PRODUCT INFORMATION

Mito-FerroGreen

Item No. 41726

Purity: ≥90%
Ex./Em.: 505/535 nm
Storage: -20°C
Stability: ≥4 years
Special Conditions: Light and moisture sensitive

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

Mito-FerroGreen is a cell-permeable fluorescent probe that binds irreversibly to mitochondrial ferrous iron (Fe^{2+}). It is selective for Fe^{2+} over 12 other metal ions. When bound to Fe^{2+}, Mito-FerroGreen displays excitation and emission maxima of 505 and 535 nm, respectively. Monitoring iron levels in cells has high relevance to various cellular processes, especially ferroptosis, which is an iron-dependent form of cell death.[1,2]

General Protocol

NOTE: This protocol is given as a general method. Please optimize the staining conditions, such as the final concentration of Mito-FerroGreen and exposure time of the stimulating agent(s).

1. Prepare solutions
   a. Prepare a 1 mM stock solution of Mito-FerroGreen in DMSO. Dissolve 50 µg of Mito-FerroGreen in 53 µl of DMSO and mix with pipetting.

      NOTE 1: DMSO can be substituted with ethanol. This ethanol solution will be stable for one month when stored at -20°C.

      NOTE 2: The Mito-FerroGreen stock solution is unstable. Once prepared, Mito-FerroGreen should be protected from light and used within one day. Do not use the remaining Mito-FerroGreen solution because the degradation of Mito-FerroGreen results in increased background.

   b. Immediately before labeling cells, dilute the 1 mM Mito-FerroGreen stock solution with HBSS to prepare a 5 µM Mito-FerroGreen working solution.

      NOTE 1: This working solution is unstable and should be used immediately.

      NOTE 2: HBSS can be substituted with serum-free cell culture medium. Serum-containing medium cannot be used because it increases background.

2. Prepare, label, and image the cells

   a. Prepare cells for the assay and incubate at 37°C with 5% CO_2.

      NOTE: Fluorescent imaging-compatible cell culture plates should be used.

   b. Discard the cell culture medium and wash the cells three times with HBSS or serum-free medium.

   c. Add the 5 µM Mito-FerroGreen working solution and incubate for 30 minutes at 37°C with 5% CO_2.

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d. Discard the Mito-FerroGreen working solution and wash the cells three times with HBSS or serum-free medium.

e. Add medium containing stimulating agents and incubate cells at 37°C with 5% CO_2.

NOTE 1: Optimize the incubation time according to stimulating conditions.

NOTE 2: The order of steps c. and e. can be changed depending on experimental conditions. For example, the 5 µM Mito-FerroGreen working solution can be added to the cells after agent stimulation.

f. Observe the cells using fluorescence microscopy with excitation and emission wavelengths of 505 and 535 nm, respectively.

Experimental Examples

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.

   NOTE 1: Please do not disturb the cells during pipetting.

   NOTE 2: 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.

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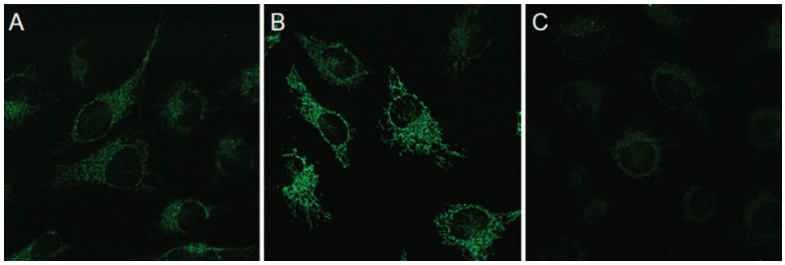
PRODUCT INFORMATION



Detection of mithochondrial ferrous iron (Fe^{2+}) using Mito-FerroGreen in HeLa cells
Ex/Em = 500/550 nm

A: Control
B: Ammonium iron (II) sulfate treated only
C: Ammonium iron (II) sulfate and deferoxamine treated

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 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.

   NOTE 1: Please do not disturb the cells during pipetting.

   NOTE 2: 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.

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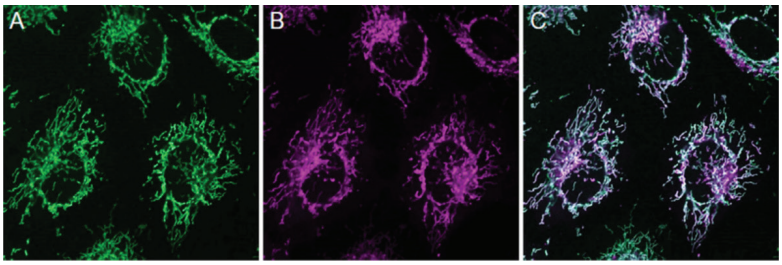

Double staining with mitochondrial staining probe

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

References

1. Angeli, J.P.F., Miyamoto, S., and Schulze, A. Ferroptosis: The greasy side of cell death. *Chem. Res. Toxicol.* **32**, 362-369 (2019).
2. Chen, Y., Guo, Y., Chang, H., *et al.* Brequinar inhibits African swine fever virus replication in vitro by activating ferroptosis. *Virol. J.* **20(1)**, 242 (2023).

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