Hydrogen Peroxide Microplate Assay Kit User Manual

Catalog # ASK1012

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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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. H_2O_2 react with titanium sulfate, the products can be measured at a colorimetric readout at 415 nm.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powder x 1	4 °C
Substrate Diluent	5 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dissolution Buffer	20 ml x 1	4 °C
Standard (100 mmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 5 ml Substrate Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 415 nm
2. Acetone
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Acetone for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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 Acetone 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 serum or plasma samples

Detect directly.

Note:

1. Acetone should be precooled before use because of its volatile, grind the sample on ice.
2. Please wear disposable gloves and masks because of the high volatile of reagents in the kit.

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V. ASSAY PROCEDURE

Warm all reagent to room temperature before use.

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	200 µl	--	--
Standard	--	200 µl	--
Acetone	--	--	200 µl
Substrate	50 µl	50 µl	50 µl
Reaction Buffer	100 µl	100 µl	100 µl
Mix, centrifuged at 4000 g, 25 °C for 10 minutes, discard the supernatant.			
Dissolution Buffer	200 µl	200 µl	200 µl
Mix, keep at room temperature for 5 minutes, add 200 µl into the microplate, record absorbance measured at 415 nm.			

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VI. CALCULATION

1. According to the protein concentration of sample

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

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

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 / $V_{Acetone}$)

= 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 / $V_{Acetone}$)

= 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, 0.2 ml;

$V_{Acetone}$: the volume of Acetone, 1 ml;

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

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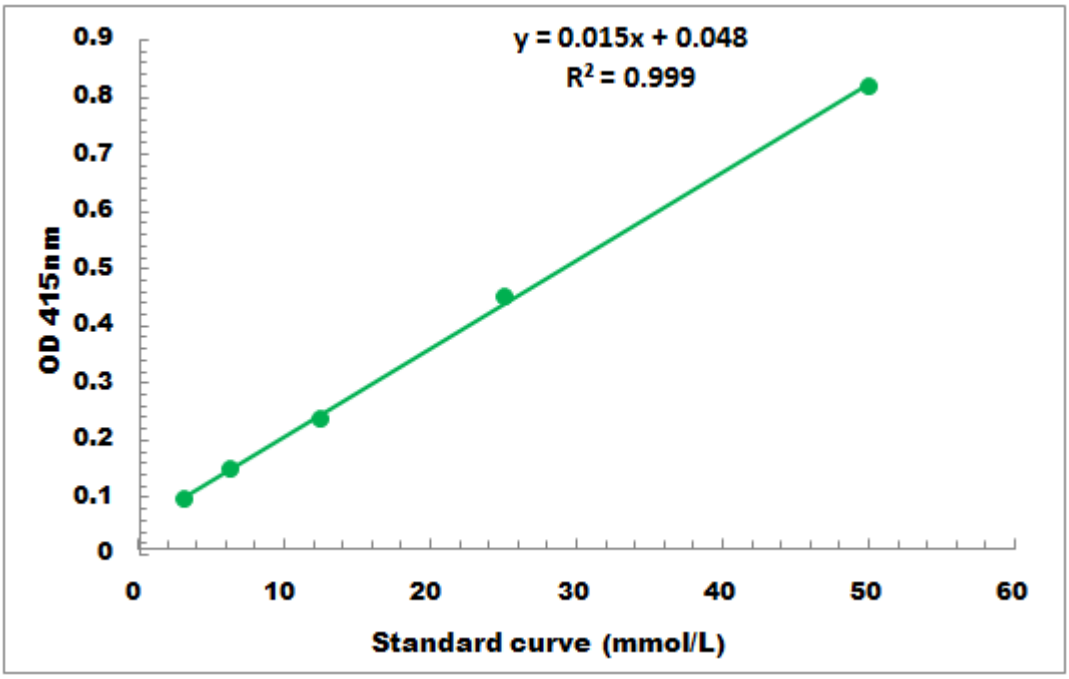
VII. TYPICAL DATA

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



Detection Range: 1 mmol/L - 100 mmol/L

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