Hydrogen Peroxide

Microplate Assay Kit

Cat #: orb219868 (manual)

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.

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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 H2O2 by Catalase. The reaction product can react with the dry reagent, and measured at a colorimetric readout at 405 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	-20 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard (100 mmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 10 ml distilled water to dissolve before use; store at -20 °C for 1 month after reconstitution.

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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.

ASSAY PROCEDURE

Warm all reagent to room temperature before use.

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Blank	Standard
Reaction Buffer	50 µl	50 µl	50 µl
Sample	40 µl	--	--
Distilled water	--	40 µl	--
Standard	--	--	40 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, incubate at room temperature for 10 minutes, measured at 405 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.

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}

= 100 × (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})

= 100 × (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})

= 100 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / N

$C_{Standard}$: the Standard concentration, 100 mmol/L = 100 μmol/ml;

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

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.04 ml;

$V_{Standard}$: the volume of sample, 0.04 ml;

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

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

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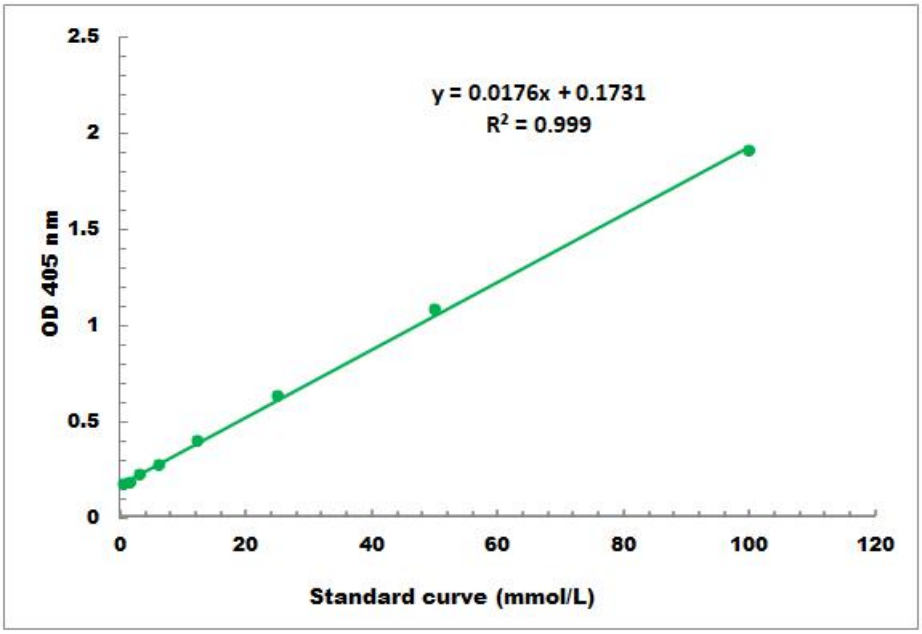
TYPICAL DATA

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



Detection Range: 0.5 mmol/L - 100 mmol/L

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