New Autophagy Detection

Detection of Autophagosome

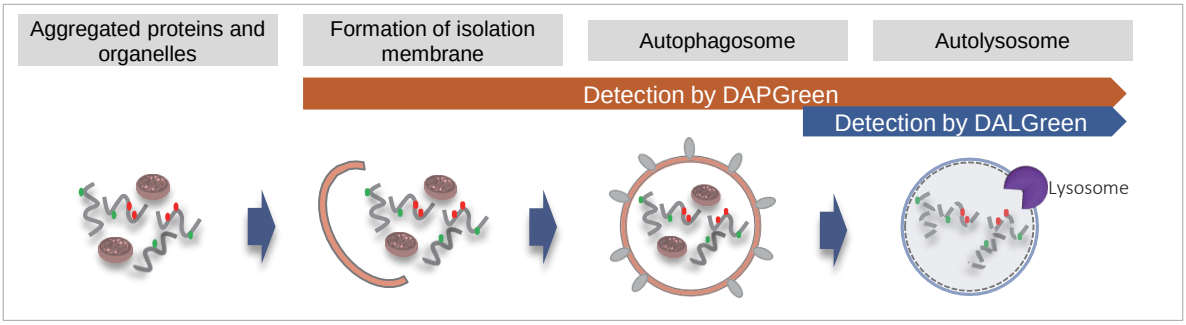
DAPGreen - Autophagy Detection

Detection of Autolysosome

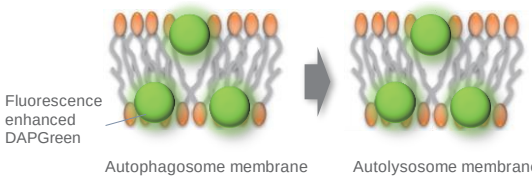
DALGreen - Autophagy Detection

How they work

Autophagy Detection Reagent "DAPGreen" emits fluorescence when it is incorporated into an autophagosome membrane, whereas DALGreen emits fluorescence during autolysosome phase, when aggregated proteins are decomposed. Thus, these reagents DAPGreen and DALGreen make it possible to monitor several phases of autophagy, from a formation of autophagosome to fusion with lysosome and decomposition of components just by adding reagents.

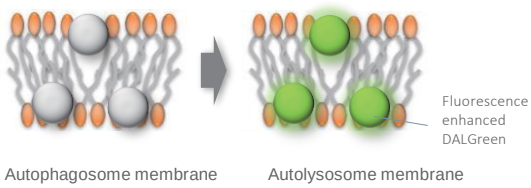


Autophagosome Detection Reagent DAPGreen



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 (see page 3).

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

Journal

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.

Contact us

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Autophagy Detection Reagent

DAPGreen - Autophagy Detection, DALGreen - Autophagy Detection

General Information

Detection is possible with a fluorescent microscope, a flow cytometer and a microplate reader using DAPGreen. DALGreen can be applied in two methods (a fluorescent microscope and a flow cytometer). Please select your most suitable method depending on your equipment.

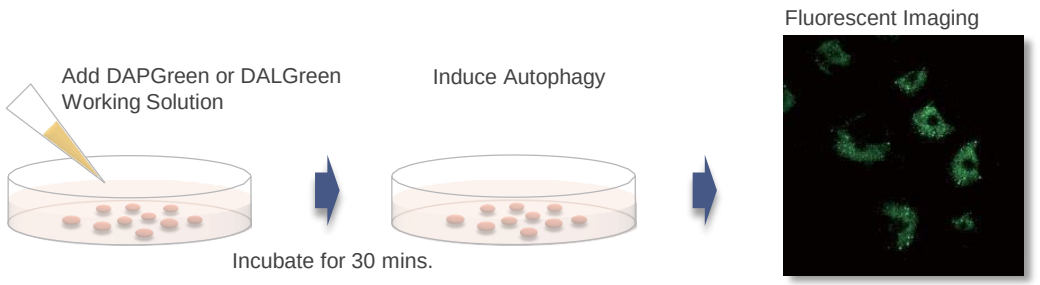
	Machines available			Fluorescent property	Volume/ the number of usable assays	Existing methods
	Fluorescent Microscope	Flow cytometer	Microplate reader			
DAPGreen	○	○	○	Ex. 425-475 Em. 500-560 ※For confocal microscope, the sample can be excited at 488 nm	5 nmol x 1 / 35 mm dish x 25 (when used with 0,1 μmol /l 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 1,0 μmol /l concentration)	LC3-GFP-RFP etc.

