Cell Meter™ Mitochondrial Autophagy Imaging Kit *Red Fluorescence*

Catalog number: 22998
Unit size: 100 Tests

Component	Storage	Amount
Component A: Mitophagy Red™	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Staining Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (50 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (50 µL)

OVERVIEW

Mitochondrial autophagy (also called "mitophagy") appears to be involved in Alzheimer and Parkinson diseases, mainly induced by the accumulation of depolarized mitochondria. Mitophagy serves as an elimination system that removes dysfunctional mitochondria caused by oxidative stress and DNA damage, causing sequestration into auto phagosome, followed by fusion to lysosome and is degraded. Cell Meter™ Mitochondrial Autophagy Mitophagy limaging Kit uses Mitophagy Red™ as the mitophagy probe, which enables the very rapid and uniform staining of mitochondria across a wide variety of mammalian cell types and translocate to lysosome upon induction of mitophagy. The Cell Meter™ Mitochondrial Autophagy Imaging Kit provides an excellent tool to be used as an indicator of mitophagy in suspended or attached live cells. The staining pattern of Mitophagy Red™in live cells is stable enough that it provides enough time for studying most live cell dynamic. The assay conditions are compatible with cell culture medium. The excitation/emission of the Mitophagy Red™ probe fits well the widely available Cy3/TRITC filter set and can easily be combined with GFP expressed cell lines if desired.

AT A GLANCE

1. Prepare cells in growth medium
2. Incubate cells with Mitophagy Red™ working solution for 20-30 minutes at 37 °C
3. Remove Mitophagy Red™ working solution
4. Analyze with a fluorescence microscope with Cy3/TRITC filter set
5. Stimulate cells to induce mitophagy and analyze it

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3/TRITC filter set
Emission	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy3/TRITC filter set

CELL PREPARATION

For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 µL for a 384-well plate.

For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 50,000-100,000 cells/well/90 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/20 µL for a 384- well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.

Note Each cell line should be evaluated on an individual basis to determine the optimal cell density

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Mitophagy Red™ stock solution (200X)
Add 50 µL of DMSO (**Component C**) into the vial of Mitophagy Red™ (**Component A**) to make 200X stock solution.

Note 50 µL of 200 X Mitophagy Red™ stock solution is enough for one 96-well plate. Unused Mitophagy Red™ stock solution can be aliquoted and stored at ≤ -20 °C with smaller aliquots. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Mitophagy Red™ working solution (200X)
Add 50 µL of 200X stock solution (**Component A**) into 10 mL of Staining Buffer (**Component B**) or cell culture medium, and mix well.

Note The Mitophagy Red™ probe is compatible with cell culture medium for most cell lines we tested. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

SAMPLE EXPERIMENTAL PROTOCOL

Staining protocol

1. Prepare cells in growth medium.
2. Remove cell culture medium and add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of Mitophagy Red™ working solution in the cell plate.

   Note The optimal concentration of the cell membrane probe varies depending on the specific application.
3. Incubate the cells at 37 °C for 20-30 minutes, protected from light.
4. Remove working solution in each well. Wash cells with Staining Buffer or buffer of your choice once.
5. Observe the fluorescence signal in cells using a fluorescence microscope with a Cy3/TRITC filter set.
6. Stimulate the cells to induce mitophagy and observe the fluorescence signal in cells using a fluorescence microscope with a Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

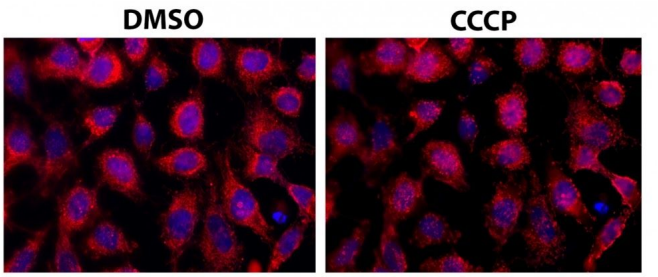

Figure 1. The fluorescence images of HeLa cells co-stained with Mitophagy Red™ and Hoechst 33342 (Cat#17535) in a 96-well black-wall clear-bottom plate. Image was acquired before (Left) and after (Right) addition of CCCP (10 uM) for 1 minute. The cells were imaged using a fluorescence microscope with a Cy3/TRITC filter.

DISCLAIMER

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