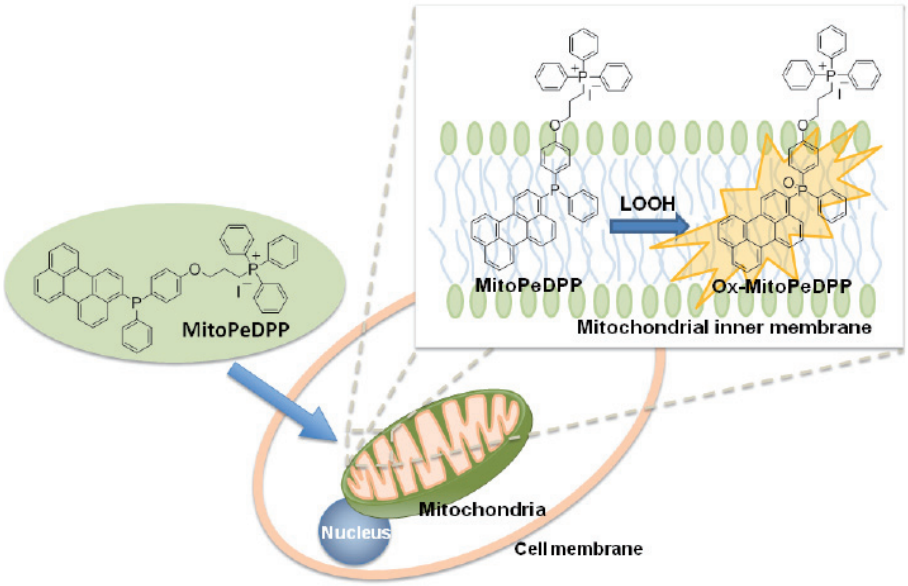
MitoPeDPP Technical Manual

General Information

MitoPeDPP is a newly developed fluorescent dye which can penetrate cell membranes and is accumulated in the mitochondria due to the triphenylphosphoniumn moiety. MitoPeDPP accumulated in mitochondrial inner membranes is oxidized by lipophilic peroxide and then emits strong fluorescence. Since the excitation and emission wavelengths of the oxidized MitoPeDPP (Ox-MitoPeDPP) are 452 nm and 470 nm, respectively, photodamage and autofluorescence of the samples can be minimized. Therefore, MitoPeDPP can be applicable for imaging of the lipophilic peroxide in living cells under a fluorescence microscope.

This product was developed by Dr. Shioji, Department of Chemistry, Fukuoka University.



Kit Contents

MitoPeDPP 5 µg x 3

Storage Condition

Store at 0-5°C and protect from light.
*Caution
Close the bag tightly and store at 0-5°C after opening the bag.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Hanks' HEPES buffer
- PBS
- Micropipettes

Preparation of Solutions

Preparation of 0.1 mmol/l MitoPeDPP DMSO solution
Add 50 µl DMSO to a tube and dissolve 5 µg MitoPeDPP with pipetting.
*Protect the MitoPeDPP solution from light and use the same day.

Preparation of MitoPeDPP working solution
Dilute the MitoPeDPP DMSO solution with Hanks' HEPES buffer to prepare 0.1-0.5 µmol/l MitoPeDPP working solution.
*Hanks' HEPES buffer is recommended to maintain cell condition.
*Use the MitoPeDPP working solution immediately to avoid auto-oxidation.

General Protocol

MitoPeDPP Staining

1) Prepare cells for the assay.
2) Discard the culture medium and wash the cells with either Hanks' HEPES buffer or PBS twice.
3) Add an appropriate volume of MitoPeDPP working solution.

Type of dishes	Volume of working sol.
35-mm dish	2000 µl
96 well plate	100 µl

4) Incubate at 37 °C for 15 minutes with protection from light.
5) Discard the solution and wash the cells with either Hanks' HEPES buffer or PBS twice.
6) Add Hanks' HEPES buffer or PBS and observe the cells under a fluorescence microscope.
*Filter (wavelength/band pass): 470/40 (Ex), 525 /50 (Em)

Notes

This protocol is given as a general method. Optimize staining conditions such as a suitable concentration of MitoPeDPP working solution.

Supplemental Information

Detection of lipophilic peroxide in mitochondria

HepG2 cells were seeded on a collagen-coated 35 mm glass-bottom dish and cultured at 37 °C in a 5%-CO_2 incubator. The cells were washed using Hanks' HEPES buffer twice, and 0.1 µmol/l MitoPeDPP working solution was added to the culture dish. The cells were then incubated at 37 °C for 15 minutes. After the washing of the cells with Hanks' HEPES buffer twice, 100 µmol/l *t*-BHP solution, a lipophilic peroxide prepared in Hanks' HEPES buffer, was added to the dish. After 15 minutes incubation, cells were observed with a fluorescence microscope. These results suggest that MitoPeDPP emits strong fluorescence due to the oxidation by *t*-BHP in mitochondria (Fig.1A). Co-staining with MitoRed indicates that MitoPeDPP is accumulated in the mitochondria (Fig. 1B and 1C).

