Hydrogen Peroxide Assay Kit

(Catalog #K265-200; 200 reactions; Store kit at –20°C)

I. Introduction:

Hydrogen Peroxide is a reactive oxygen metabolic byproduct that serves as a key regulator for a number of oxidative stress-related states. Functioning through NF-KB and other factors, hydroperoxide-mediated pathways have been linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, neuro-degenerative diseases, Down's syndrome and immune system diseases. amsbio's Hydrogen Peroxide Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric and fluorometric assay for measuring H_2O_2 in biological samples. In the presence of Horse Radish Peroxidase (HRP), the OxiRed Probe reacts with H_2O_2 to produce product with color (λ_{max} = 570 nm) and red-fluorescent (Ex/Em=535/587 nm). The kit can perform 200 reactions by fluorometric method or 100 reactions by colorimetric method. The detection limit can be as low as 2 pmol per assay (or 40 nM concentration) of H_2O_2 in the sensitive fluorometric assay.

II. Kit Contents:

Components	K265-200	Cap Code	Part No.
H_2O_2 Assay Buffer	25 ml	WM	K265-200-1
OxiRed™ Probe	1 vial	Red	K265-200-2
Dimethylsulfoxide (DMSO, anhydrous)	0.4 ml	Brown	K265-200-3
HRP	1 vial	Green	K265-200-4
H_2O_2 Standard (0.88M)	0.1 ml	Yellow	K265-200-5

III. Storage and Handling:

Warm the assay buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

OxiRed™ Probe: Dissolve in 220 µl DMSO (provided), pipeting up and down. The OxiRed™ Probe solution is stable for 1 week at 4°C and 1 month at -20°C.

HRP: Dissolve in 220 µl assay buffer, pipetting up and down. The HRP solution is stable for 1 week at 4°C and 1 month at -20°C.

V. Hydrogen Peroxide Assay:

1. Sample Preparations:

Collect cell culture supernatant, serum, plasma, urine and other biological fluids (contains 0.8-6 µM H_2O_2). Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Remove particulate pellet. Samples, especially those such as culture medium, tissue lysate or plasma should be filtered through a 10Kd mw spin filter (amsbio, Cat # 10kc-20) to remove all proteins then kept at -80°C for storage. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C. Avoid repeated freeze-thaw cycles. Add 2-50 µl samples into each well, bring the volume to 50 µl with assay buffer.

2. H_2O_2 Standard Curve:

For the Colorimetric Assay: Dilute 10 µl 0.88M H_2O_2 standard into 870 µl dH_2O to generate 10 mM H_2O_2 standard, then dilute 10 µl 10 mM H_2O_2 standard into 990 µl dH2O to generate 0.1 mM H_2O_2 standard. Add 0, 10, 20, 30, 40, 50 µl of the 0.1 mM H_2O_2 standard into 96-well plate in duplicate to generate 0, 1, 2, 3, 4, 5 nmol/well H_2O_2 standard.

For the Fluorometric Assay: Dilute 100 µl of the 0.1 mM H_2O_2 standard into 900 µl dH2O to generate 10 µM H_2O_2 Standard. Add 0, 10, 20, 30, 40, 50 µl of the 10 µM H_2O_2 standard into 96-well plate in duplicate to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well H_2O_2 standard.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

Colorimetric Assay	Fluorometric Assay
46 µl Assay Buffer	48 µl Assay Buffer
2 µl OxiRed™ Probe solution	1 µl OxiRed™ Probe solution
2 µl HRP solution	1 µl HRP solution

Add 50 µl of the Reaction Mix to each test samples and H_2O_2 standards. Mix well. Incubate at room temperature for 10 min.

*For a more sensitive assay, you can dilute the standard 10 fold further, decrease OxiRed™ amount to 0.2 µl and HRP amount to 0.4 µl per well, it will decrease the fluorescence background and detects as low as 2 pmol/well (or 40 µM concentration) H_2O_2.

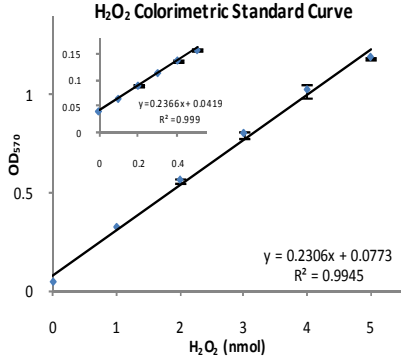
4. Measure OD(570 nm) or fluorescence (Ex/Em = 535/587 nm) in a micro-plate reader.

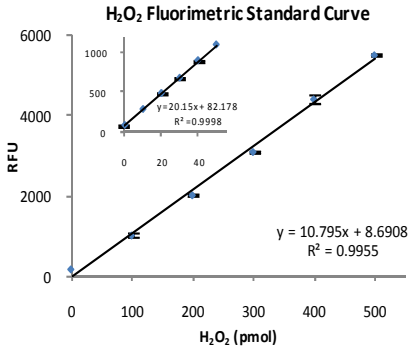
5. Calculation:

Correct background by subtracting the value derived from the 0 nmol H_2O_2 control from all sample and standard readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot the H_2O_2 standard curve. Apply your sample readings to the standard curve. H_2O_2 concentrations of the test samples can then be calculated, where:

C=Sa/Sv (pmol/µl or µM),

Sa is the sample amount from your standard curve (in pmol),
Sv is sample volume (µl).





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