Mitophagy Detection Kit

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/md01.pdf

General Information

Mitochondria is one of the cytoplasmic organelle that plays a crucial role in cells such as production of energy for cell viability. Recently, Mitophagy appears to be related to Alzheimer and Parkinson disease induced by the accumulation of depolarized mitochondria. Mitophagy serves as a specific elimination system that dysfunctional mitochondria caused by oxidative stress and DNA damage are sequestered into autophagosome, fused to lysosome and degraded by digestion.
This kit is composed of Mtphagy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtphagy Dye accumulates in 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.

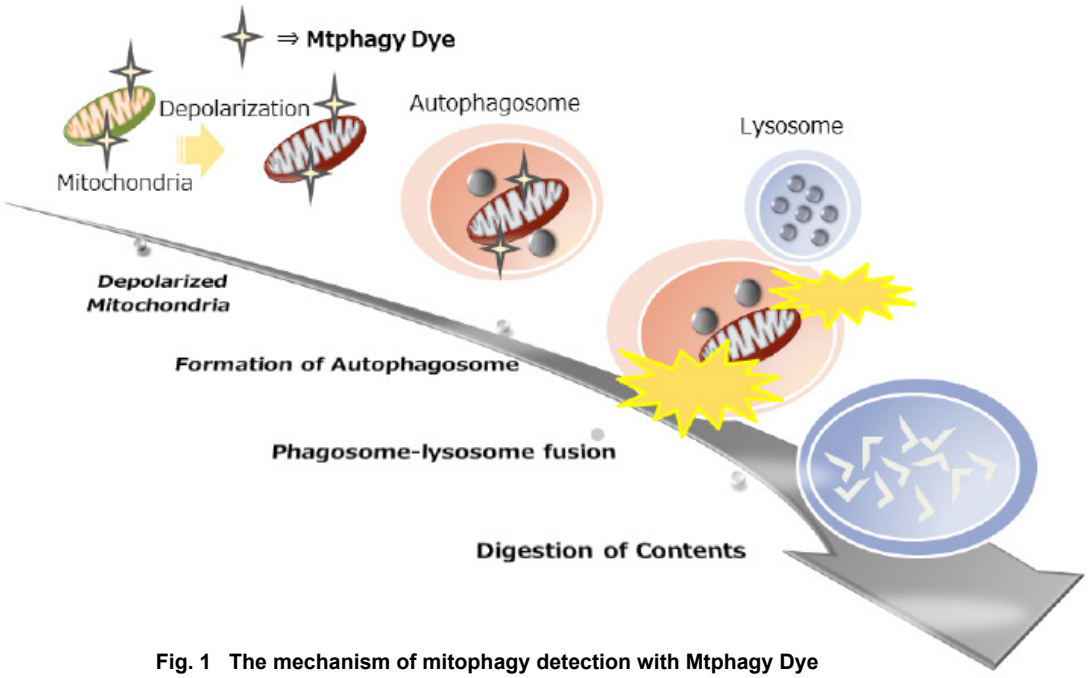


Fig. 1 The mechanism of mitophagy detection with Mtphagy Dye

Kit Contents

Mtphagy Dye	5 µg x 1
Lyso Dye	30 µg x 1

Storage Condition

Store at 0-5°C and protect from light.

Required Equipment and Materials

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

Preparation of Solutions

Preparation of 100 µmol/l Mtphagy Dye DMSO stock solution

Add 50 µl of DMSO to a tube of Mtphagy Dye (5 µg) and dissolve it with pipetting.
*Store reconstituted DMSO solution at -20°C. The reconstituted solution is stable at -20°C for 1 month.

Preparation of 1 mmol/l Lyso Dye DMSO stock solution

Add 55 µl of DMSO to a tube of Lyso Dye (30 µg) and dissolve it with pipetting.
*Store reconstituted DMSO solution at -20°C. The reconstituted solution is stable at -20°C for 1 month.

Preparation of 100 nmol/l Mtphagy Dye working solution

Dilute the 100 µmol/l Mtphagy Dye DMSO stock solution with Hanks' HEPES buffer or serum-free medium to prepare 100 nmol/l Mtphagy working solution.
*Use Hanks' HEPES buffer or serum-free medium to the dilution because serum in medium is interference with Mtphagy.

Preparation of 1 µnmol/l Lyso Dye working solution

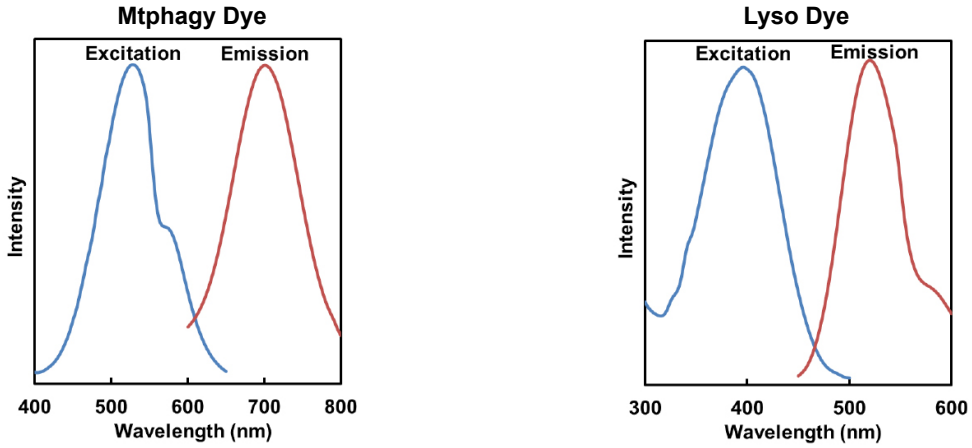
Dilute the 1 mmol/l Lyso Dye DMSO stock solution with Hanks' HEPES buffer or serum-free medium to prepare 1 µmol/l Lyso Dye working solution.
*Use Hanks' HEPES buffer or serum-free medium to the dilution because serum in medium is interference with Lyso Dye.

General Protocol

Mitophagy detection

1. Prepare cells on dish for assay.
2. Discard the culture medium and wash the cells with Hanks' HEPES buffer or serum-free medium twice.
3. Add an appropriate volume of 100 nmol/l Mtphagy Dye working solution and then incubate at 37°C for 30 minutes.
4. Discard the supernatant and wash the cells with Hanks' HEPES buffer or serum-free medium twice.
5. Add medium containing mitophagy-inducing agent and incubate at 37°C for appropriate time. Confirm the mitophagy phenomenon on a fluorescence microscope.
6. To observe the co-localization of Mtphagy Dye and lysosome, incubate at 37°C for 30 minutes with 1 µmol/l Lyso Dye working solution.
7. Discard the supernatant, wash the cells with Hanks' HEPES buffer or serum-free medium twice and observe on a fluorescence microscope.

Excitation and emission spectra of Mtphagy Dye and Lyso Dye



Induction of mitophagy by carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) as a mitochondrial-uncoupling reagent with Parkin expressed HeLa cells

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 transfected with Parkin plasmid vector by HilyMax transfection reagent from Dojindo (Code#:H357), and incubated at 37°C overnight. The Parkin expressed HeLa cells were washed with Hanks' HEPES buffer twice and then incubated at 37°C for 30 minutes with 250 µl of 100 nmol/l Mtphagy Dye working solution. After the washing of the cells with Hanks' HEPES buffer twice, the culture medium containing 10 µmol/l CCCP was added to the well. After 24 hours incubation, mitophagy was observed by a fluorescence microscopy. After removing the supernatant, 250 µl of 1 µmol/l Lyso Dye working solution were added to the cells and incubated at 37°C for 30 minutes. The cells were washed with Hanks' HEPES buffer twice and then co-localization of Mtphagy and Lyso Dye was observed.

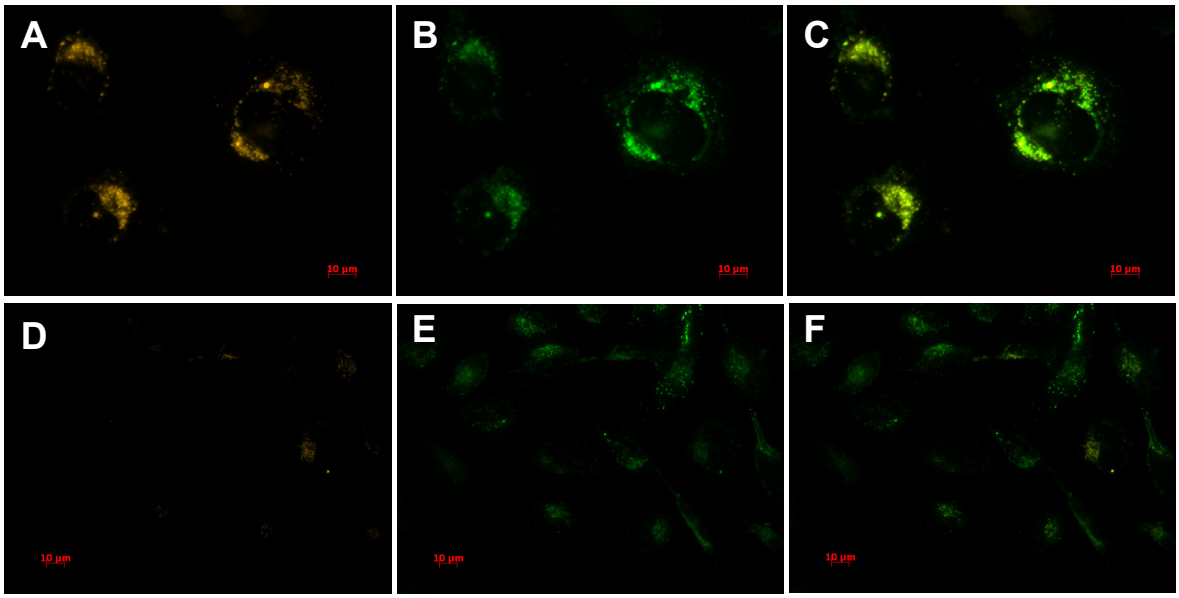


Fig. 2 Observation of mitophagy using Parkin expressed HeLa cells (upper panel) and normal HeLa cells (lower)

A, D) Fluorescent images of Mtphagy Dye; B, E) Fluorescent images of Lyso Dye; C, F) Co-localized fluorescent images of Mtphagy and Lyso Dye

*Mtphagy Dye: excitation filters 550 ± 25 nm, emission filters 605 ± 35 nm
Lyso Dye: excitation filters 470 ± 20 nm, emission filters 525 ± 25 nm

Mtphagy Dye and Lyso Dye are Patent Pending.
If you need more information, please contact Dojindo technical service.

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