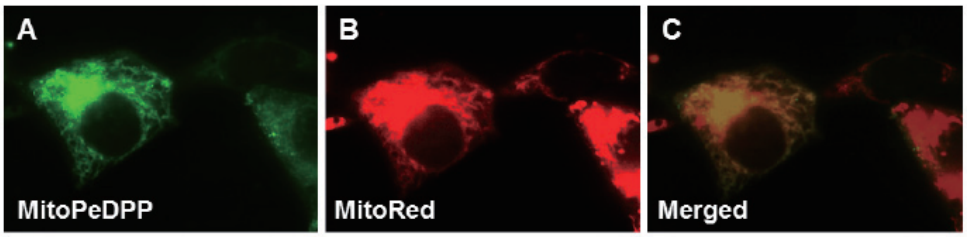


Figure 1. The detection of peroxide introduced with *t*-BHP in HepG2 cell
A) Cells were exposed to MitoPeDPP and then treated with *t*-BHP
B) Cells were stained by MitoRed, mitochondria staining reagent
C) Merged image of A and B

Selectivity of MitoPeDPP reaction to various ROS (reactive oxygen species) and RNS (reactive nitrogen species)

In a homogeneous system, MitoPeDPP reacts with various peroxides such as H_2O_2, *t*-BHP, and $ONOO^-$. On the other hands, Fig. 2A and 2B show that MitoPeDPP accumulated in mitochondria is oxidized by *t*-BHP but not with other ROS or RNS.

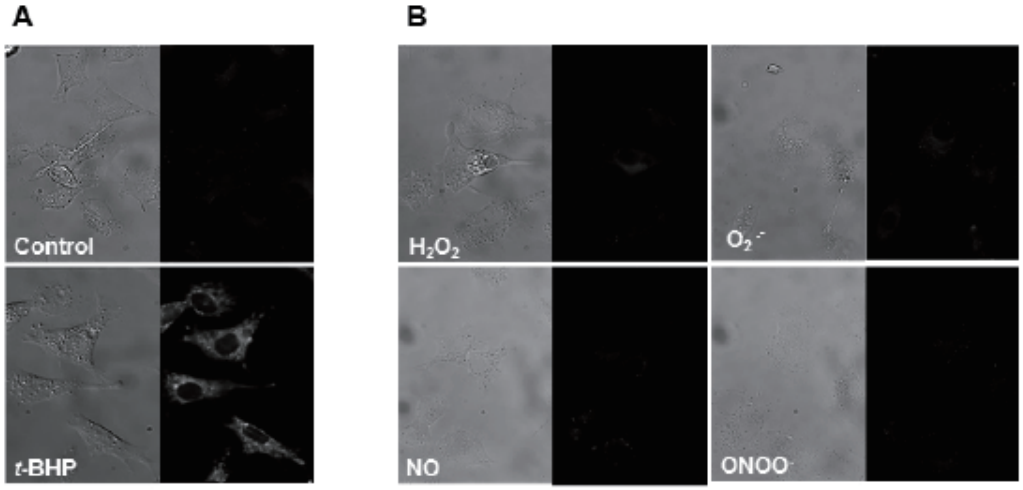


Figure 2.
A) Cells were incubated with MitoPeDPP for 15 minutes and then treated with 100 µmol/l *t*-BHP. The fluorescent image was taken after 15 minutes incubation. These photos shows the phase contrast (left) and fluorescence (right) images of cells with or without *t*-BHP.
B) Cells exposed to MitoPeDPP were treated with the ROS and RNS generating reagents. The concentrations of each ROS and RNS were 100 µmol/l (H_2O_2, NO, and $ONOO^-$) and 10 µmol/l ($O_2^{\cdot -}$). PMA was used as a $O_2^{\cdot -}$ generator.
**t*-BHP, *tert*-Butylhydroperoxide; PMA, Phorbol myristate acetate; SIN-1, 3-(Morpholinyl)sydnonimine, hydrochloride; NOC 7, 1-Hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene
Filter (wavelength/band pass): 470/40 (Ex), 525 /50 (Em)

Reference

1) K. Shioji *et al.*, ''Synthesis and properties of fluorescence probe for detection of peroxides in mitochondria'', *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 3911-3915.
2) K. Shioji *et al.*, ''Fluorescence imaging of accumulated lipid peroxidation in mitochondria by oxidative stress'', *Bioorg. Med. Chem.*, submitted.

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