FerroOrange (Fe^{2+} indicator)

Introduction

Iron is the most abundant transition metal in the human body, and it plays a key role in a variety of biological processes, including respiration, oxygen delivery, and energy metabolism with oxygen. In living cells, iron is mainly present in the form of ferric ions (Fe^{2+}) and ferric ions (Fe^{3+}). Fe^{2+} is considered to be one of the main factors of cellular oxidative damage due to its ability to react with oxygen, superoxide and hydrogen peroxide (H_2O_2) to generate harmful reactive oxygen species, which is closely related to the occurrence of serious diseases such as cancer and neurodegenerative diseases.

FerroOrange is an orange fluorescent probe specifically designed to detect unstable Fe^{2+} in living cells, with a fluorescent signal localized to the endoplasmic reticulum. FerroOrange is highly specific for Fe^{2+} and does not bind Fe^{3+}, other labile metal ions, ferritin or other iron complexes. At the same time, FerroOrange is particularly suitable for live cells due to its excellent cell penetration and low toxicity.

Components and Storage

This product should be stored at -20°C away from light and moisture. Stable for 1 year.

Protocol

1. **The stock solution preparation:** Add 35 μL of DMSO to the tube containing 24 μg of FerroOrange, dissolve and mix well to make a 1 mM stock solution. Store the storage solution at -20°C away from light, stable for 1 month. The stock solution can be aliquoted in single-use tubes to avoid repeated freeze-thaw cycles.

***Note:** Warm the FerroOrange to room temperature and centrifuge for a few seconds before use to allow the powder to fully aggregate at the bottom of the tube.

2. **The working solution preparation:** Take an appropriate amount of stock solution and dilute it with a suitable buffer (e.g., HBSS) or serum-free medium to make a 1 μM FerroOrange working solution. Prepare a fresh working solution every time.

***Note:** The optimal concentration of the working solution can be adjusted.

3. **Cell staining:**

   1) For adherent cells, when the cells have grown to an appropriate density, remove the medium and wash cells with HBSS or serum-free medium three times.

   2) Treat the cells with interested drugs for a certain period according to the experimental design.

***Note:** To obtain more reliable results, it is recommended to prepare a sample with iron chelator 2,2'-Bipyridine (Cat. No. A9060) or ammonium ferrous sulfate as a control.

3) Remove the medium and wash cells 3 times with HBSS or serum-free medium.

4) Add an appropriate amount of FerroOrange working solution to cover the cells, and then incubate in a 37°C incubator protected from light for 30 min.

5) Observe the cells with a fluorescent microscope (Ex/Em: 543/580 nm).

***Note:** Washing is not necessary after the incubation as it may cause the FerroOrange to leak out of the cells.

Note

1. Fluorescent probes are easy to quench, so please protect them from light when used.
2. FerroOrange is a live-cell fluorescent probe and is not suitable for the detection of dead cells.
3. FerroOrange is suitable for the detection of labile iron.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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