Hydrogen Peroxide

Microplate Assay Kit

User Manual

Catalog # CAK1012

(Version 3.1G)

Detection and Quantification of Hydrogen Peroxide (H_2O_2) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....2
II. KIT COMPONENTS.....3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....3
IV. SAMPLE PREPARATION.....4
V. ASSAY PROCEDURE.....5
VI. CALCULATION.....6
VII. TYPICAL DATA.....7
VIII. TECHNICAL SUPPORT.....7
IX. NOTES.....7

I. INTRODUCTION

Hydrogen Peroxide (H_2O_2) is a reactive oxygen metabolic byproduct that serves as a key regulator for a number of oxidative stress-related states. Functioning through NF-kappaB 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. Hydrogen Peroxide Microplate Assay Kit provides a simple and direct procedure for measuring Hydrogen Peroxide levels in a variety of samples. The assay is initiated with the enzymatic hydrolysis of H_2O_2 by Catalase. The reaction product can react with the dye reagent, and measured at a colorimetric readout at 570 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Dye Reagent Diluent	1 ml x 1	4 °C
Standard (400 µmol/L)	1 ml x 1	4 °C, keep in dark
Technical Manual	1 Manual	

Note:

Dye Reagent: Warm Dye Reagent Diluent to RT prior to use to melt frozen Dye Reagent Diluent; then add 1 ml Dye Reagent Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Enzyme: add 1 ml Reaction Buffer to dissolve before use; store at -80 °C for 1 month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagent to room temperature before use.

Add following reagents into the microplate.

Reagent	Sample	Blank	Standard
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Distilled water	--	10 µl	--
Standard	--	--	10 µl
Dye Reagent	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 10 minutes s, measured at 570 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

H_2O_2 (μmol/ml) = ($C_{Standard}$ × V_{Sample}) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / V_{Sample}

= 0.4 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

2. According to the weight of sample

H_2O_2 (μmol/g) = ($C_{Standard}$ × V_{Sample}) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})/ (V_{Sample} × W / V_{Assay})

= 0.4 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / W

3. According to the quantity of cells or bacteria

H_2O_2 (μmol/10^4) = ($C_{Standard}$ × V_{Sample}) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})/ (V_{Sample} × N / V_{Assay})

= 0.4 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / N

$C_{Standard}$: the Standard concentration, 400 μmol/L = 0.4 μmol/ml

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

V_{Sample}: the volume of sample, 0.01 ml

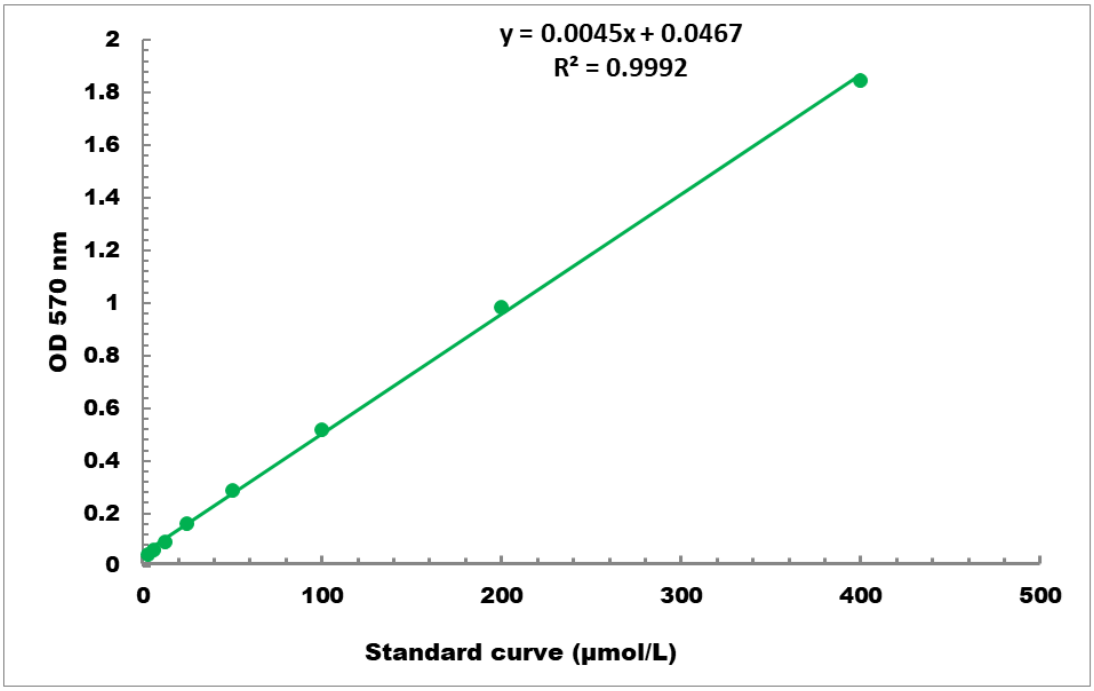
$V_{Standard}$: the volume of sample, 0.01 ml

V_{Assay}: the volume of Assay buffer, 1 ml

N: the quantity of cell or bacteria, N × 10^4

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 μmol/L - 400 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES