FerroBrite™ Green

Catalog number: 20205
Unit size: 1 mg

Component	Storage	Amount (Cat No. 20205)
FerroBrite™ Green	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Ferroptosis is an iron-dependent form of regulated cell death associated with the increase in lipid peroxides. Divalent iron (Fe2+) can lead to spontaneous lipid peroxidation through the Fenton reaction. Ferroptosis is regulated by signaling pathways that control iron storage and oxidative stress. FerroBrite™ Green has been developed for detecting ferroptosis via fluorescence imaging. FerroBrite™ Green has superior photostability and responds quickly to ferroptosis. The dye is permeable to live cells. Upon induction of ferroptosis, the imbalance of Fe2+ causes the reduction in the fluorescence intensity of FerroBrite™ Green. The fluorescence of FerroBrite™ Green can be readily monitored using the common FITC filter, which is equipped in most of fluorescence instruments. FerroBrite™ Green enables the real-time tracking of ferroptosis.

AT A GLANCE

Important Note

Before initial use, thaw FerroBrite™ Green at room temperature and briefly centrifuge to collect the dried pellet.

Protocol Summary

1. Treat the cells as desired.
2. Remove the treatment and add 100 µL of FerroBrite™ Green working solution.
3. Incubate at 37 °C for 30 minutes.
4. Use an Ex/Em = 450/550 filter set to monitor the fluorescence signal.

KEY PARAMETERS

Fluorescence microscope

Emission	550 nm
Excitation	450 nm
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

FerroBrite™ Green Stock Solution

1. Prepare a 5 to 10 mM FerroBrite™ Green stock solution in DMSO. For example, to make a 10 mM FerroBrite™ Green stock solution add 284 µL of DMSO to the vial of FerroBrite™ Green, and mix thoroughly.

   Note: Prepare single-use aliquots of any remaining FerroBrite™ Green stock solution. Store aliquots at ≤ -20 ° C, protected from light. Avoid freeze/thaw cycles.

PREPARATION OF WORKING SOLUTION

FerroBrite™ Green Working Solution

1. Prepare a 10 to 20 µM working solution by diluting the FerroBrite™ Green stock solution into Hanks' solution with 20 mM Hepes buffer (HHBS, AAT Cat No. 20011).

   Note: For optimal results, use this solution within 2 hours of preparation.

   Note: Protect the working solution from light by covering it with foil or placing it in the dark.

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate the cells as desired in a 96-well black wall-clear bottom plate.
2. Add the drug of interest to the cells at the necessary concentration.

   Note: For a positive control, add Erastin at a concentration of 10 µM to the cells. Incubate the cells with Erastin for 5 to 6 hours at 37°C in a 5% CO2 incubator to induce ferroptosis.
3. Remove the cell culture medium and add 100 µL of FerroBrite™ Green working solution to the cells.
4. Incubate the cells at 37°C for 20 to 30 minutes, keeping them protected from light.

   Note: The optimal concentration and incubation time for FerroBrite™ Green may vary depending on the cell line used. It is recommended to test various concentrations to determine the most effective dose.
5. Remove the FerroBrite™ Green working solution and wash the cells twice using HHBS buffer.
6. Add HHBS buffer and analyze the cells using a fluorescence microscope with an Ex/Em = 450/550 nm filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

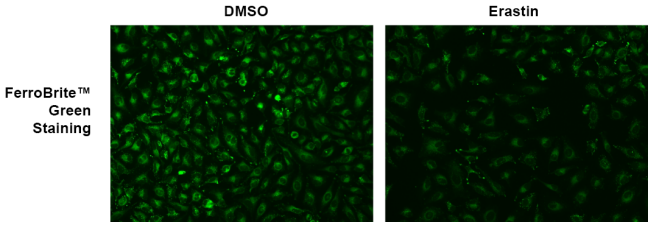

Figure 1. Erastin induced ferroptosis in Hela cells. The fluorescence response of FerroBrite™ Green (20 µM) was evaluated in HeLa cells, in the absence and presence of 6-hour Erastin treatment at 37°C, 5% CO2 incubator. Fluorescence intensities were measured using fluorescence microscopy equipped with a FITC filter.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.