DALGreen
-Autophagy Detection

FEATURES

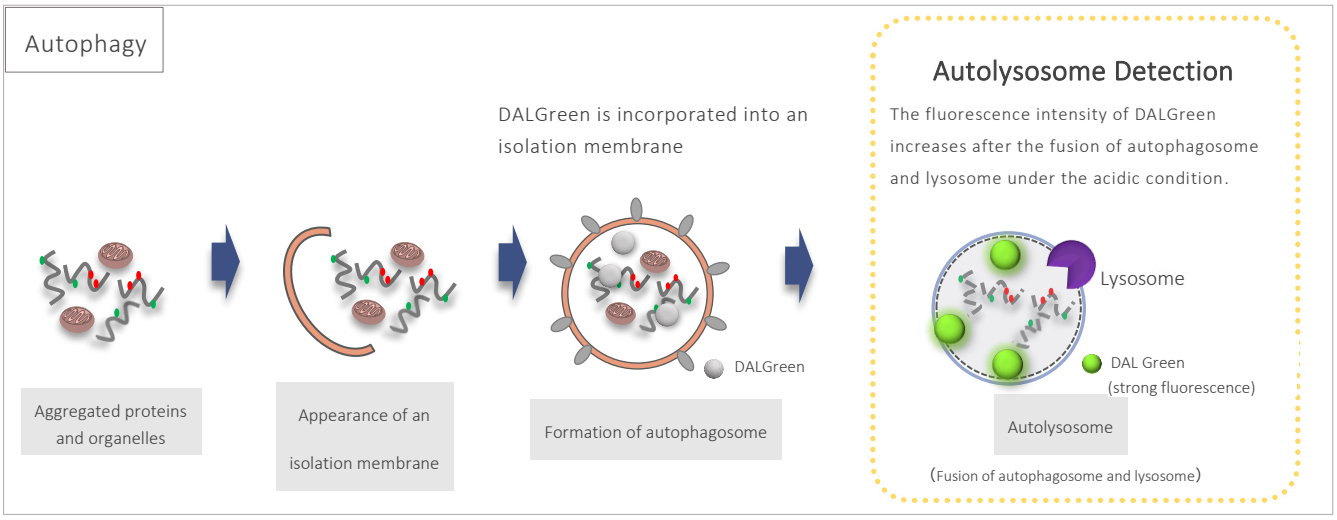
- ✧ Easy Procedure
- ✧ Better Signal
- ✧ Good correlation with LC3

D675-10 1 set (20 nmol)

PRODUCT DESCRIPTION

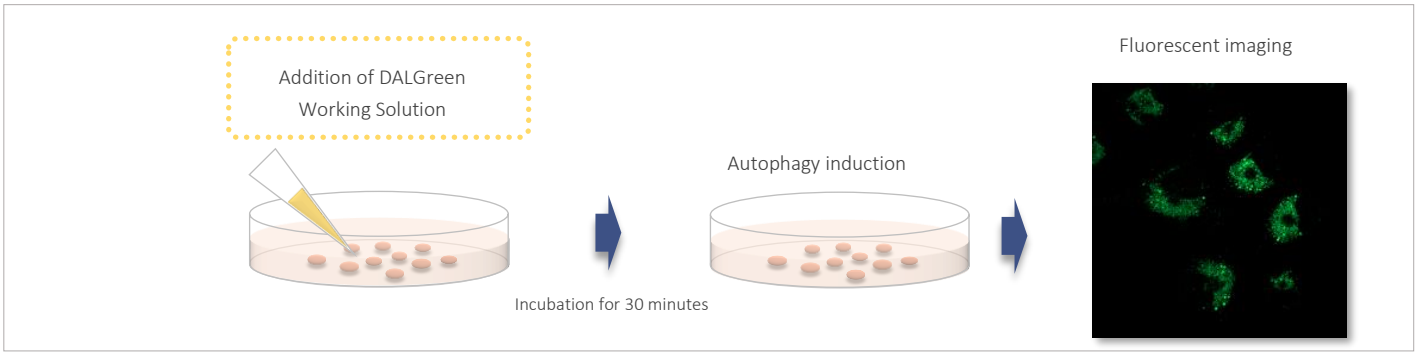
DALGreen is a cell permeable fluorescent dye, which detects autophagy. Autophagy is a degradation process of cytoplasmic dysfunctional proteins and organelles. DALGreen added in the culture medium penetrates the cell membrane and fused to autolysosome formed after autophagy induction. The fluorescence intensity of DALGreen increases after the fusion of autophagosome and lysosome under the acidic condition.

ASSAY PRINCIPLE



EASY PROCEDURE

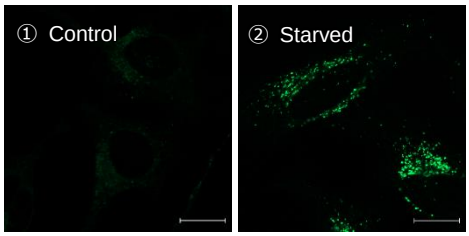
Gene transfection is not necessary. Only need to do is adding reagent, and you can get fluorescent image.



Comparison with LC3-II expression

LC3-II is known as a marker of early stage of autophagosome formation. After addition of DALGreen, HeLa cells were incubated with a growth medium (①) or an amino acid-free medium (②).

Fluorescenct imaging by DALGreen



Western Blot Analysis of LC-II expression

Result

The fluorescent intensity of DALGreen increased while the amount of LC3-II expression increased, thus the result indicates that DALGreen has a good correlation with LC-II

Imaging condition by DALGreen

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

The condition of autophagy induction

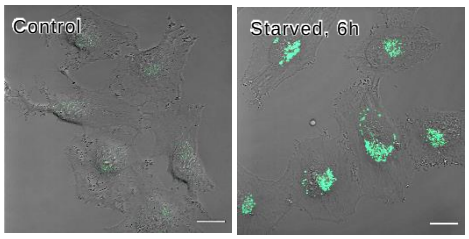
① Control
6 hrs incubation in growth medium

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

Comparison with MDC

The autophagy of HeLa cells in the starved condition was monitored using DALGreen and MDC (Monodansylccadaverine).

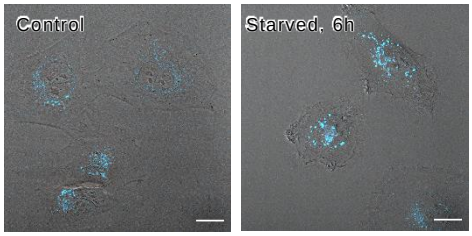
Imaging by DALGreen



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.

Imaging by 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 both DALGreen and MDC was increased in the starved HeLa cells.

Wavelength

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

Procedure

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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