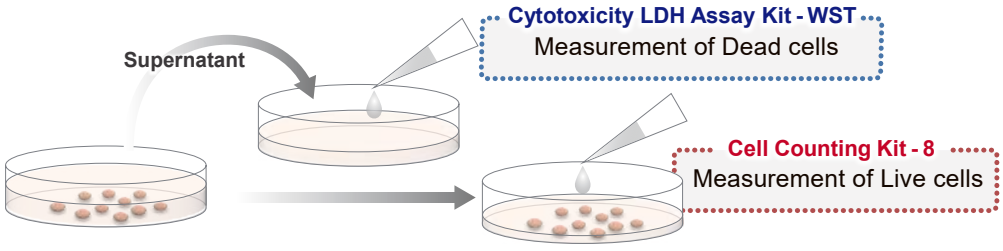


・Cytotoxicity LDH Assay Kit-WST



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	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
Viability/Cytotoxicity Multiplex Assay Kit	500 tests	CK17-10

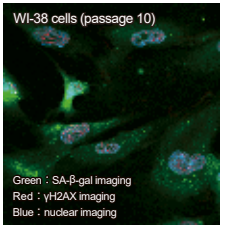
Senescence Detection

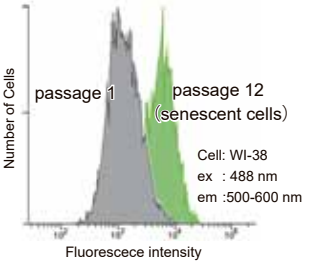
Cellular Senescence Detection Kit - SPiDER-βGal

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

SPiDER-βgal

Compatible with quantitative analysis

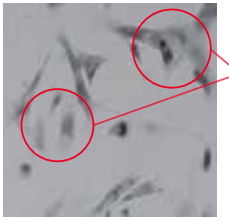




Compatible Instruments:
✓ Microscope ✓ Flow Cytometer ✓ Plate Reader

X-Gal

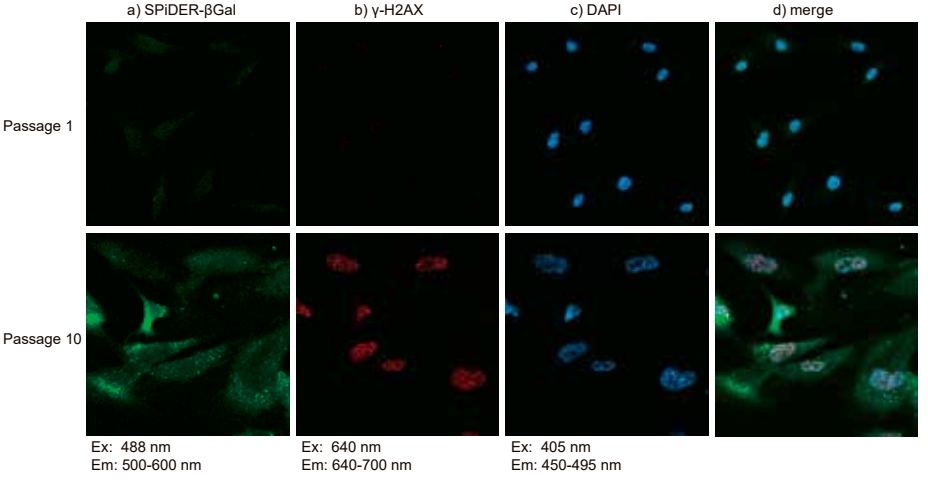
Difficult to quantify



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

Compatible Instruments:
✓ Microscope

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



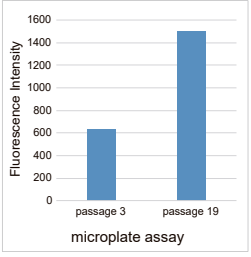
WI-38 cells were treated with anti- γ-H2AX antibody and observed under a confocal microscope. The procedure involved several steps, including fixing the cells, permeabilizing, blocking, adding primary and secondary antibodies, staining with DAPI, and washing the cells. The experiment aimed to detect DNA damage and study DNA repair pathways.

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

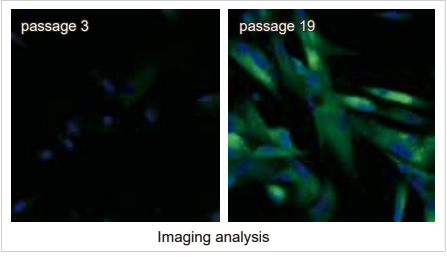
Cellular Senescence Plate Assay Kit - SPiDER-βGal

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

Correlation with Imaging Data



microplate assay



Imaging analysis

Plate Assay
Ex. 535nm / Em. 580nm

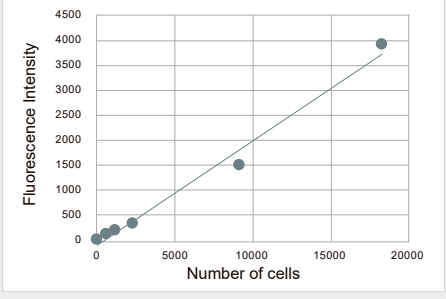
Imaging data
Green: Ex. 488nm / Em. 500-600nm (SA-β-Gal staining with Cellular Senescence Detection Kit – SPiDER-βGal(Code SG04))
Blue: Ex. 405nm / Em. 450-495nm (Nuclear staining with -Cellstain- DAPI solution(Code D523))

As a result, it was confirmed that in both kits, SA-β-gal staining increased in the high-passage WI-38 cells. Bear in mind that although initial cell seeding densities are the same, cell densities at the time of plate assay differ due to low proliferation rate of senescent cells at higher passage levels. Therefore, in this experiment, we used SA-β-Gal activity values normalized by the results obtained using the Cell Count Normalization Kit in which cell number is determined by a nuclear marker.

