DALGreen - Autophagy Detection

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/d675.pdf

General Information

Autophagy is a degradation process of cytoplasmic dysfunctional proteins and organelles. In this process, an isolation membrane composed of a double membrane appears in cytosol, expands gradually, enfolds with the aggregated proteins and damaged organelles, and closes to form autophagosomes. The autophagosomes are fused with lysosomes to form autolysosomes, in which an acidic environment exists. The contents in autolysosomes are decomposed by digestive enzymes in lysosomes. Since this cellular function is said to be related to aging as well as neurodegenerative diseases such as Parkinson's disease, a simple autophagy detection method is required.
DALGreen is a small fluorescent molecule. Because it has unique properties which emits fluorescence under hydrophobic and acidic conditions, DALGreen can detect the autolysosomes. DALGreen is cell permeable, has no requirement of transfection method, and enables live cell imaging with fluorescence microscopy and quantitative assay by flow cytometry.

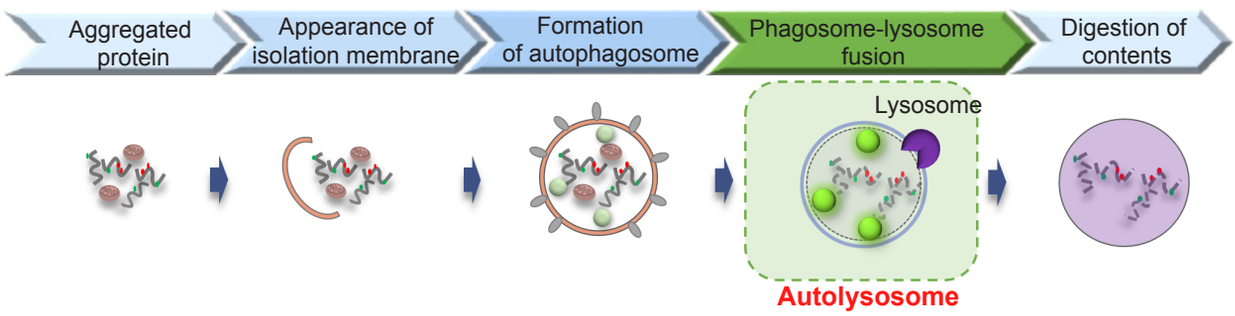


Fig. 1 The detection of autophagy with DALGreen

Contents

DALGreen - Autophagy Detection 20 nmol x 1

Storage Condition

Store at -20°C and protect from light.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Culture medium
- Hanks' HEPES buffer or serum-free medium
- Micropipettes

Preparation of Solutions

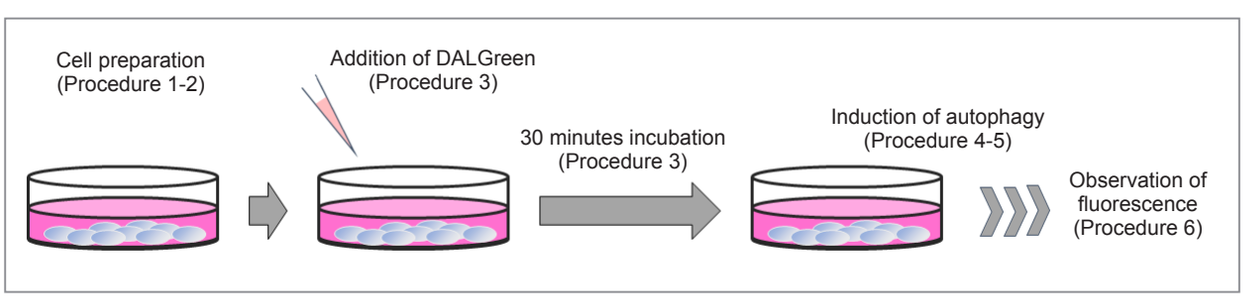
Preparation of 1 mmol/l DALGreen DMSO stock solution

Add 20 µl of DMSO to a tube of DALGreen (20 nmol) and dissolve it with pipetting.
*Store the reconstituted DMSO solution at -20°C. The solution is stable at -20°C for 1 month.

Preparation of DALGreen working solution

Dilute the 1 mmol/l DALGreen DMSO stock solution with culture medium to prepare 0.1-1.0 µmol/l DALGreen working solution.
*Please optimize the final concentration of DALGreen depeneding on the cell lines.

General Protocol



Autophagy detection

1. Prepare cells on dish for assay.
2. Discard the supernatant and wash the cells with culture medium.
3. Add an appropriate volume of DALGreen working solution and then incubate at 37°C for 30 minutes.
4. Discard the supernatant and wash the cells with culture medium twice.
5. Add medium containing autophagy-inducing agent and incubate at 37°C.
   *Please optimize the incubation time according to autophagy-inducing conditions.
6. Observe fluorescence with a fluorescence microscope or flow cytometer.

Experimental Example

Observation on Confocal Fluorescence Microscopy

HeLa cells were seeded on μ-slide 8 well (Ibidi) and cultured at 37°C overnight in a 5% CO_2 incubator. The cells were washed with culture medium and then incubated at 37°C for 30 minutes with 250 μl of 1 μmol/l DALGreen working solution. After the cells were washed with the culture medium twice, the culture medium or amino acid-free mediumthe (Wako Pure Chemical Industries, Ltd., Code: 048-33575) was added to the well. After 6 hours incubation, the cells were washed with Hanks' HEPES buffer twice and then DALGreen was observed by confocal fluorescence microscopy.

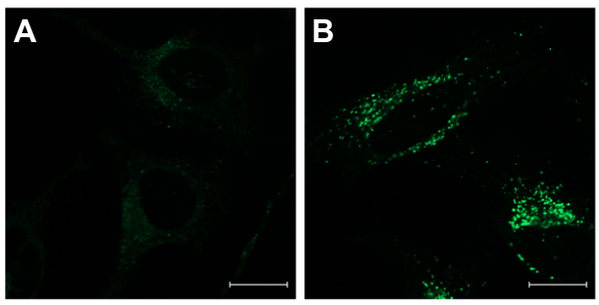


Fig. 2 Confocal microscopic images of various autophagy induced conditions.
After the addition of DALGreen, the cells were incubated with the culture medium (A) or the amino acid-free medium (B). Fluorescence images were obtained using confocal microscopy at an excitation wavelength of 488 nm and a 500-563 nm emission filter. Scale bar: 20 μm.

Analysis by Flow Cytometry

HeLa cells were seeded on 24 well plate and cultured at 37°C overnight in a 5% CO_2 incubator. The cells were washed with culture medium and then incubated at 37°C for 30 minutes with 1 μmol/l DALGreen working solution. After the cells were washed with the culture medium twice, the culture medium or amino acid-free medium was added to the well. After 20 hours incubation, the cells were washed with PBS, treated with trypsin and centrifuged. The pellets were suspended in Hanks' HEPES buffer, and detected by flow cytometry.

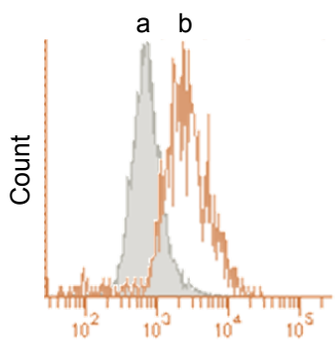
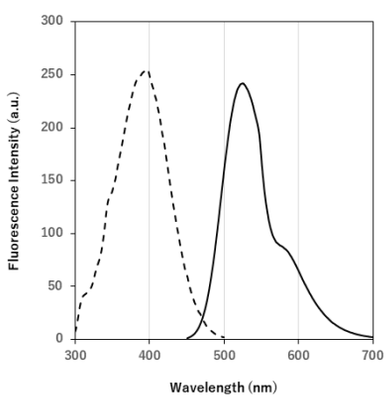


Fig. 3 Detection by flow cytometry.
After the addition of DALGreen, the cells were incubated with the culture medium (a) or the amino acid-free medium (b). These data were obtained using flow cytometer at an excitation wavelength of 405 nm and a 485-535 nm emission filter.

Supplemental Information

Excitation and emission spectra of DALGreen



λ_{ex} : 405 nm
λ_{em} : 525 nm

<Recommended filter>
Ex : 350 ～ 450 nm
Em : 500 ～ 560 nm

DALGreen is Patent Pending.
If you need more information, please contact Dojindo technical service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto 861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies,Inc.
Tel: +1-301-987-2667 Web:http://www.dojindo.com/
Dojindo EU GmbH
Tel: +49-89-3540-4805 Web: http://www.dojindo.eu.com/
Dojindo China Co., Ltd
Tel: +86-21-6427-2302 Web:http://www.dojindo.cn/