

Version 1 Last updated 13 September 2018

ab239701 Hydrogen Peroxide Assay Kit (Cell-based, Orange)

For the identification of hydrogen peroxide in adherent cells.

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

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1. Overview

Hydrogen Peroxide Assay Kit (Cell-based, Orange) (ab239701) uses a dye that reacts with intracellular hydrogen peroxide to produce an orange color and fluorescence, which is proportional to the concentration of intracellular hydrogen peroxide.

The kit allows for screening/studying/characterizing stimulators/inhibitors that affect intracellular levels of hydrogen peroxide.

2. Protocol Summary

Seed 2-3 x 10⁴ cells/well in desired media. Grow overnight in 37°C incubator containing 5% CO₂.



On day two, treat cells with compounds of interest in 100 µL media.



Dilute staining dye 1/1000 in assay buffer just before use.



Add 20 µL of diluted staining dye per well. Incubate in the dark at 37°C.



Remove media containing staining dye without disturbing the cells. Gently wash the cells 2-3 times with 100 µL assay buffer.



Examine cells using light and fluorescence microscope (Ex/Em = 543 nm/545-750 nm).

3. 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.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C immediately upon 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.
- Please note that the staining dye is light sensitive.

Item	Quantity	Storage condition
Assay Buffer	100 mL	-20°C
Staining Dye	10 µL	-20°C

5. Materials Required, Not Supplied

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

- 96-well plate
- Light and fluorescence microscope with Ex/Em = 543/545-750 nm
- 37°C Incubator with 5% CO₂

6. Reagent Preparation

- Equilibrate staining dye to room temperature (18-25°C) prior to use and the assay buffer to 37°C before use.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Both reagents are ready to use.

7. Assay Procedure

- This kit can only work on freshly prepared samples.
- We recommend that you assay all samples in duplicate.

7.1 Cell Culture and Staining:

- 7.1.1 Seed $2-3 \times 10^4$ cells/well in a 96-well plate in desired media. Grow cells overnight in 37°C incubator containing 5% CO₂.
- 7.1.2 On day two, treat cells with compounds of interest in 100 µL media. As a control, we recommend treating cells with vehicle alone.
- 7.1.3 Dilute staining dye 1/1000 in assay buffer just before use. Dilute as much as required.
- 7.1.4 Add 20 µL of diluted staining dye per well. Incubate in the dark for 24 hrs or desired time period at 37°C.
- 7.1.5 Carefully remove the media containing staining dye using a pipette, without disturbing the cells. Gently wash the cells 2-3 times with 100 µL assay buffer.

7.2 Detection:

- 7.2.1 Examine cells using light and fluorescence microscope (Ex/Em = 543 nm/545-750 nm). Acquire several images per well for analysis.
- 7.2.2 Since staining dye photo-bleaches very rapidly, we recommend analyzing samples immediately.

8. Typical Data

Typical data provided for demonstration purposes only.

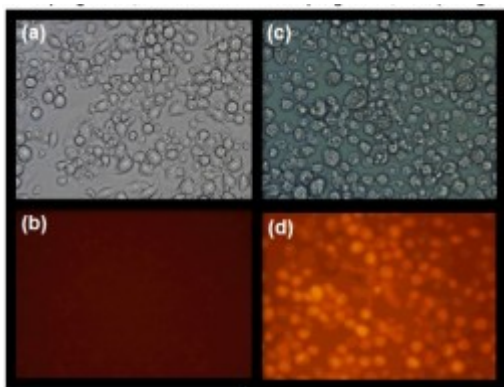


Figure 1. Hydrogen Peroxide staining of Macrophage cells (J774A.1). Macrophage cells were cultured overnight and next day treated with LPS (10 mg/ml) or vehicle control for 24 hrs in the presence of staining dye. Light and fluorescence images of cells were taken using microscope. (a) and (b) are control cells, and (c) and (d) are cells treated with LPS (10 mg/ml). Treatment with LPS caused increased production of intracellular hydrogen peroxide in cells, which is demonstrated by increase fluorescent signal.

10. Notes

Technical Support

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