※ Please visit our website for Spectrum data

※ Double staining imaging by DAPGreen and DALGreen is not possible

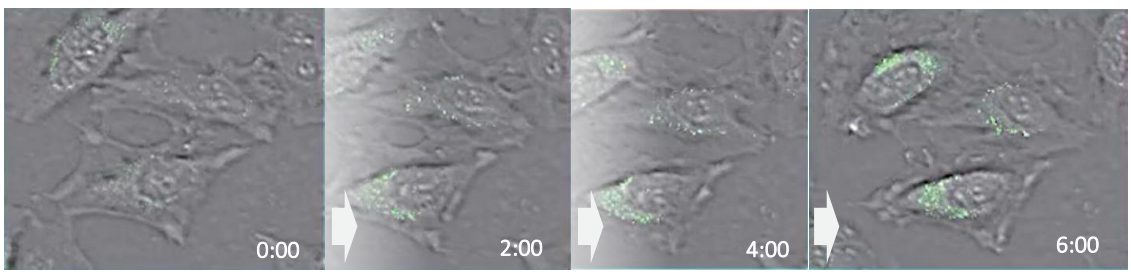
Just adding reagent

Gene transfection is not necessary. You only need to add the reagent to cultured cells, and you can get a fluorescent image.



Time-lapse imaging of autophagy

After staining with DALGreen, HeLa cells in the starved condition were observed for 6 hrs.



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

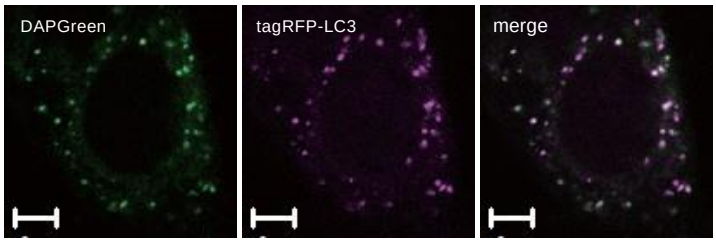
Fluorescence of DALGreen is enhanced after autophagy is induced.
Please visit our website to watch the

DAPGreen – Autophagy Detection

Autophagosome Detection Reagent (D676-10, 5 nmol)

Good correlation with LC3

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



Result

Almost all DALGreen signals were colocalized with LC3-II.

Imaging condition

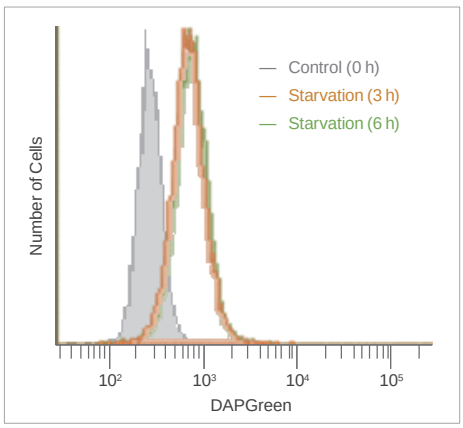
DAPGreen : Ex. 488 nm / Em. 500-563 nm
Scale bar : 10 μm

The condition of autophagy induction

After adding DAPGreen to the RFP-LC3 expressed Hela cells, the cells were treated with rapamycin to induce autophagy.
Fluorescent imaging was conducted by a confocal microscopy after 4 hrs. from autophagy induction.

