Instruction Manual

FastMitophagy™
Detection Kit

Designed for mitophagy detection
in mammalian cells

Storage: Store at 0-5 °C with protection from light.

Cat. No. 092690201

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Product Description

FastMitophagy™ Detection Kit is specifically designed for mitophagy detection in mammalian cells. Mitophagy is the selective degradation of defective mitochondria by autophagy following DNA damage or oxidative stress. It serves as a specific elimination system by sequestering dysfunctional mitochondria into autophagosomes, fusing the phagosome to lysosomes, and degrading them by digestion. Since its first observation in 1962, mitophagy has attracted intense research for cell homeostasis and development in mammalian cells. Recent research efforts are directed towards further elucidating the role of induced autophagy as a cell survival response to stress, microbial infection, and disease (e.g., cancer, Alzheimer's disease, and Parkinson's disease).

This kit is composed of a mitophagy dye (Mtphagy Dye, a reagent for detection of mitophagy) and lysosome dye (Lyso Dye). Mtphagy Dye initially accumulates in intact mitochondria and becomes immobilized via chemical bonding, resulting in a very weak fluorescence due to the influence of the surrounding environment. When mitophagy is induced, the damaged mitochondria fuses to the lysosome and the Mtphagy Dye emits a high fluorescence. To confirm the fusion of Mtphagy Dye–labeled mitochondria and lysosome, Lyso Dye is also included with the kit.

For 96 well plate format (100 µL/well), one FastMitophagy™ Detection Kit is sufficient for 5 plates. For 35 mm dish format (2 mL), the kit is sufficient for 25 assays.

Key Benefits

- Simple procedure: just add the Mtphagy dye - no need for transfection
- More sensitive than other autophagy markers

Kit Contents

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

Storage

Store at 0-5°C with protection from light.

Working solution preparation

1. As shown in Figure 1, prepare 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.

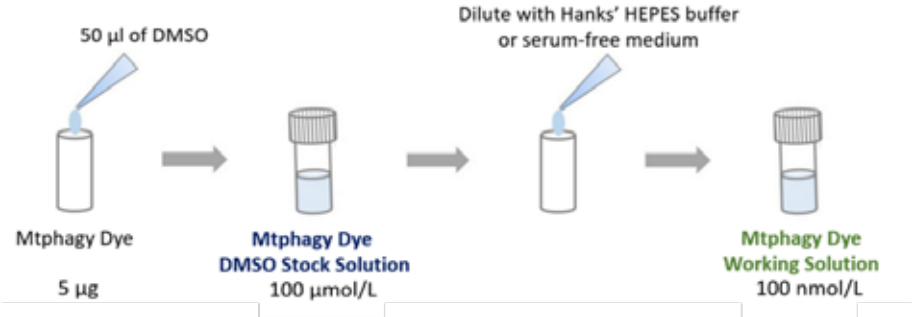

Figure 1. Preparation of Mtphagy Dye working solution.

2. 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.

3. Preparation of 100 nmol/L Mtphagy Dye working solution (Figure 1): 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.

4. Preparation of 1 µmol/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 for the dilution because serum in the medium will interfere with Mtphagy Dye. To confirm the fusion of Mtphagy Dye–labeled mitochondria and lysosome, Lyso Dye (included in this kit) can be used. Lyso Dye should be added after confirmation of the mitophagy induction to avoid excess staining (the fluorescence of Lyso Dye fades after long periods of staining).

Mitophagy detection protocol (Figure 2)

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 the appropriate time. Confirm the mitophagy phenomenon on a fluorescence microscope. To observe the co-localization of Mtphagy Dye and lysosome, add 1 μmol/L Lyso Dye working solution and incubate at 37°C for 30 minutes.

6. 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 are recommended below (Figure 3).

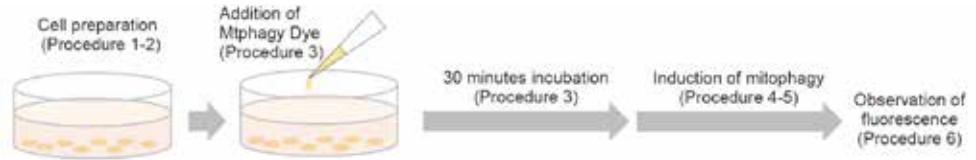

Figure 2. General mitophagy detection procedure.

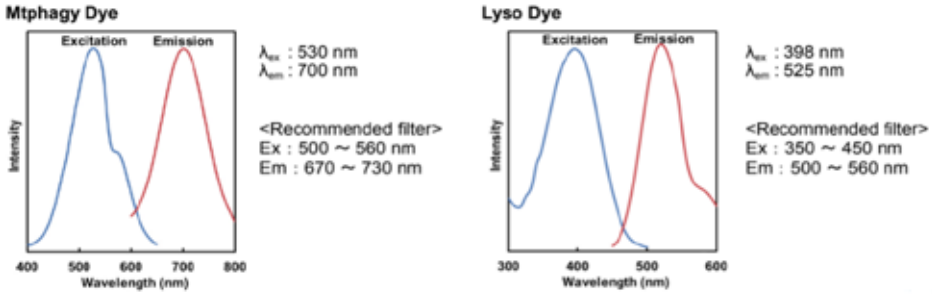

Figure 3. Excitation and emission spectra of Mtphagy Dye and Lyso Dye.

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