

Si-DMA for Mitochondrial Singlet Oxygen Imaging

Technical Manual

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/mt05.pdf>

General Information

Singlet oxygen (1O_2) is one of the Reactive Oxygen Species (ROS). 1O_2 is known to be a cause of spots and wrinkles of the skin due to its very strong oxidizing potential. In the field of cancer research, 1O_2 is of a particular importance because of its key role in photodynamic therapy (PDT), an emerging anticancer treatment using photoirradiation and photosensitizers. Therefore, the monitoring of 1O_2 in living cells is highly important for understanding of anti-cancer mechanism of PDT. However, the existing fluorescence probe for the detection of 1O_2 cannot be used in living cells because of its cell membrane impermeability.

Majima et. al. synthesized a new far-red fluorescence probe composed of silicon-containing rhodamine and anthracene moieties, namely Si-DMA, as a chromophore and a 1O_2 reactive site, respectively. In the presence of 1O_2 , fluorescence of Si-DMA increases due to endoperoxide formation at the anthracene moiety.¹⁾ Among seven different ROS, Si-DMA is able to selectively detect 1O_2 (Fig. 3). In addition, Si-DMA is able to visualize the generation of 1O_2 from protoporphyrin IX in mitochondria with 5-aminolevulinic acid (5-ALA), a precursor of heme (Fig. 4).

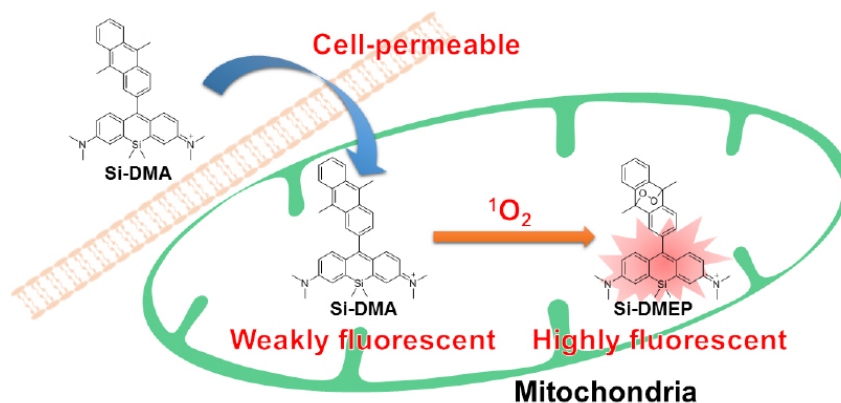


Fig.1 Cell staining mechanism by Si-DMA

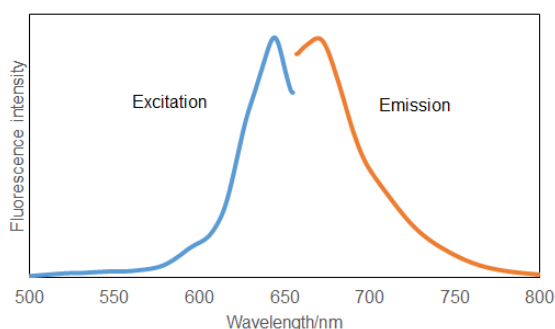


Fig.2 Excitation and Emission spectra of Si-DMA after reaction with 1O_2

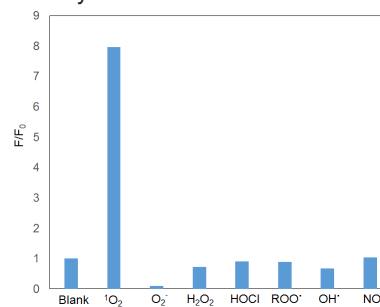


Fig.3 Selectivity of Si-DMA toward various ROS

Kit Contents

Si-DMA 2 μ g x 1

Storage Condition

Store at $-20^{\circ}C$ and protect from light.

Caution:

Si-DMA is sensitive to light. Store unused Si-DMA in the bag at $-20^{\circ}C$.

- Dimethyl sulfoxide (DMSO)
- Hanks' HEPES buffer or HBSS
- Micropipettes

Preparation of 100 μ mol/l Si-DMA DMSO stock solution

Add 36 μ l of DMSO to a tube of Si-DMA (2 μ g) and dissolve it with pipetting.

*Store the Si-DMA DMSO stock solution at $-20^{\circ}C$.

Si-DMA DMSO stock solution is stable at $-20^{\circ}C$ for up to a month.

Preparation of Si-DMA working solution

Dilute the Si-DMA DMSO stock solution with Hanks' HEPES buffer to prepare 25-100 nmol/l Si-DMA working solution.

*Protect the solution from light and use it within the same day, since Si-DMA is not stable in Hanks' HEPES buffer.

Notes

The recommended concentration of Si-DMA working solution for use is 25-100 nmol/l. Once the final concentration of Si-DMA becomes more than 1 μ mol/l, Si-DMA may be accumulated to organelle. In addition, the final concentration of Si-DMA becomes more than 5 μ mol/l, cytotoxicity may be seen.

Si-DMA staining

1. Prepare cells for the assay.
2. Discard the culture medium and wash the cells with Hanks' HEPES buffer twice.
3. Add an appropriate volume of Si-DMA working solution.
4. Incubate for 45 minutes at 37°C.
5. Discard the supernatant and wash the cells with Hanks' HEPES buffer twice.
6. Add Hanks' HEPES buffer and observe the cells under a fluorescence microscope.