Cell Count Normalization Kit

Cell Count Normalization Kit includes nucleic acid staining dye, Hoechst 33342 which binds to nuclear DNA to emit blue fluorescence. By measuring this blue fluorescence, correction of the measured value can easily be carried out in simple steps whereas the visual cell counting method requires complicated procedure. Moreover, unlike the correction by protein or ATP amount, the kit requires no lysis procedure. In addition, Quenching Buffer included in the kit enables a direct measuring of fluorescence signal without any background.

Highly correlated to cell number



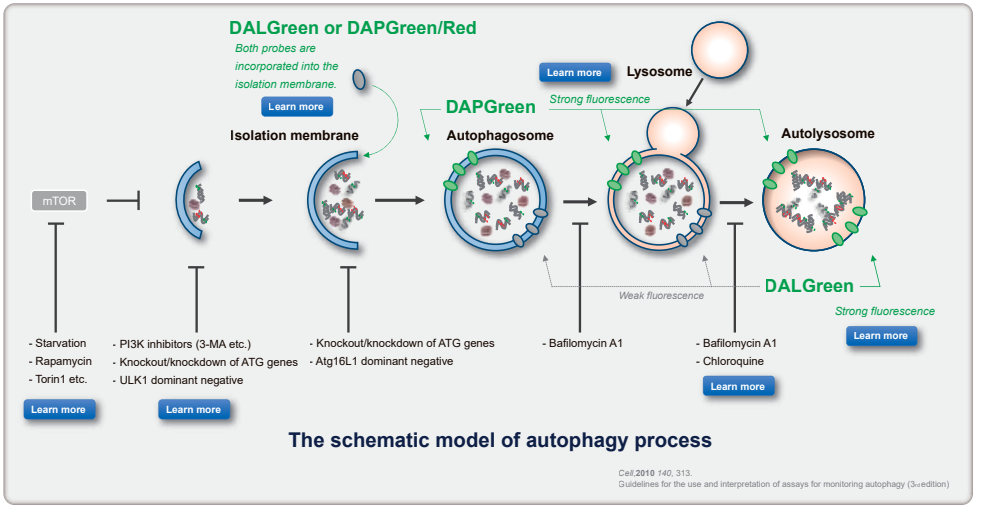
Description	Unit	Code
Cellular Senescence Plate Assay Kit - SPiDER-βGal	20 tests	SG05-01
	100 tests	SG05-05
Cell Count Normalization Kit	200 tests	C544-02
	1000 tests	C544-10

Autophagy

DAPGreen / Red - Autophagy Detection
DALGreen - Autophagy Detection

DALGreen DAPGreen DAPRed

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



Feature of Each Dye

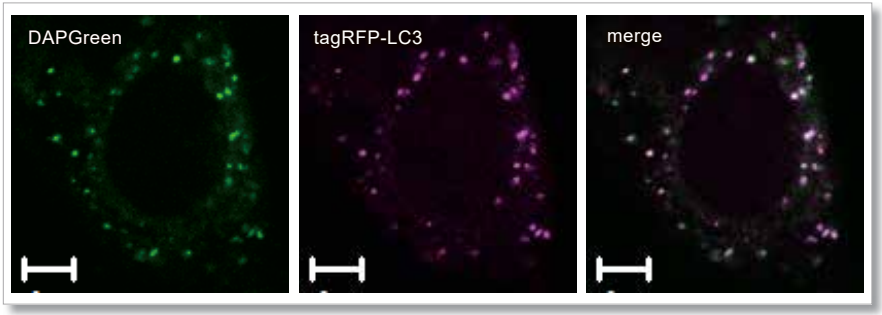
	Applicable instruments			Fluorescent properties	Volume / the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	○	○	○	Ex = 425-475 nm Em = 500-560 nm * For confocal microscope,the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish: 25 (when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

*Double staining imaging by DAPGreen and DALGreen is not possible

High Correlation with LC3 DAPGreen

The Condition of Autophagy Detection

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, 4 hours after autophagy induction.



Result

Almosto all DAPGreen signals were colocalized with LC3.

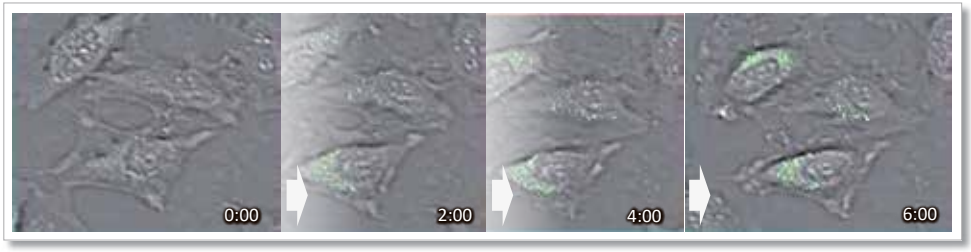
Imaging Condition

DAP Green: Ex = 488 nm, Em = 500 – 563 nm

Scale bar: 10 μm

Time-lapse imaging DALGreen

The fluorescence intensity of DALGreen increased in autophagy-induced cells.



Detection Condition

Ex = 405 nm, Em = 500 – 550 nm

Confocal quantitative image cytometer CQ1, Yokogawa Electric Corporation

HeLa cells were stained with DALGreen, and autophagy was induced in an amino acid-free medium. Time-lapse observation was performed up to 6 hours after the induction of autophagy.

Description	Unit	Code
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)