

## General Information

Mitochondria are one of the cytoplasmic organelles that play 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 Mtpahgy Dye, reagent for detection of mitophagy, and Lyso Dye. Mtpahgy Dye accumulates in intact 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 fuse to lysosome and then Mtpahgy Dye emits a high fluorescence. To confirm the fusion of Mtpahgy Dye-labeled mitochondria and lysosome, Lyso Dye included in this kit can be used.

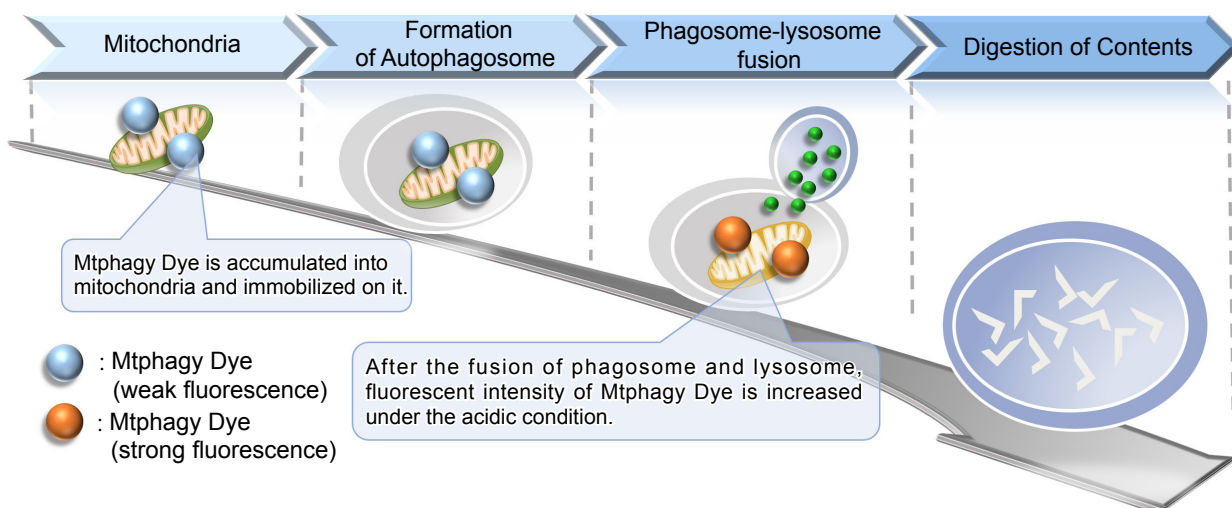


Fig. 1 The mechanism of mitophagy detection with Mtpahgy Dye

## Kit Contents

Mtpahgy Dye 5  $\mu\text{g}$  x 1  
Lyso Dye 30  $\mu\text{g}$  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 $\mu\text{mol/l}$ Mtpahgy Dye DMSO stock solution

Add 50  $\mu\text{l}$  of DMSO to a tube of Mtpahgy Dye (5  $\mu\text{g}$ ) 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  $\mu\text{l}$  of DMSO to a tube of Lyso Dye (30  $\mu\text{g}$ ) 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 Mtpahgy Dye working solution

Dilute the 100  $\mu\text{mol/l}$  Mtpahgy Dye DMSO stock solution with Hanks' HEPES buffer or serum-free medium to prepare 100 nmol/l Mtpahgy working solution.

\*Use Hanks' HEPES buffer or serum-free medium to the dilution because serum in medium is interference with Mtpahgy Dye.

### Preparation of 1 $\mu\text{mol/l}$ Lyso Dye working solution

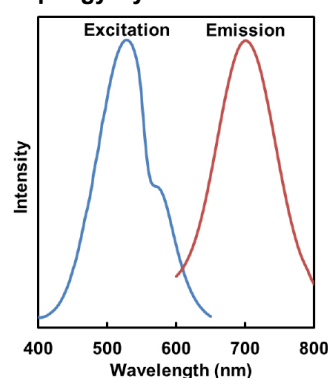
Dilute the 1 mmol/l Lyso Dye DMSO stock solution with Hanks' HEPES buffer or serum-free medium to prepare 1  $\mu\text{mol/l}$  Lyso Dye working solution.

\*Use Hanks' HEPES buffer or serum-free medium to the dilution because serum in medium is interference with Lyso Dye.

## Supplemental Information

### Excitation and emission spectra of Mtpahgy Dye and Lyso Dye

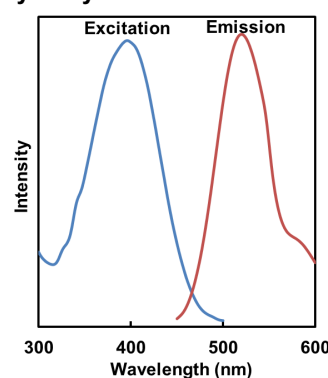
#### Mtpahgy Dye



$\lambda_{\text{ex}}$  : 530 nm  
 $\lambda_{\text{em}}$  : 700 nm

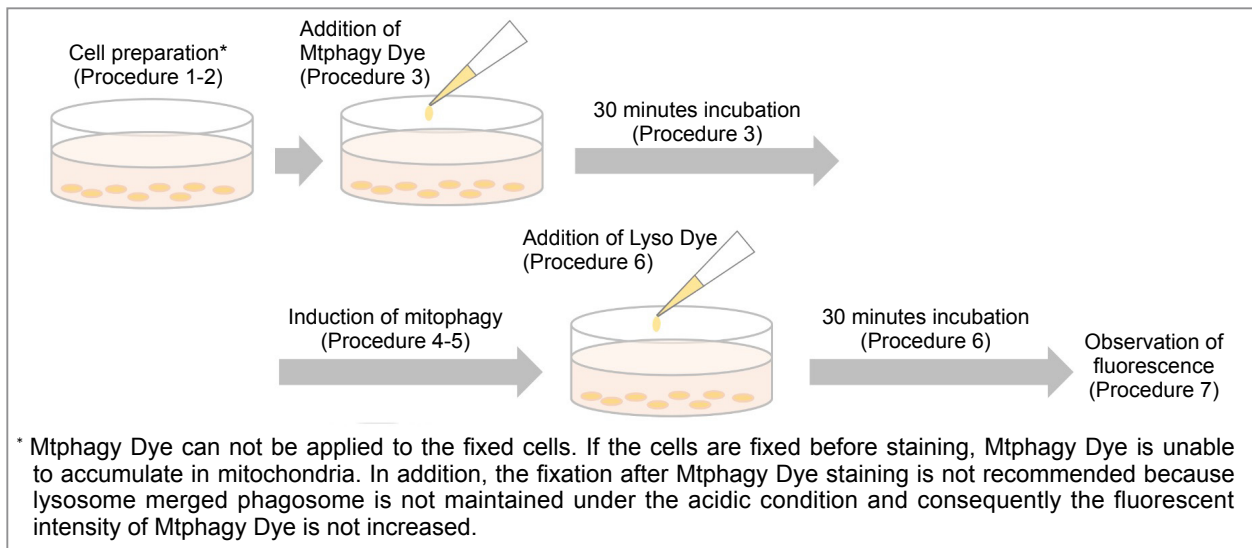
<Recommended filter>  
Ex : 500 ~ 560 nm  
Em : 670 ~ 730 nm

#### Lyso Dye



$\lambda_{\text{ex}}$  : 398 nm  
 $\lambda_{\text{em}}$  : 525 nm

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



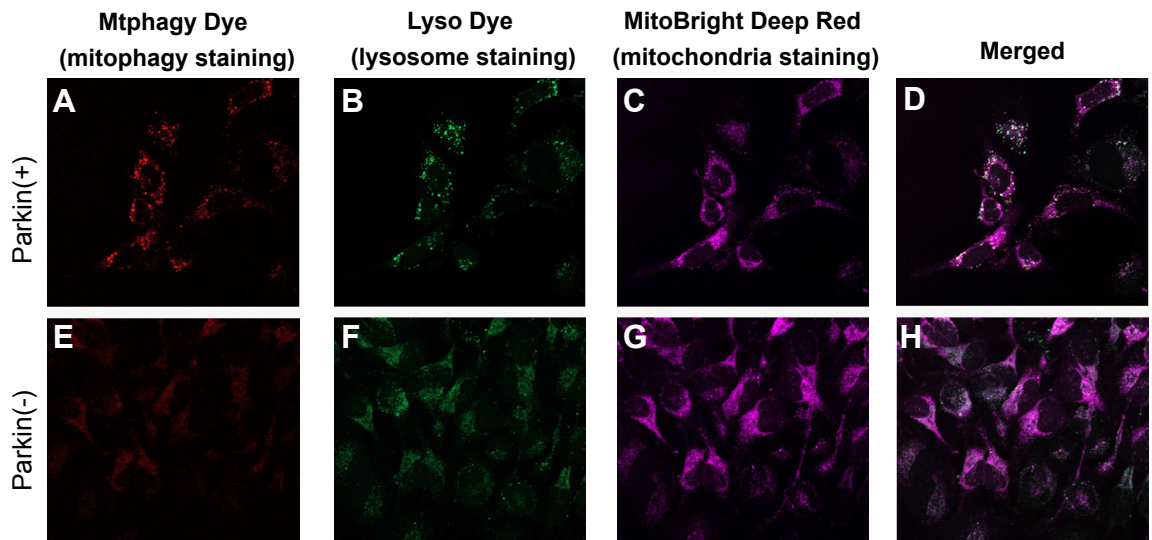
### Mitophagy detection

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 Mtpagy 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 Mtpagy 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.

### Experimental Example

#### 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<sub>2</sub> incubator. The cells were transfected with Parkin plasmid vector by HilyMax transfection reagent (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 Mtpagy Dye working solution containing 100 nmol/l MitoBright Deep Red (Dojindo, Code#:MT08). 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 Mtpagy, Lyso Dye and MitoBright Deep Red was observed by confocal fluorescence microscopy.



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

A, E) Fluorescent images of Mtpagy Dye; B, F) Fluorescent images of Lyso Dye; C, G) Fluorescent images of MitoBright Deep Red; D, H) Co-localized fluorescent images of Mtpagy, Lyso Dye and MitoBright Deep Red;

- Mtpagy Dye: 561 nm (Ex), LP 650 nm (Em)
- Lyso Dye: 488 nm (Ex), 502-554 nm (Em)
- MitoBright Deep Red: 640 nm (Ex), 656-700 nm (Em)

*Mtpagy Dye and Lyso Dye are Patent Pending.*

*If you need more information, please contact Dojindo technical service.*

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