Usage Examples**Fluorescence microscopic detection of $^1\text{O}_2$ in HeLa cells treated with 5-aminolevulinic acid (5-ALA)**

1. HeLa cells at 2.4×10^5 cells/ml (200 μl) were seeded on a μ -slide 8 well (Ibidi) in DMEM (10% fetal bovine serum, 1% penicillin-streptomycin) and cultured in a 5% CO_2 incubator overnight at 37 °C.
2. The cells were washed with 200 μl of Hanks' HEPES buffer twice.
3. 5-ALA in Hanks' HEPES buffer (150 $\mu\text{g}/\text{ml}$, 200 μl) was added to the μ -slide, and the cells were cultured in a 5% CO_2 incubator for 4 hours at 37 °C.
4. The cells were washed with 200 μl of Hanks' HEPES buffer twice.
5. Si-DMA working solution (40 nmol/l, 200 μl) was added, and the cells were cultured in a 5% CO_2 incubator for 45 minutes at 37 °C.
6. The cells were washed with 200 μl of Hanks' HEPES buffer twice.
7. Hanks' HEPES buffer (200 μl) were added, and the cells were observed under a fluorescence microscope.

Filter (wavelength/band pass)

Fluorescence imaging: 600/50 nm (Ex), 685/50 nm (Em)

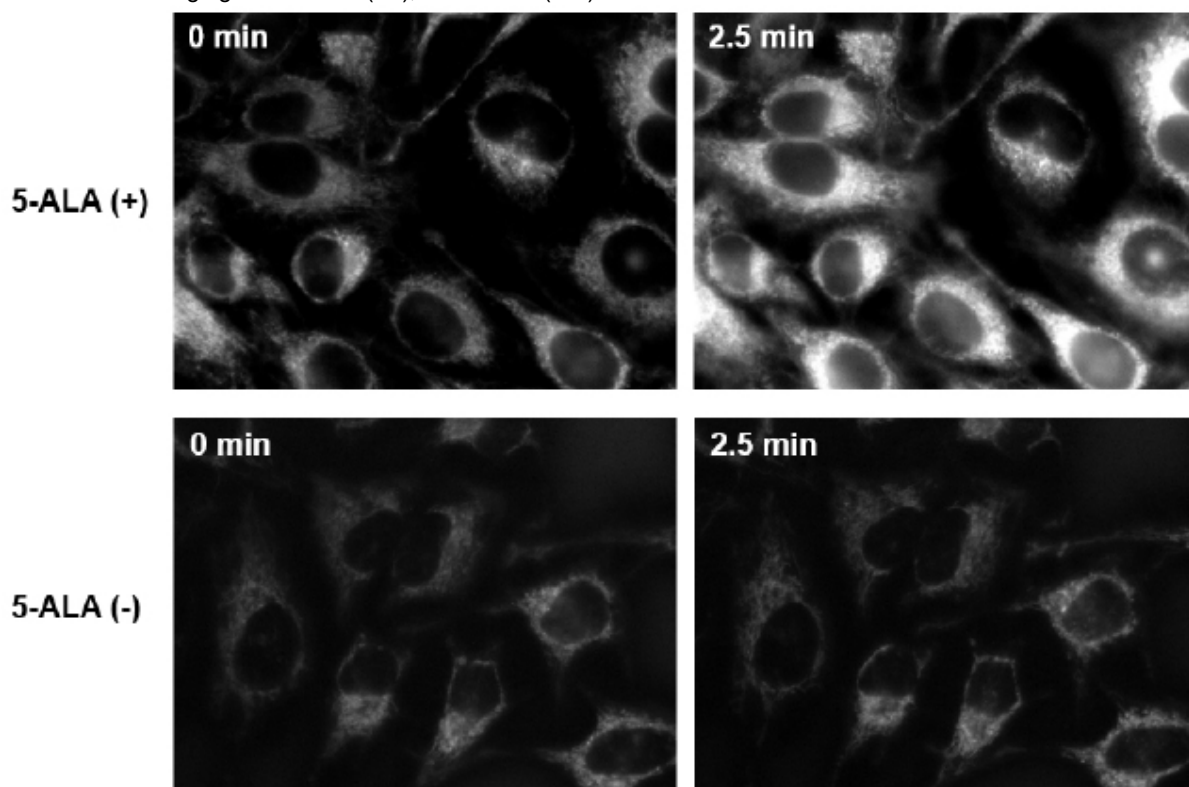


Fig.4 Fluorescence imaging of mitochondrial $^1\text{O}_2$ with Si-DMA in HeLa cells treated with 5-ALA.

Fluorescent of Si-DMA in 5-ALA-treated HeLa cells increased after 2.5 minutes irradiation. It was found that Si-DMA was able to visualize in real time the $^1\text{O}_2$ generated from protoporphyrin IX in mitochondria.

References

- 1) S. Kim, T. Tachikawa, M. Fujitsuka, T. Majima, "Far-Red Fluorescence Probe for Monitoring Singlet Oxygen during Photodynamic Therapy", *J. Am. Chem. Soc.*, **2014**, 136 (33), 11707-11715.

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

Dojindo Laboratories

2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies, Inc.

Tel: +1-301-987-2667 Web: <http://www.dojindo.com/>

Dojindo EU GmbH

Tel: +49-89-3540-4805 Web: <http://www.dojindo.eu.com/>

Dojindo China Co., Ltd

Tel: +86-21-6427-2302 Web: <http://www.dojindo.cn/>

MT05 : Si-DMA for Mitochondrial Singlet Oxygen Imaging