ABS_Bio™ Catalase Activity Detection Kit

(Cat# K225-200; 200 assays; store kit at -20°C)

Introduction

Catalase is an antioxidant enzyme omnipresent in mammalian and non-mammalian cells that destroys hydrogen peroxide by dismutation. Hydrogen Peroxide is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events and intracellular pathways that have been linked to several diseases. Measurements of reactive oxygen species help to determine how oxidative stress modulates varied intracellular pathways.

The ABS_Bio™ Catalase Activity Detection Kit provides a simple, sensitive, one-step colorimetric and fluorimetric assay to detect catalase activity in various sample. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, the remaining H_2O_2 in the reaction mixture convert the colorless probe into a colored products which can be measured by spectrophotometry or fluorometry. The intensity of color, measured at 570 nm or fluorescence at Ex/Em=530/590nm, is directly proportional to the catalase activity in the sample. The kit is supplied with enough reagents for 200 tests in 96-well plate assay, linear detection range of 0.02-17 U/L. It could easily be modified for use in 384-well assay.

Kit Components (200 tests)

Assay Buffer:	30 mL	H_2O_2 Standard (0.88 M):	0.2 mL	HRP:	0.22 mL	Probe:	0.22 mL
Positive Control:	0.01 mL	Stop Solution:	5 mL				

Storage and Handling: Store kit at -20°C. Shelf Life: 12 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample & reagents preparation

Add 500 µL assay buffer to positive control tube. Aliquot and store at -20°C. Diluted positive control solution is stable for 2 months at -20°C. Add 20 µL of reconstitute positive control for the assay directly (option).

A variable dilution will be required depending on the catalase present in the sample. Clear cell culture supernatant, serum, plasma, urine, and other biological fluids can be assayed directly. Homogenize cell (10^6) or tissue (10 mg) in 200 µL cold assay buffer, and centrifugation to correct clear supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.
Transfer 20 µL of sample into a 96-well plate in duplicate (one as sample, one as sample blank control). Add 20 µL of stop solution into sample blank control well, incubate for 5 min at room temperature to inhibit sample catalase activity.

2. Catalase reaction

Fresh prepare 500 µM H_2O_2 dilution as below each time since the dilution is unstable.
Add 20 µL of fresh prepared 500 µM H_2O_2 into each sample, sample blank control and positive control wells, incubate 30 minutes at room temperature, and then add 20 µL of stop solution into sample and positive control wells.

3. Standard Preparation

Transfer 10 µL of H_2O_2 standard (0.88 M) into 870 µL dH_2O to prepare 10 mM of H_2O_2, then transfer 50 µL of 10 mM H_2O_2 into 950 µL dH_2O to generate a 500 µM H_2O_2 standard, use the following table to generate 500, 300, 150, 50 and 0 µM H_2O_2 standards.

500 µM H_2O_2 std(µL)	dH_2O(µL)	final H_2O_2 concentration (µM)
50	0	500
30	20	300
15	35	150
5	45	50
0	50	0

Transfer 20 µL of appropriate standards into the 96-well plate in duplicate. The blank control contains dH_2O only.
Add 20 µL of assay buffer and 20 µL of stop solution into each standard wells.

4. Assay reaction

Prepare enough working reagent by mixing 60 µL assay buffer, 1 µL HRP, 1 µL probe for each reaction (samples, sample blanks, positive control & standards).
Transfer 60 µL prepared working reagent into each reaction well. Tap plate to mix well. Incubate 10 min. at room temperature, protected from light.

5. Measurement

Read the optical density at 570 nm.

6. Calculation

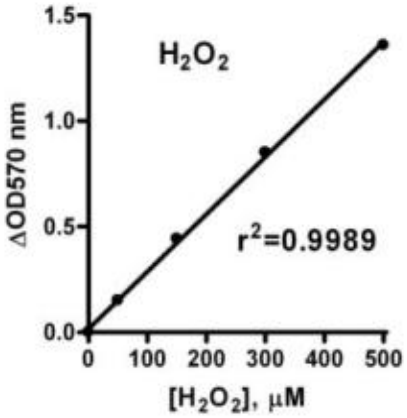
Average the duplicate OD570 nm reading for standard. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the H_2O_2 concentration of the standards. Determine the slope and calculate the catalase activity of samples.

[Catalase activity]= ($OD_{sample\ blank}$-OD_{sample}) / (Slope x *t*) x *n* (U/L)

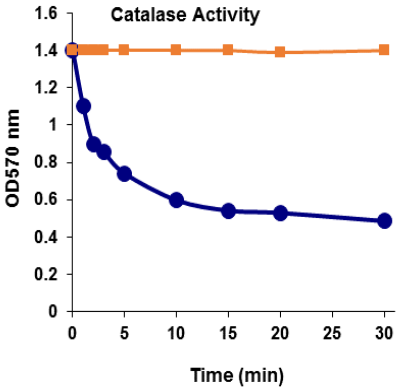
$OD_{sample\ blank}$ and OD_{sample} are optical density readings of the sample blank control and sample. Slope is determined from the standard curve. *t* is the catalase reaction time 30 mins. *n* is the sample dilution factor.

Unit Definition: One unit of catalase is the amount of enzyme decomposes 1.0 µmol of H_2O_2 per min at assay condition.

Typical Standard Curve



H_2O_2 standard in 96 wells-plate assay (colorimetric).



Catalase activity in 96 wells-plate assay.

Always run your own standard curves for calculation of results.

Fluorimetric assay has around a 10-fold sensitivity of the colorimetric assay.
The prepared H_2O_2 standard from above should be diluted 10-fold more with dH_2O to generate 50, 30, 15, 5 and 0 µM H_2O_2 standards.

Prepare enough working reagent by mixing 60 µL assay buffer, 1 µL HRP, 0.5 µL probe for each reaction. Transfer 60 µL prepared working reagent into each reaction well. Tap plate to mix well. Incubate 10 min. at room temperature, protected from light.

Read fluorescence at Ex=530 nm and Em=590 nm.

Plot the $\Delta RFU_{standard}$ against standard concentration. Determine the slope and calculate the catalase activity of samples.

[Catalase activity]= ($RFU_{sample\ blank}$-RFU_{sample})/ (Slope x *t*) x *n* (U/L)

$RFU_{sample\ blank}$ and RFU_{sample} are related fluorescence units of the sample blank and sample. Slope is determined from the standard curve. *t* is the catalase reaction time 30 mins. *n* is the sample dilution factor.

Sensitivity and Limit of Detection

The Limit of Detection was determined at 0.02 U/L, with a linear detection range up to 17 U/L in 96-well plate assay for 30 minutes reaction. Samples with values above 20 U/L should be diluted with dH_2O or assay buffer, re-assayed, and multiplied by dilution factor.

Interferences

DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM.

References

Slaughter, MR. et al. 2000, Clinical Biochemistry. 33:525–534
Sinha AK. 1972, Analytical Biochemistry. 47:389-394
Johansson LH. et al. 1988, Analytical Biochemistry. 174:331-336

Related Products:

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HRP Substrate system (#C8042)
Creatinine Detection Kit (#K148-250)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
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