

# 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- $\kappa$ B 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  $H_2O_2$  in biological samples. In the presence of Horse Radish Peroxidase (HRP), the OxiRed Probe reacts with  $H_2O_2$  to produce product with color ( $\lambda_{max} = 570$  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  $\mu$ l 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  $\mu$ l 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  $\mu$ M  $H_2O_2$ ). 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  $\mu$ l samples into each well, bring the volume to 50  $\mu$ l with assay buffer.

### 2. $H_2O_2$ Standard Curve:

**For the Colorimetric Assay:** Dilute 10  $\mu$ l 0.88M  $H_2O_2$  standard into 870  $\mu$ l dH<sub>2</sub>O to generate 10 mM  $H_2O_2$  standard, then dilute 10  $\mu$ l 10 mM  $H_2O_2$  standard into 990  $\mu$ l dH<sub>2</sub>O to generate 0.1 mM  $H_2O_2$  standard. Add 0, 10, 20, 30, 40, 50  $\mu$ l 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  $\mu$ l of the 0.1 mM  $H_2O_2$  standard into 900  $\mu$ l dH<sub>2</sub>O to generate 10  $\mu$ M  $H_2O_2$  Standard. Add 0, 10, 20, 30, 40, 50  $\mu$ l of the 10  $\mu$ M

$H_2O_2$  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  $\mu$ l Reaction Mix:

#### Colorimetric Assay

46  $\mu$ l Assay Buffer  
2  $\mu$ l OxiRed™ Probe solution  
2  $\mu$ l HRP solution

#### Fluorometric Assay

48  $\mu$ l Assay Buffer  
1  $\mu$ l OxiRed™ Probe solution  
1  $\mu$ l HRP solution

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

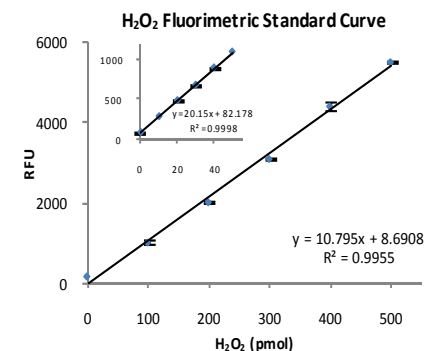
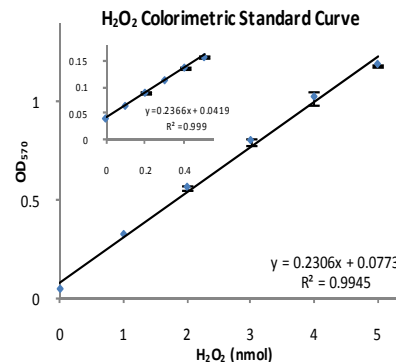
\*For a more sensitive assay, you can dilute the standard 10 fold further, decrease OxiRed™ amount to 0.2  $\mu$ l and HRP amount to 0.4  $\mu$ l per well, it will decrease the fluorescence background and detects as low as 2 pmol/well (or 40  $\mu$ M concentration)  $H_2O_2$ .

**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:

$$C = Sa/Sv \text{ (pmol/}\mu\text{l or } \mu\text{M)},$$

where Sa is the sample amount from your standard curve (in pmol),  
Sv is sample volume ( $\mu$ l).



## VI. Related Products:

Glutathione Reductase Assay Kit  
Colorimetric Glutathione Detection Kit  
KitGlutathione Kit (GSH, GSSG and Total)  
GST Colorimetric Assay Kit  
Acid Phosphatase Assay Kit  
Phosphate Fluorescence Assay Kit  
NAD/NADH Quantification Kit  
Pyruvate Assay Kit  
Ammonia Assay Kit  
Glucose Assay Kit

Glutathione Peroxidase Assay Kit  
ApoGSH Glutathione Detection  
GST Fluorometric Assay Kit  
Triglyceride Assay Kit  
ADP/ATP Ratio Assay Kit  
Phosphate Colorimetric Assay Kit  
NADP/NADPH Quantitation Kit  
Lactate Assay Kit/ II  
Glutamate Assay Kit  
Fatty Acid Assay Kit