FOR RESEARCH USE ONLY!

Intracellular Hydrogen Peroxide Detection Kit (cell-based)

(Catalog # K204-200; 200 assays; Store at -20°C)

I. Introduction:

Hydrogen Peroxide (H_2O_2) is a reactive oxygen species and by-product of oxygen metabolism. H_2O_2 serves as a key regulator for a number of oxidative stress related pathways and is linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, and a number of neurodegenerative diseases. BioVision's Intracellular Hydrogen Peroxide Detection kit 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. This kit provides a simple, easy to perform, histochemical method for identification of intracellular H_2O_2 in the samples. The kit provides sufficient reagents for 200 assays for 96-well, 96 assays for 24-well and 18 assays for 6-well plate.

II. Application:

- Screening/studying/characterizing stimulators/inhibitors that affect intracellular levels of hydrogen peroxide.

III. Sample Type:

- Adherent cells

IV. Kit Contents:

Components	K204-200	Cap Code	Part Number
Assay Buffer	100 ml	NM	K204-200-1
Staining Dye	10 µl	Red	K204-200-2

V. User Supplied Reagents & Equipment:

- 6-well, 12-well, 24-well or 96-well plate
- 37°C Incubator with 5% CO_2
- Light and fluorescence microscope with Ex/Em = 543/545-750 nm.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Read entire protocol before performing the assay. Open all reagents under sterile conditions (e.g. cell culture hood).

- **Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C before use.
- **Staining Dye:** Light sensitive, do not expose to intense light. Ready to use. Store at -20°C.

VII. Intracellular H_2O_2 Detection Protocol:

This protocol is for a 96-well plate. Adjust the volume according to the plate size.

1. Cell Culture and Staining:

a. Seed 2-3 x 10^4 cells/well in a 96-well plate in desired media. Grow cells overnight in 37°C incubator containing 5% CO_2. On day two, treat cells with compounds of interest in 100 µl media. As a control, we recommend treating cells with vehicle alone.

b. Dilute Staining Dye 1:1000 in Assay Buffer just before use. Dilute as much as required. Add 20 µl of diluted Staining Dye per well. Incubate in dark for 24 hrs or desired time period at 37°C. 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.

Note: We recommend treating cells with compound of interest in the presence of staining dye and adding 100 µl of diluted Staining Dye per well of 24-well and 500 µl per well of 6-well plate.

2. Detection: Examine cells using light and fluorescence microscope (Ex/Em = 543 nm/545-750 nm). Acquire several images per well for analysis.

Note: Since Staining Dye photo-bleaches very rapidly, we recommend analyzing samples immediately.

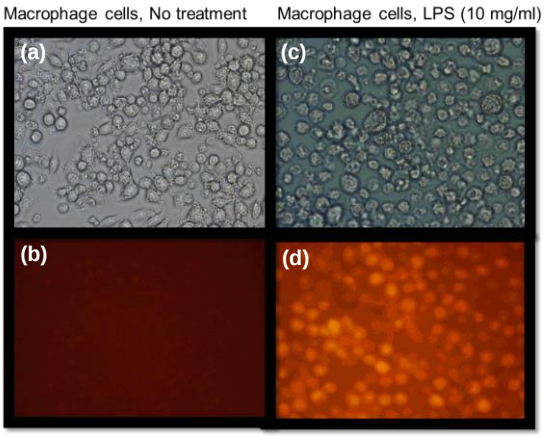

Figure: Macrophage cells (J774A.1) Hydrogen Peroxide staining: Macrophage cells were cultured overnight and next day treated with LPS (10 mg/ml) or vehicle control for 24 hrs in the presence of Staining Dye. Light and fluorescence images of cells were taken using Nikon TiE microscope. (a) and (b) are 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.

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