Hydrogen Peroxide Assay Kit (Catalog #K265-200; 200 reactions; Store kit at –20oC)

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, neurodegenerative diseases, Down's syndrome and immune system diseases. BioVision's Hydrogen Peroxide Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric and fluorometric assay for measuring H2O2 in biological samples. In the presence of Horse Radish Peroxidase (HRP), the OxiRed Probe reacts with H2O2 to produce product with color (λmax = 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 H2O2 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. 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 dH_2O 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 dH_2O 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:

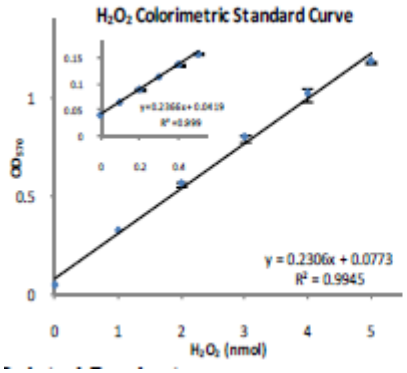
Colorimetric Assay	**Fluorometric Assay**
46 µl Assay Buffer	48 µl Assay Buffer
2 µl OxiRed™ Probe solution	1 µl OxiRed™ Probe solution
2 µl HRP solution	1 µl HRP solution

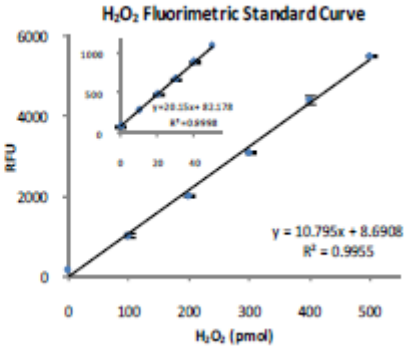
Add 50 µl of the Reaction Mix to each test samples and H_2O_2 standards. Mix well.
Incubate at room temperature for 10 min.
*For a more sensitive assay, you can dilute the standard 10 fold further, decrease OxiRed™ amount to 0.2 µl and HRP amount to 0.4 µl per well, it will decrease the fluorescence 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:

C=Sa/Sv (pmol/µl or µM),
where Sa is the sample amount from your standard curve (in pmol), Sv is sample volume (µl).





VI. Related Products:

Glutathione Reductase Assay Kit Glutathione Peroxidase Assay Kit
Colorimetric Glutathione Detection Kit ApoGSH Glutathione Detection KitGlutathione Kit (GSH, GSSG and Total) GST Fluorometric Assay Kit
GST Colorimetric Assay Kit Triglyceride Assay Kit
Acid Phosphatase Assay Kit ADP/ATP Ratio Assay Kit
Phosphate Fluorescence Assay Kit Phosphate Colorimetric Assay Kit
NAD/NADH Quantification Kit NADP/NADPH Quantitation Kit
Pyruvate Assay Kit Lactate Assay Kit/ II
Ammonia Assay Kit Glutamate Assay Kit
Glucose Assay Kit Fatty Acid Assay Kit

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GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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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