Catalase Microplate Assay Kit

User Manual

Catalog # FTA0060

(Version 1.2F)

Detection and Quantification of Catalase (CAT) Activity 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

Catalase is an antioxidant enzyme ubiquitously present in mammalian and non-mammalian aerobic cells containing a cytochrome system. It was initially isolated from ox liver and later from blood, bacterial, and plant sources.

The enzyme contains 4 ferrihemoprotein groups per molecule. The enzyme has a molecular mass of 240 kDa. Catalase activity varies greatly between tissues. The activity is highest in the liver and kidney, and lowest in connective tissues. In eukaryotic cells the enzyme is concentrated in the subcellular organelles called peroxisomes (microbodies). Catalase catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell as a by-product of various oxidase and superoxide dismutase reactions. Hydrogen peroxide is highly deleterious to the cell and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell. It's role in oxidative stress related diseases has been widely studied.

The assay is initiated with the enzymatic hydrolysis of H_2O_2 by CAT. The reaction product can be react with the dry reagent, and measured at a colorimetric readout at 405 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	8 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard (50 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 10 ml distilled water to dissolve before use.

Positive Control: add 1 ml distilled water to dissolve before use.

III. 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

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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 20 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 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm the Substrate to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Blank	Standard	Positive Control
Substrate	80 µl	80 µl	--	80 µl
Sample	20 µl	--	--	--
Distilled water	--	20 µl		--
Positive Control	--	--	--	20 µl
Incubate at room temperature for 3 minutes.				
Standard	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, 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) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of catalase activity is defined as the enzyme decomposes 1 μmol of hydrogen peroxide per minute.

1. According to the protein concentration of sample

$$CAT\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / (V_{Sample} \times C_{Protein}) / T$$

$$= 83.3 \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / C_{Protein}$$

2. According to the weight of sample

$$CAT\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 83.3 \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / W$$

3. According to the quantity of cell or bacteria

$$CAT\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 83.3 \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / N$$

4. According to the volume of sample

$$CAT\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / OD_{Standard} / V_{Sample} / T$$

$$= 83.3 \times (OD_{Blank} - OD_{Sample}) / OD_{Standard}$$

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

$C_{Standard}$: the concentration of Standard, 50 mmol/L = 50 μmol/ml;

W: the weight of sample, g;

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

V_{Sample}: the volume of sample, 0.02 ml;

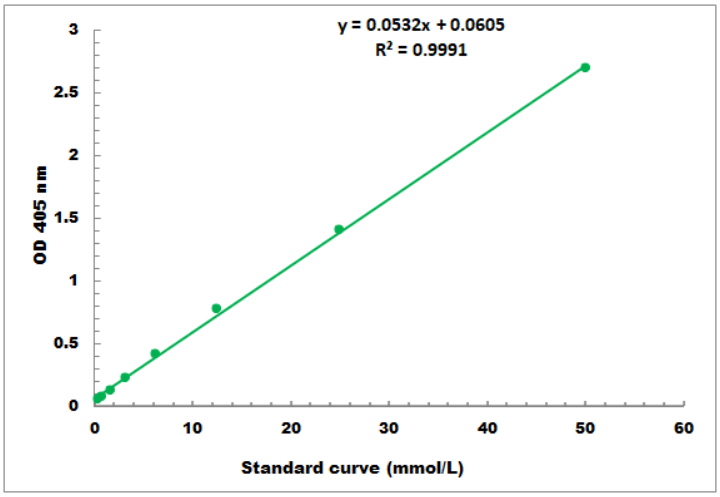
$V_{Standard}$: the volume of sample, 0.1 ml;

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

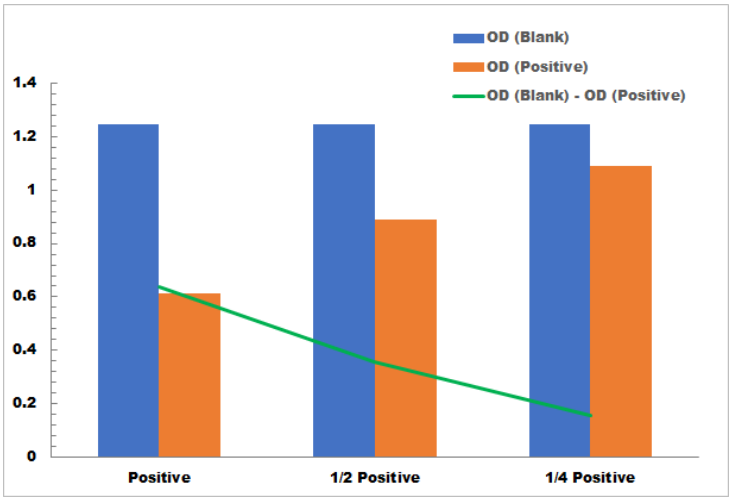
T: the reaction time, 3 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.5 mmol/L - 50 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES