Product Information

Hydrogen Peroxide Assay Kit

I. Kit Contents:

Components	K2110-200 200 assays	Cap Color	Part Number
H_2O_2 Assay Buffer	25 ml	WM	K2110-C-1
OxiRed™ Probe (in anhydrous DMSO)	0.2 ml	Red	K2110-C-2
HRP	1 vial	Green	K2110-C-3
H_2O_2 Standard (0.88 M)	0.1 mg	Yellow	K2110-C-4

II. Introduction:

Hydrogen Peroxide plays an important role in various oxidative stress-associated conditions. Hydroperoxide-mediated NF-κB is related to asthma, neurodegenerative disease, atherosclerosis and immune system etc. Hydrogen Peroxide Assay Kit provides a sensitive, easy and direct way 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 (λmax = 570 nm) and red-fluorescent (Ex/Em = 535/587 nm). 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.

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: Ready to use as supplied. Briefly warm at 37℃ to melt frozen DMSO. The OxiRed™ Probe solution is stable for 1 week at 4℃ and 1 month at -20℃.

HRP: Dissolve in 220 µl Assay Buffer, pipetting up and down. The HRP solution is stable for 1 week at 4℃ and 1 month at -20℃.

V. Hydrogen Peroxide Assay Protocol:

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 10 kDa MW spin filter to remove all proteins then kept at -80℃ for storage. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80℃. 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 0.88 M H_2O_2 standard into 870 µl dH_2O to generate a 10 mM H_2O_2 standard, then dilute 10 µl of the 10 mM H_2O_2 standard into 990 µl dH_2O to generate a 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 dH_2O to generate a 10 µM H_2O_2 Standard. Add 0, 10, 20, 30, 40, 50 µl of the 10 µM H_2O_2 standard into a 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
Assay Buffer	46 µl	48 µl
OxiRed™ Probe solution	2 µl	1 µl
HRP solution	2 µl	1 µl

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) H_2O_2.

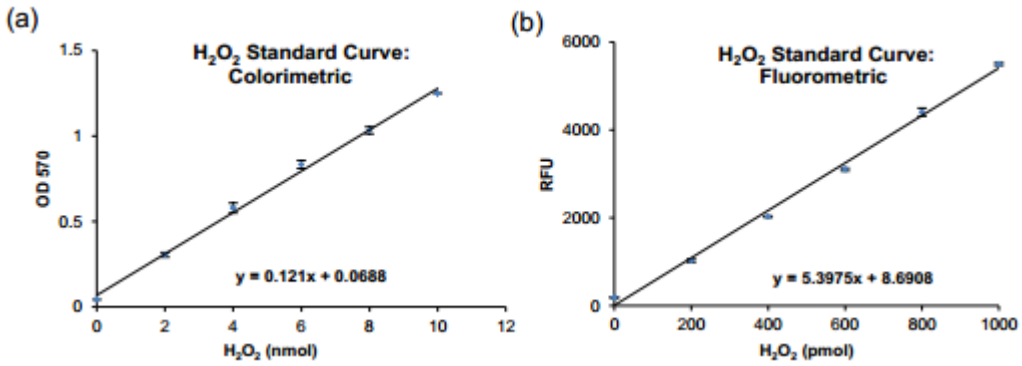
4. Measure OD (570 nm) or fluorescence (Ex/Em = 535/587 nm) in a micro-plate reader.

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,

C=Sa/Sv (pmol/µl or µM),

Where: Sa is the sample amount from your standard curve (in pmol),

Sv is sample volume (µl).



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample	• Refer data sheet for details about incompatible samples • Use the reaction buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes);

	• Use of old or inappropriately stored samples	observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and re-suspend all components before preparing the reaction mix • Avoid pipetting small volumes • Pipette gently against the wall of the tubes • Prepare a master reaction mix whenever possible • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com