Cell Meter™ Fluorimetric Cellular Lipid Peroxidation Assay Kit

Catalog number: 22906
Unit size: 200 Tests

Component	Storage	Amount
Component A: Lipoxite™ R590/G525 (500X)	Freeze (<-15 °C), Minimize light exposure	50 uL
Component B: HHBS	Refrigerate (2-8 °C)	50 mL
Component C: 3% H2O2 (4000X, ~ 1M)	Refrigerate (2-8 °C)	100 µL

OVERVIEW

Lipid peroxidation is the oxidative degradation of cellular lipid by reactive oxygen species (ROS). This process can lead to not only disruption of the cellular membrane integrity, but also inactivation of membrane-bound receptors. It is one of the main causes of free radical-mediated damages in cells. Cell Meter™ Fluorimetric Cellular Lipid Peroxidation Assay Kit provides a sensitive method for monitoring lipid peroxidation. The kit uses our sensitive ratiometric lipid peroxidation sensor, Lipoxite™ R590/G520 that changes its red fluorescence from red to green upon peroxidation by ROS in cells, this peroxidation-dependent shift enables the ratiometric measurement of lipid peroxidation. Our kit includes H2O2 as a positive control treatment to induce lipid peroxidation.

AT A GLANCE

Protocol summary

1. Plate cells at desired confluency.
2. Treat cells with compound of interest and incubate.
3. Add Lipoxite™ R590/G525.
4. Remove media, and wash with PBS.
5. Analyze sample by fluorescence microscope or flow cytometer through FITC/TRITC or FITC/PE channels.

Important

Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	490 nm (FITC) and 545 nm (TRITC)
Emission:	530 nm (FITC) and 600 nm (TRITC)
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC and TRITC channels
Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm, 575/26 nm filter
Instrument specification(s):	FITC channel

PREPARATION OF WORKING SOLUTION

Prepare 10X working solution of Lipoxite™ R590/G525 by making 1:50 dilution of 500X Lipoxite™ R590/G525 (Component A) into HHBS (Component B)

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Grow cells at desired density and incubate overnight in a humidified chamber at 37°C with 5% CO_2.
2. Treat cells with test compounds as desired.

   Note For a positive control, add hydrogen peroxide (Component C) to the cells at a final concentration of ~250 µM (1X) for 30 minutes.
3. Add 10X Lipoxite™ R590/G525 to the cells at a final concentration of 1X (for example add 10 uL to 90 uL of the cells).
4. Incubate the cells for 30 min at 37°C with 5% CO_2 cell incubator.
5. Remove media and wash cells with HHBS (Component B) or DPBS for three times.
6. Monitor fluorescence of cells with a fluorescence microscope or flow cytometer through FITC/TRITC or FITC/PE channels within 2 hours of staining.

EXAMPLE DATA ANALYSIS AND FIGURES

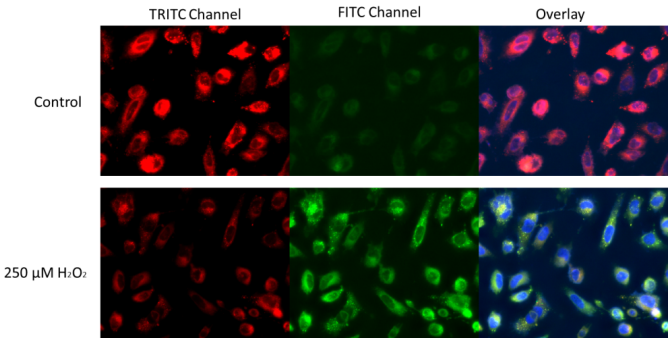

Figure 1. HeLa cells were stained with 1X Lipoxite™ R590/G525 for 30 mins in complete growth medium at 37°C. For H_2O_2 treatment, approximately 250 µM of H_2O_2 were added to the cells and incubated for 30 mins. The cells were then incubated with 1X Lipoxite™ R590/G525, and stained with Hoechst 33342 during the last 10 mins of incubation. The cells were washed 3 times with HHBS and imaged with a Keyence fluorescent microscope. With H_2O_2 treatment, a clear shift of fluorescence signal of red to green was observed.

DISCLAIMER

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