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ab243377 Lipid Peroxidation Assay Kit (Cell-based)

View Lipid Peroxidation Assay Kit (Cell-based) datasheet: www.abcam.com/ab243377

For the measurement of lipid peroxidation in cultured mammalian cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Lipid Peroxidation Assay Kit (Cell-based) (ab243377) uses a sensitive ratiometric Lipid Peroxidation Sensor that changes its 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 H_2O_2 as a positive control treatment to induce lipid peroxidation.

Grow cells at desired density and incubate overnight in a humidified chamber at 37°C with 5% CO_2 .

Treat cells with test compounds or H_2O_2 (positive control).

Add Lipid Perixidation Sensor to the cells.

Incubate the cells for 30 minutes at 37°C with 5% CO_2 cell incubator.

Wash cells 3 times with HHBS (or DPBS).

Monitor fluorescence of cells with a fluorescence microscope or flow cytometer through FITC/TRITC or FITC/PE channels within 2 hours of staining.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Lipid Peroxidation Sensor	50 µL	-20°C
HHBS	50 mL	+4°C
3% H_2O_2 (1 M, 4000X)	100 µL	+4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microscope capable of reading at Ex/Em = 490 nm (FITC)/530 nm (FITC) and 545 nm (TRITC)/600 nm (TRITC)
- Flow cytometer with FITC and PE channels (Ex = 488 nm and Em = 530 nm (FITC) or 572 nm (PE))
- Cells of interest

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Prepare only as much reagent as is needed on the day of the experiment.

5.1 Lipid Peroxidation Sensor:

Prepare a 10X working solution by diluting the 500X stock solution 1:50 in HHBS.

5.2 HHBS:

Ready to use as supplied.

5.3 H_2O_2 (1 M, 4000X):

Ready to use as supplied.

6. Sample Preparation

Grow cells of interest under standard conditions.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all controls and samples in duplicate.

7.1 Grow cells at desired density and incubate overnight in a humidified chamber at 37°C with 5% CO_2 .

7.2 Treat cells with test compounds as desired.

ΔNote: For a positive control, add hydrogen peroxide to the cells at a final concentration of ~250 µM (1X) for 30 minutes.

7.3 Add Lipid Peroxidation Sensor the cells at a final concentration of 1X (for example add 10 µL to 90 µL of cells).

7.4 Incubate the cells for 30 min at 37°C with 5% CO_2 cell incubator.

7.5 Remove media and wash cells with HHBS or DPBS three times.

7.6 Monitor fluorescence of cells with a fluorescence microscope or flow cytometer through FITC/TRITC or FITC/PE channels within 2 hours of staining.

8. Data Analysis

Monitor fluorescence of cells with a fluorescence microscope or flow cytometer through FITC/TRITC or FITC/PE channels within 2 hours of staining, as described in Section 7.6.

9. Typical Data

Data provided **for demonstration purposes only**.

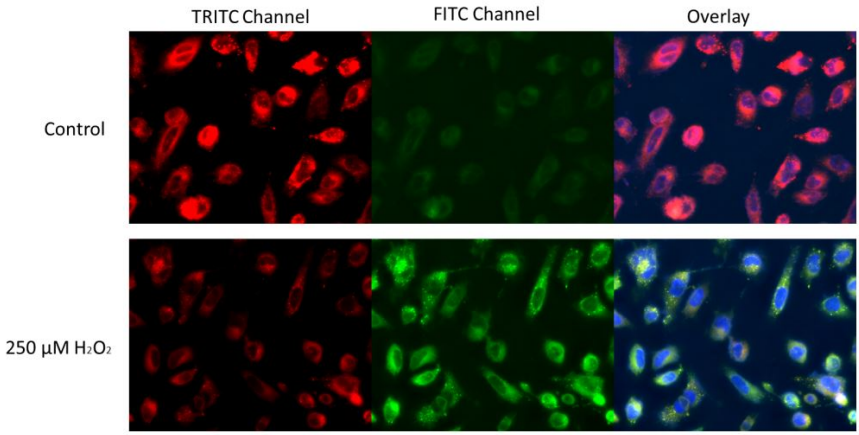

Figure 1. HeLa cells were stained with 1X Lipid Peroxidation Sensor for 30 minutes in complete growth medium at 37°C. For H_2O_2 treatment, approximately 250 µM H_2O_2 was added to the cells and incubated for 30 minutes. The cells were then incubated with 1X Lipid peroxidation Sensor, and stained with Hoechst 33342 during the last 10 minutes 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.

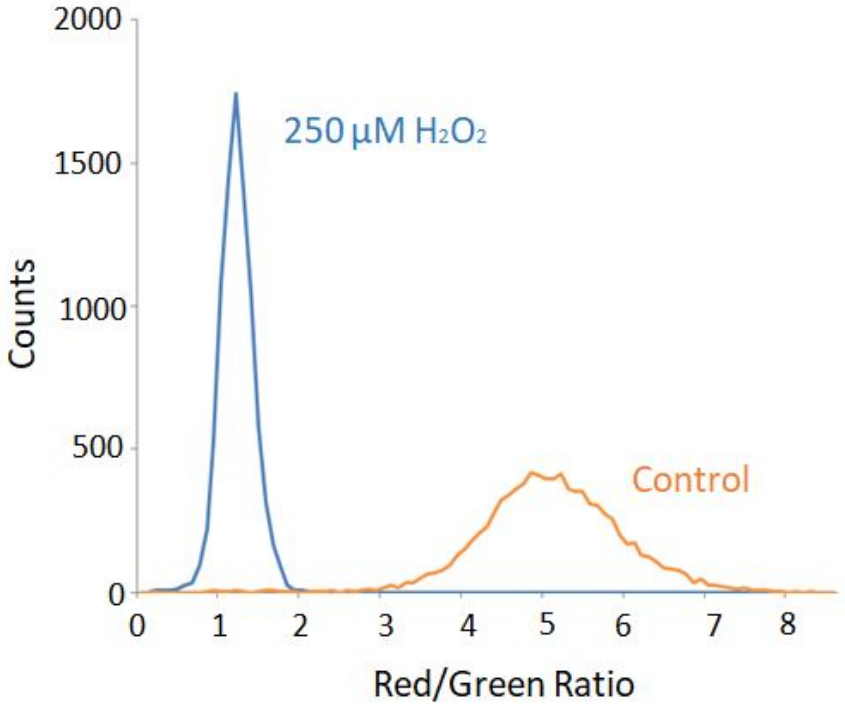

Figure 2. Jurkat cells were stained with 1X Lipid Peroxidation Reagent for 30 minuts in complete growth medium at 37°C. For H_2O_2 treatment, approximately 250 µM H_2O_2 was added to the cells and incubated for 30 minutes. The cells were then incubated with 1X Lipid Peroxidation Sensor, and analyzed with a flow cytometer through FITC (488/530 nm) and PE (488/572 nm) channels. The data are represented as the ratios of red (PE)/green (FITC) fluorescence intensities. The ratio of red/green decreases in H_2O_2 treated cells indicating the presence of H_2O_2-induced lipid peroxidation.

10. Notes

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