Mito-FerroGreen

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/M489.pdf

General Information

It is reported that iron is the most abundant transition metal element within an organism and shows various physiological activities. Recently, free iron in living cells is getting attention because its high reactivity is suggested to be related to cellular damage or death. Free iron exists in its stable redox states, ferrous ion (Fe^{2+}) and ferric ion (Fe^{3+}). In living cells, it is considered that understanding the behavior of Fe^{2+} is more important than that of Fe^{3+} because of the intracellular reductive environment, metal transporters and water solubility of Fe^{2+}. Mito-FerroGreen is a novel fluorescent probe for the detection of ferrous ion (Fe^{2+}) in mitochondria where Fe-S clusters and heme proteins are synthesized, and enables live cell fluorescent imaging of intracellular Fe^{2+}.

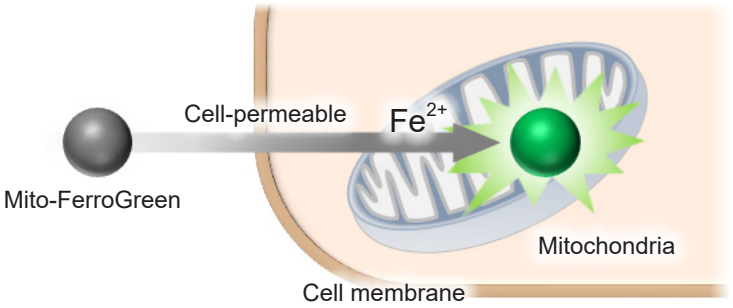

Figure 1 Detection of mitochondrial ferrous ion (Fe^{2+}) using Mito-FerroGreen

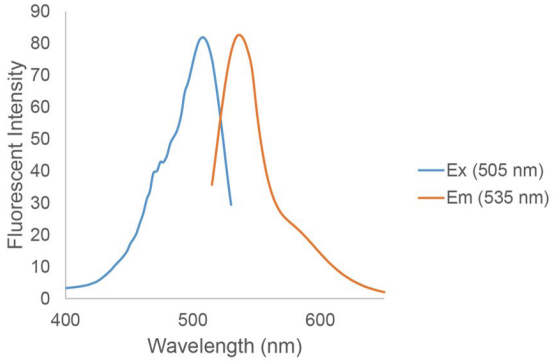

Figure 2 Excitation and emission spectra of Mito-FerroGreen

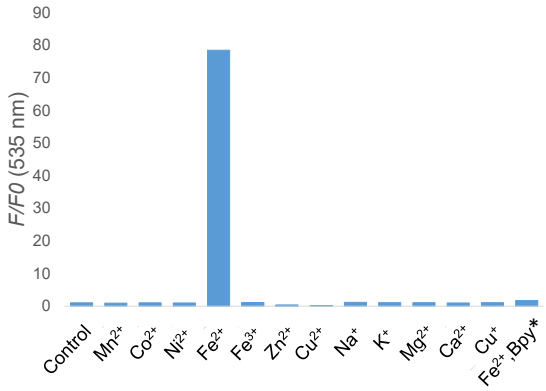

Figure 3 Metal ion selectivity of Mito-FerroGreen
*Bpy; 2,2'-Bipyridyl

Contents

- Mito-FerroGreen 50 µg x 2

Storage Condition

Store at -20°C and protect from light.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO) or Ethanol
- HBSS
- Serum-free medium
- Micropipettes

Preparation of Solution

Preparation of Mito-FerroGreen working solution

Add 53 µl of DMSO to a tube containing of 50 µg of Mito-FerroGreen and dissolve it by pipetting to prepare 1 mmol/l Mito-FerroGreen solution. Dilute the 1mmol/l Mito-FerroGreen solution with HBSS to prepare 5 µmol/l Mito-FerroGreen working solution.

※ 1 mmol/l Mito-FerroGreen DMSO solution and 5 µmol/l Mito-FerroGreen working solution are unstable. Please prepare the solutions just before the staining experiment and use them immediately. Do not use the rest of the Mito-FerroGreen solutions due to the increase of background signal resulted from degradation of the Mito-FerroGreen solution. Ethanol can also be used for preparation. The Ethanol solution is stable at -20°C for 2 weeks.

