Catalase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1061

(Version 1.3G)

Detection and Quantification of Catalase(CAT)Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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.

Catalase Activity Colorimetric Microplate Assay Kitprovides a simple and sensitive method for monitoring catalase activity in various samples.The assay is initiated with the enzymatic hydrolysis of H_2O_2 by catalase. The reaction product can react with the dry reagent, and measured at a colorimetric readout at 405 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 mlx 1	4 °C
Substrate(150X)	20µlx 1	4 °C, keep in dark
Stop Solution	4 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard (50 mmol/L)	1 ml x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be 50/25/12.5/6.25/3.12/ 1.56/0.78mmol/L.

Substrate: Prepare Substrate (1X) by dilutingSubstrate (150X)with Reaction Bufferfor immediate use.Keep the diluted solutions (1X) in dark and useit within 2 hours. The volumes of reagent could be enlarged according to the specified numbers of well. The table below gives some examples.

No. of wells	Substrate (150X) (µl)	Reaction Buffer (µl)
1	0.2	30
5	1	150
10	2	300
50	10	1500
100	20	3000

Dye Reagent:Add 3 ml distilled waterto dissolve before use.Store at 4°C for 1 week or -20 °C for 3-4 months.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Reaction Bufferto dissolve before use.Store at-20 °C for 3-4 months.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm theSubstrateto room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl	--	--
Sample	20 µl	--	--	--	--
Distilled water	--	20 µl	--	30 µl	130 µl
Positive Control	--	--	20 µl	--	--
Substrate(1X)	30 µl	30 µl	30 µl	--	--
Incubate at room temperature for 5 minutes.					
Standard	--		--	100 µl	--
Stop Solution	40 µl	40 µl	40 µl	40 µl	40 µl
Dye Reagent	30µl	30µl	30µl	30µl	30µl
Mix, measured at 405 nm and record the absorbance.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Control}-OD_{Sample}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times V / (V + V_{Assay})\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, mmol/L =μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

V_{Sample}: the volume of sample in assay procedure, mL

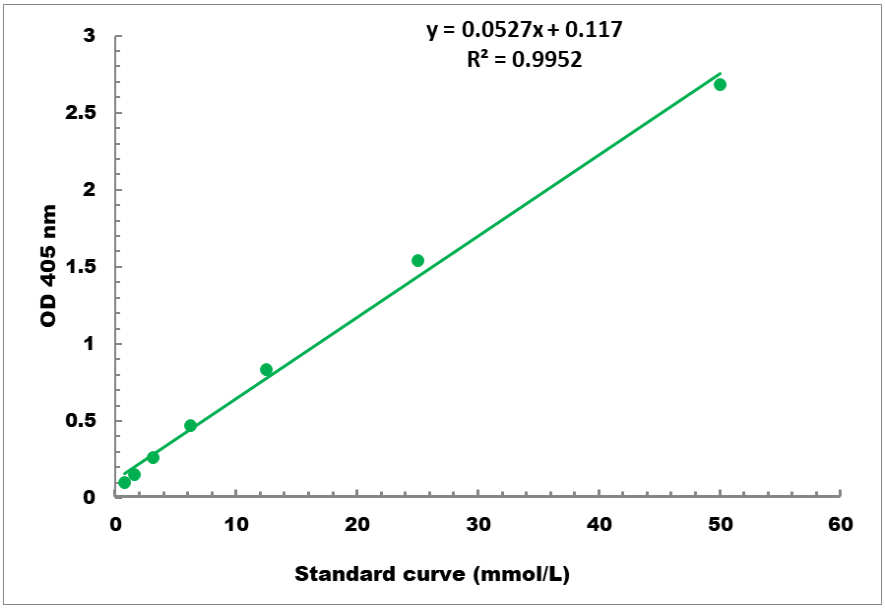
V_{Assay}: the volume of Assay Buffer in sample preparation, mL

V: the volume of sample in sample preparation, mL

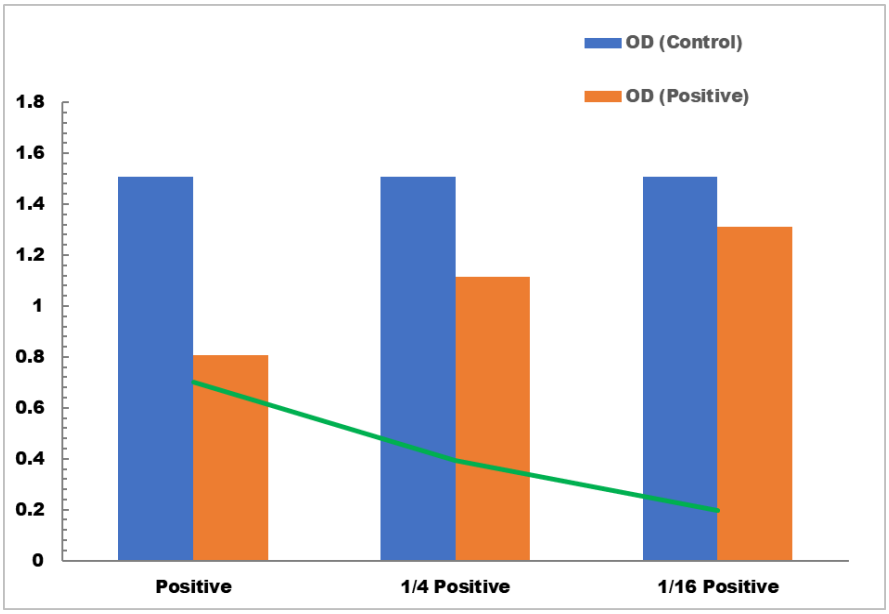
T: the reaction time, minute

VIII. TYPICAL DATA

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



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



Positive Control reaction in 96-well plate assay with decreasing theconcentration