mtSOX Deep Red - Mitochondrial Superoxide Detection

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/MT14.pdf

General Information

Mitochondria are major cellular organelles. They synthesize ATP via energy metabolism that depends on redox reactions. Superoxide, a reactive oxygen species (ROS), is produced in mitochondria by electron leaking during redox reactions. Cellular control of ROS is important because ROS are associated with disease, cellular damage and senescence. The mtSOX Deep Red is oxidized by mitochondrial superoxide and accumulated in mitochondria.

Content

mtSOX Deep Red 100 nmol ×1
100 nmol ×3

Storage Condition

Store at 0–5°C

Required Equipment and Materials

- Fluorescence microscope, fluorescence plate reader, or flow cytometer
- Incubator (37°C), - Microtubes
- Micropipettes (100–1000 µl, 0.5–10 µl)
- Hanks' Balanced Salt Solution (HBSS)
- Dimethyl sulfoxide (DMSO)

Precaution

Allow the mtSOX Deep Red to equilibrate to room temperature before use.
Centrifuge the tube briefly before opening the cap because the content may be on the tube wall or the cap.
Refer to Table 1 to check suitable fluorescence wavelengths for each application.

Table 1. Recommended filter settings for using mtSOX Deep Red

Applications	Fluorescence plate reader	Fluorescence microscope	Flow cytometer
Measurement wavelength	Ex: 535–565 nm Em: 660–690 nm	(Confocal microscope) ・Ex/Em: 561/640–700 nm (for Single staining) ・Ex/Em: 633/640–700 nm (for co-staining with red fluorescence*) (Fluorescence microscope) ・TxRed filter	APC filter

* When co-stain with another red fluorescence probe, use Ex 633 nm for mtSOX Deep Red to avoid signal overlap.

Preparation of Solutions

Preparation of 10 mmol/l mtSOX Deep Red DMSO stock solution

Add 10 µL of DMSO to the provided tube containing mtSOX Deep Red (100 nmol), and dissolve by pipetting.
Note: The mtSOX Deep Red is difficult to see because it is present in a small amount.
Store the reconstituted mtSOX Deep Red DMSO stock solution at −20°C until use. The solution is stable at −20°C for 1 month.

Preparation of mtSOX Deep Red working solution

Dilute the 10 mmol/l mtSOX Deep Red DMSO stock solution with culture medium or HBSS to prepare 10 µmol/l mtSOX Deep Red working solution.
Note: The working solution cannot be stored and must be freshly prepared each day and used within the day.
Refer to Table 2 for preparation of the working solution by vessel type.

Table 2. The required amount of mtSOX Deep Red working solution by vessel type

Vessel	35-mm dish	ibidi 8-well plate	96-well clear black plate (clear bottom)	Flow cytometer
Appropriate amount	2 ml/dish	200 µl/well	100 µl/well	0.5 ml/tube

General protocol

Mitochondrial superoxide detection

1. Seed cells on dishes or plates and incubate in an incubator set at 37°C equilibrated with 95% air and 5% CO_2.
Note: Refer to Table 3 to check the recommended vessels for each detector.

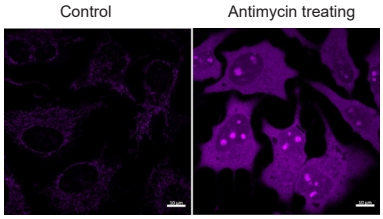
Table 3. Recommended vessels by a detector

Detector	Fluorescence plate reader	Fluorescence microscope	Flow cytometer
Vessel	96-well black plate (clear bottom)	96-well black plate (clear bottom) ibidi 8-well plate 35-mm dish	6-well plate

2. Discard the culture medium and wash the cells with the culture medium.
3. Add an appropriate volume of mtSOX Deep Red working solution (*Table 3.) and incubate at 37°C for 30 minutes.
4. Remove the supernatant and wash the cells twice with HBSS.
5. Add medium or HBSS containing stimulation agents and incubate at 37°C for an appropriate time.
Note: Optimize the incubation time according to the stimulation conditions.
6. Observe fluorescence signals.
Note: Do not wash the cells after step 5 as it may result in dye leakage.

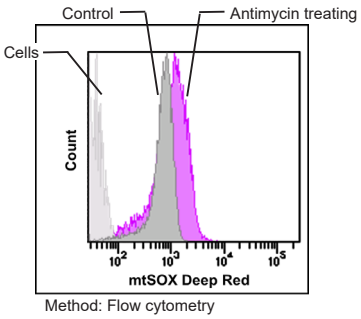
Detection of superoxide in HeLa cells treated with Antimycin

1. HeLa cells (1×10^5 cells/ml) in MEM (supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded on vessels and incubated overnight in an incubator set at 37°C and equilibrated with 95% air and 5% CO_2.
2. The culture medium was removed and the cells were washed with HBSS twice.
3. mtSOX Deep Red working solution containing Antimycin (10 µmol/l) was added to the cells.
4. The cells were again incubated at 37°C for 30 minutes.
5. Fluorescence signals were measured by a plate reader, a flow cytometer and imagings were performed with fluorescence microscopy.



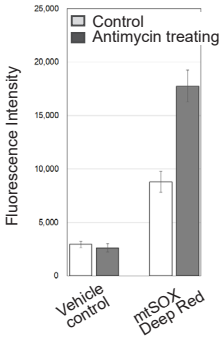
Method: Confocal laser microscopy

Ex: 561 nm, Em: 640–700 nm



Method: Flow cytometry

APC filter



Method: Plate reading

Ex: 550 nm, Em: 675 nm

Figure 1. Fluorescence signals from HeLa cells in the presence and absence of the Complex III inhibitor Antimycin detected with a confocal laser microscope, flow cytometer and plate reader

Simultaneous analysis of mitochondrial mass, mitochondrial membrane potential and mitochondrial superoxide in HeLa cells.

1. HeLa cells (3×10^4 cells/ml) in MEM (supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin) were seeded on ibidi-8-well plates and incubated overnight in an incubator (5% CO_2, 37°C).
2. The culture medium was removed and the cells were washed twice with HBSS.
3. The supernatant was replaced with a working solution [Hoechst33342: 1 µg/ml, MitoTracker™ Green FM: 100 nmol/l, and tetramethylrhodamine methyl ester (TMRE): 150 nmol/l]and the cells were incubated at 37°C for 30 minutes.
4. The supernatant was removed and the cells were washed twice with HBSS.
5. The supernatant was replaced and mtSOX Deep Red working solution containing Antimycin (10 µmol/l) was added to the cells.
6. The cells were again incubated at 37°C for 30 minutes.
7. The cells were observed under a fluorescence microscope.

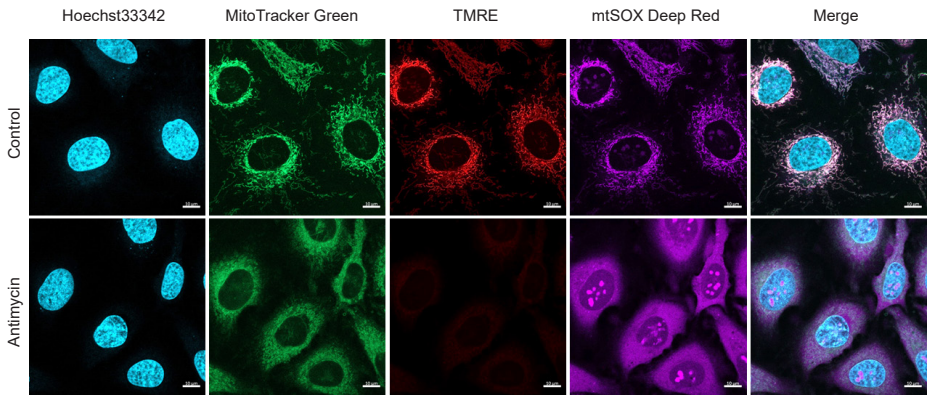


Figure 2. Simultaneous fluorescence imaging of nuclear, mitochondrial mass, mitochondrial membrane potential and mitochondrial superoxide in HeLa cells

Method: Confocal laser microscopy
(Blue) Nuclear stain: Hoechst33342 (Ex: 405 nm, Em: 450–495 nm)
(Green) Mitochondrial mass stain: MitoTracker™ Green FM (Ex: 488 nm, Em: 500–550 nm)
(Red) Mitochondrial membrane potential stain: TMRE (Ex: 561 nm, Em: 560–620 nm)
(Purple) Mitochondrial superoxide stain: mtSOX Deep Red (Ex: 633 nm, Em 640–700 nm)

Excitation and emission spectra of mtSOX Deep Red

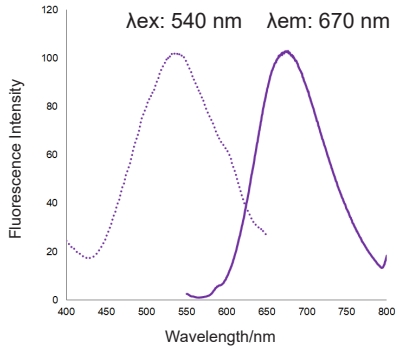


Figure 3. Excitation and emission spectra of mtSOX Deep Red after reaction with superoxide

Selectivity of mtSOX Deep Red for ROS

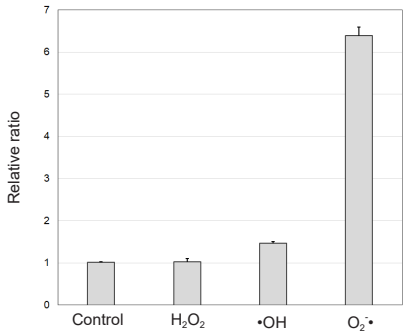


Figure 4. Selectivity of mtSOX Deep Red for ROS

Comparison of detection sensitivity of mtSOX Deep Red with a commonly used probe for mitochondrial superoxide indication

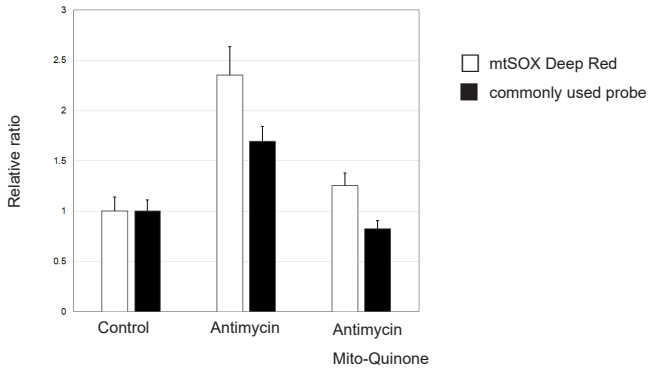


Figure 5. Comparison of sensitivity for mitochondrial superoxide

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

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