※ Substitute serum-free medium for HBSS if needed. However, please note that serum-containing medium cannot be used due to causing high background.

General Protocol

1. Inoculate cells to a dish for assay and culture the dish at 37°C in a 5% CO_2 incubator.
2. Discard the supernatant and wash the cells with HBSS or serum-free medium three times.
3. Add 5 µmol/l Mito-FerroGreen working solution to the cells and incubate at 37°C for 30 minutes in a 5% CO_2 incubator.
4. Discard the supernatant and wash the cells with HBSS or serum-free medium three times.
5. Add medium containing stimulating agents and incubate at 37°C in a 5% CO_2 incubator.

※ Please optimize the incubation time according to stimulating conditions.

6. Observe cells by fluorescence microscopy.

※ The order of step 3 and step 5 can be changed in response to experimental conditions, i.e. 5 µmol/l Mito-FerroGreen working solution can be added to the cells after agent stimulation.

Experimental Example 1

Detection of mitochondrial ferrous iron (Fe^{2+}) using Mito-FerroGreen.

1. HeLa cells (2.0×10^4 cells/well) were seeded on µ-slide 8 well (ibidi) and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. The cells were washed with HBSS (200 µl) three times.
3. Mito-FerroGreen working solution (5 µmol/l, 200 µl) were added to the cells, and the cells were incubated at 37°C for 30 minutes in a 5% CO_2 incubator.
4. After supernatant was discarded, 10 mmol/l deferoxamine mesylate salt (sigma) prepared with HBSS (200 µl) was added to the cells, and the cells were incubated at 37°C for 30 minutes in a 5% CO_2 incubator.
5. The supernatant was discarded and the cells were washed with HBSS (200 µl) three times. After the HBSS was removed, serum-free medium (200 µl) was added to the cells.
6. Ammonium iron (II) sulfate (10 mmol/l) was prepared with purified water.
7. Ammonium iron (II) sulfate (2 µl) was added to wells (The final concentration:100 µmol/l). To mix Ammonium iron (II) sulfate and serum-free medium, the entire medium was pipetted up from wells and then immediately pipetted back one time.

*Please do not disturb the cells during pipetting.

*When adding 10 mmol/l Ammonium iron (II) sulfate to well, please exactly follow step 7 as described. Do not add pre-prepared 100 µmol/l Ammonium iron (II) sulfate to cells. It may result in precipitation of Ammonium iron (II) sulfate during the experiment due to a vortex or a pipetting.

8. The cells were incubated at 37°C for 1 hour in a 5% CO_2 incubator, and the cells were washed with HBSS (200 µl) three times.
9. The cells were observed by confocal fluorescence microscopy.

