BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Peroxide Assay Kit (Cat #: A-113)

COMPONENTS: Peroxide Assay Solution-I- 0.5 ml, store at 4ºC (for 250 wells)
Peroxide Assay Solution-II- 50 ml, store at 4ºC
4 mM H_2O_2 Standard- 0.5 ml, store at 4ºC

PRODUCT DESCRIPTION: Major reactive oxygen species (ROS) include hydrogen peroxide, superoxide, and hydroxyl radical. The Peroxide Assay Kit is based on peroxide-mediated oxidation of Fe^{+2} to Fe^{+3} in the presence of the xylenol orange (Free Radical Res. 37:1209-1213, 2003), which can be conveniently quantified by spectroscopy. Molar extinction coefficient of xylenol orange-Fe^{+3} complex is 1.5 x $10^4/M^{-1}cm^{-1}$. As low as 0.2 μM peroxides can be detected using the assay kit in 30 min. The kit is compatible with serum/plasma and urine samples. The assay kit is stable for at least 1 year if stored and handled properly.

PROTOCOLS

H_2O_2 STANDARDS

Dilute the 4 mM H_2O_2 Standard with dH_2O 100-fold to 40 μM, e.g., 198 μl dH_2O + 2 μl 4 mM H_2O_2. Perform 1:1 serial dilution to generate 20 μM, 10 μM, and 5 μM H_2O_2. Store the H_2O_2 standard set at 4℃.

PREPARATION OF FRESH WORKING SOLUTION

Calculate the amount of working solution required for each assay (0.2 ml per well). Prepare a fresh enough working solution by mixing one part of Peroxide Assay Solution-I and 100 parts of Peroxide Assay Solution-II. Mix well and use immediately. Discard unused portion after each assay.

SAMPLE PREPARATION

Liquid sample: Culture medium, serum/plasma, and urine can be used for the assay. Samples containing insoluble materials should be clarified by centrifugation prior to assay. Store samples at -20ºC.

Cell sample: Pellet at least ~10^6 normal saline-washed cells and remove liquid completely. Freeze-thaw cells once. Bring up cells in 50 - 100 μl ice-cold dH_2O by thorough pipetting. The use of a homogenizer for cell lysis can be helpful. Centrifuge lysate in a microfuge at ~13,000 rpm at 4℃ for 5 min. Harvest supernatant and store at -80℃.

Tissue sample: Tissue should be washed with saline thoroughly to remove blood cells, which can cause assay variation. Tissue (20 mg) is homogenized in 0.2 ml of ice-cold dH_2O. Centrifuge lysate in a microfuge at ~13,000 rpm at 4℃ for 5 min. Harvest supernatant and store at -80℃.

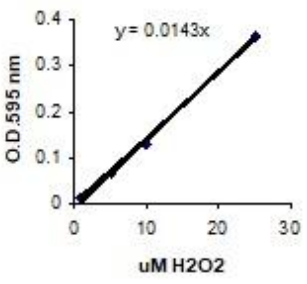
ASSAY

1. Add 40 μl of H_2O_2 standards and samples to a plain (uncoated) 96-well plate.

2. Add 0.2 ml freshly prepared working solution to each well. Mix contents by gentle agitation for 10 sec. Cover plate and incubate at room temperature for 30 min.

3. Read absorbance (O.D.) at 595 nm using a plate reader.

4. Plot H_2O_2 standards vs. $O.D._{595\ nm}$. Generate a trend line equation on chart. Calculate sample peroxide concentration using the derived equation (x = peroxide concentration in μM; y = sample $O.D._{595\ nm}$). A new plot should be generated for each assay.



Note:

Avoid skin contact with Peroxide Assay Solution-I, which contains H_2SO_4 (sulfuric acid). Please contact us or visit the product webpage for SDS information on sulfuric acid, hydrogen peroxide, and xylenol orange.