Quantitative analysis by flow cytometer

The fluorescence of DAPGreen was observed by a flow cytometer after autophagy induction.



Result

After 3 hrs. of incubation under starved condition, the strong fluorescence of DAPGreen was detected.

Detection

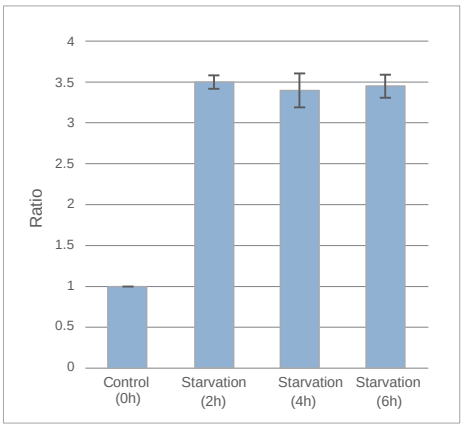
Wavelengths: Ex. 488 nm / Em. 500-560 nm

The condition of autophagy induction

After staining with DAPGreen, HeLa cells were incubated for 0, 3, 6 hrs. with amino acid-free medium and detected by a flow cytometer.

Quantitative analysis by microplate reader

The fluorescence of DAPGreen was observed by a microplate reader after autophagy induction.



Result

After 2 hrs. of incubation under starved condition, the enhanced fluorescence of DAPGreen was observed. It was ca. 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 hrs. with amino acid-free medium and detected by a microplate reader.

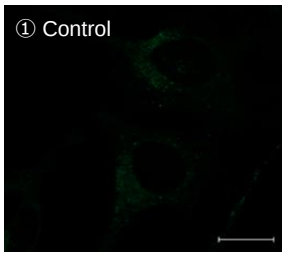
NEXT Autolysosome detection reagent

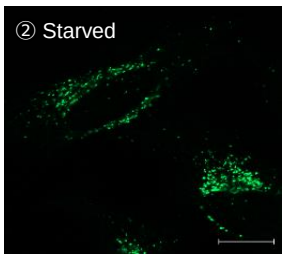
DALGreen – Autophagy Detection

Autolysosome Detection Reagent (D675-10, 20 nmol)

Autolysosome analysis by microscopy

After addition of DALGreen, 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 the staved HeLa cells.

Imaging condition

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

The condition of autophagy induction

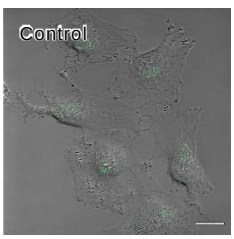
① Control
6 hrs incubation in growth medium

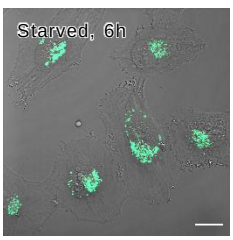
② Starved
6 hrs incubation in amino acid-free growth medium

Comparison with MDC

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

Live cell imaging with DALGreen

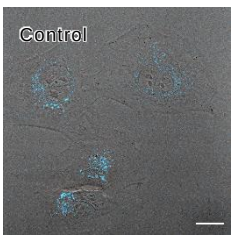


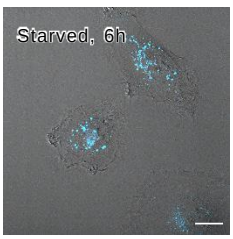


Dye concentration：1 µmol/l
Incubation time：30 min
Detection:
Confocal fluorescence microscope
Ex. 488 nm/ Em. 500-563 nm
Scale bar：40 µm

The reagent was added
BEFORE starvation for 6 hrs.

Live cell imaging with MDC





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 hrs.

Results

The fluorescent intensity of DALGreen was increased in the starved HeLa cells. Whereas the fluorescent intensity of MDC was not changed significantly.

Wavelength

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

Procedure

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

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

REMARKS: Please add DALGreen BEFORE autophagy induction.

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