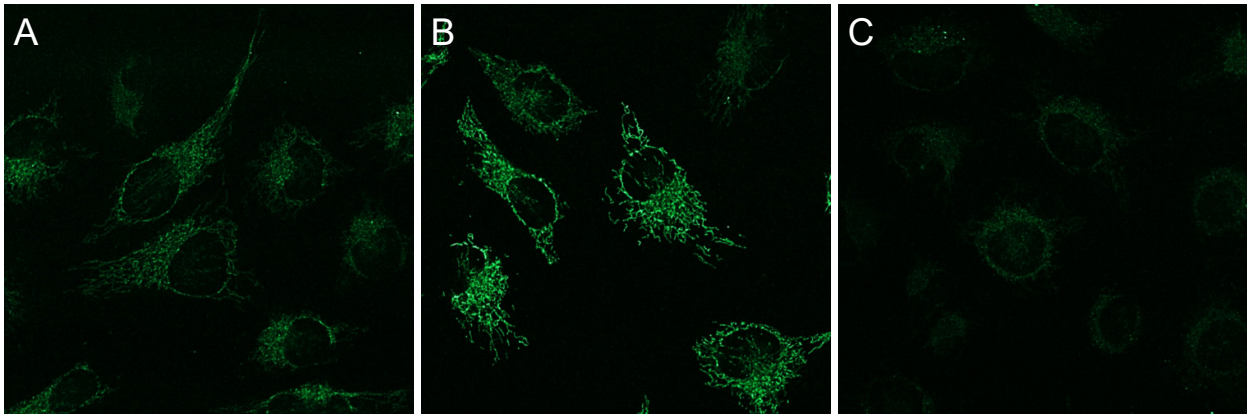

Figure 4 Detection of mitochondrial ferrous ion (Fe^{2+}) using Mito-FerroGreen in HeLa cells
Ex/Em = 488 nm/ 500-550 nm
A Control
B Ammonium iron (II) sulfate treated only
C Ammonium iron (II) sulfate and Deferoxamine treated

Experimental Example 2

Double staining with mitochondrial staining probe

1. HeLa cells (2.0×10^4 cells/well) were seeded on µ-slide 8 well (ibidi) and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. The cells were washed with HBSS (200 µl) three times.
3. HBSS (200 µl) containing the final concentration of 5 µmol/l Mito-FerroGreen and 200 nmol/l MitoBright Deep Red (Dojindo, Code: MT08) were added to the cells and the cells were incubated at 37°C for 30 minutes in a 5% CO_2 incubator.
4. The supernatant was discarded and the cells were washed with HBSS (200 µl) three times. After the HBSS was removed, serum-free medium (200 µl) was added to the cells.
5. Ammonium iron (II) sulfate (10 mmol/l) was prepared with purified water.
6. Ammonium iron (II) sulfate (2 µl) was added to wells (The final concentration:100 µmol/l). To mix Ammonium iron (II) sulfate and serum-free medium, the entire medium was pipetted up from wells and then immediately pipetted back one time.

*Please do not disturb the cells during pipetting.

*When adding 10 mmol/l Ammonium iron (II) sulfate to well, please exactly follow step 6 as described. Do not add pre-prepared 100 µmol/l Ammonium iron (II) sulfate to cells. It may result in precipitation of Ammonium iron (II) sulfate during the experiment due to a vortex or a pipetting.

7. The cells were incubated at 37°C for 1 hour in a 5% CO_2 incubator, and the cells were washed with HBSS (200 µl) three times.
8. The cells were observed by confocal fluorescence microscopy.

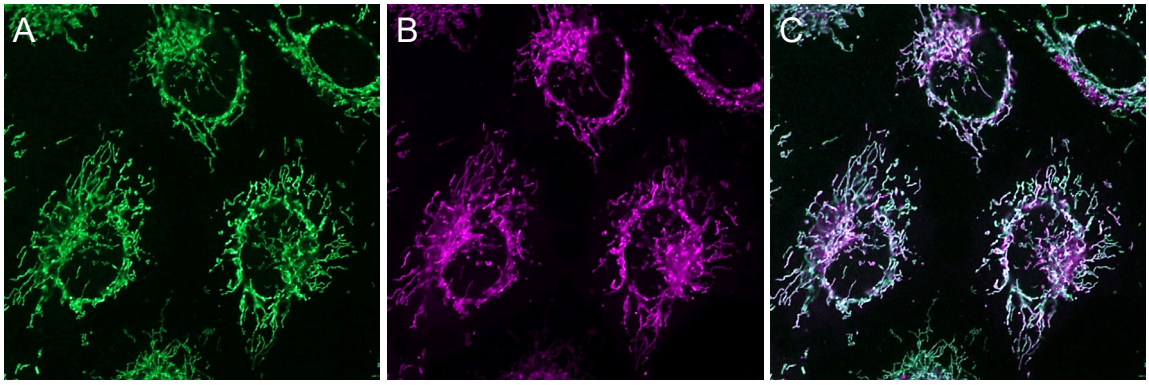

Figure 5 Double staining with mitochondrial staining probe
Mito-FerroGreen (5 μmol/l) Ex/Em = 488 nm/ 500-550 nm
MitoBright Deep Red (200 nmol/l) Ex/Em = 640 nm/ 656-700 nm
A Mito-FerroGreen
B MitoBright Deep Red
C Merge

This product was commercialized under the advisory of Dr. Hideko Nagasawa and Dr. Tasuku Hirayama (Gifu Pharmaceutical University).

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/