EZ-Catalase Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-CAT400

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Catalase is a representative antioxidant enzyme found in most living organisms, both animals and plants. It is generally known to be involved in the decomposition of toxic substances such as H_2O_2 and reactive oxygen species (ROS) that are produced during aerobic metabolism. The decomposition activities catalyzed by catalase include both catalytic activity and peroxidatic activity. In catalytic activity, catalase facilitates the conversion of two molecules of H_2O_2 into one molecule of oxygen and two molecules of water. In peroxidatic activity, it serves as an electron donor in the depolymerization of alcohols. In the human body, catalase is abundant in the liver, kidneys, and red blood cells, and it is primarily involved in the breakdown of H_2O_2.

The EZ-Catalase Assay Kit is a highly sensitive product that allows for accurate measurement of catalase with a simple experimental procedure. It can measure catalase as low as 50 mU/㎖, and both fluorescence and absorbance methods can be used for detection.

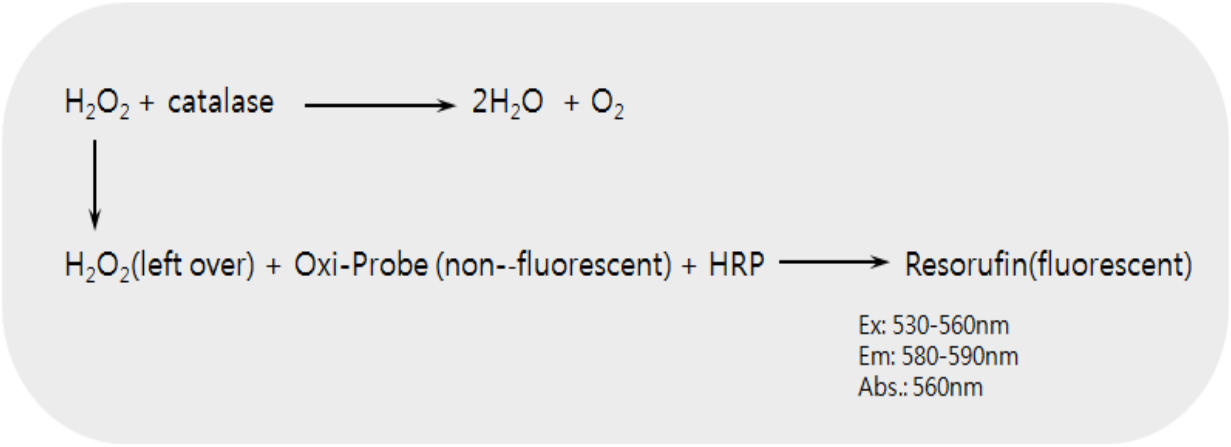

Fig. Detection mechanism with EZ-Catalase assay kit

- Catalase in the sample breaks down H_2O_2 into water (H_2O) and oxygen (O_2). The amount of H_2O_2 decomposed is proportional to the catalase activity. The remaining, undecomposed H_2O_2 reacts 1:1 with the Oxi-Probe reagent in the presence of horseradish peroxidase (HRP), resulting in the formation of resorufin, an oxidative fluorescent product.
- In the results of this reaction, a lower amount of resorufin indicates increased catalase activity, meaning that the higher the catalase activity, the lower the fluorescence or absorbance measurement.

▪ Kit Contents and Storage Conditions

Components	400 assay	Storage
Oxi-Probe	2 vial	-20℃
Dimethylsulfoxide (DMSO), anhydrous	500 ㎕	
Horseradish peroxidase (HRP, 20U)	1 vial	
Hydrogen peroxide (H_2O_2, 3%)	500 ㎕	
5X Reaction Buffer (pH 7.5)	20 ㎖	
Catalase (1,000 U/㎖)	1 vial	

* The kit remains stable at -20°C for 6 months before opening.
* Oxi-Probe reagent is highly sensitive to air. It is recommended to use it promptly after opening and to avoid exposure to light.

▪ Preparation of Stock Solution

Solution	Preparation	Storage
Oxi-Probe	Allow the Oxi-Probe and DMSO vials to fully dissolve at room temperature. Add 100 ㎕ of DMSO to one Oxi-Probe vial and mix thoroughly.	Store any remaining solution at -20°C and mix it just before use. **Protect from light *One vial of Amplex Red is sufficient for 200 assays.
1X Reaction Buffer	Mix 4 ㎖ of 5X Reaction Buffer with 16 ㎖ of deionized water (dH_2O) thoroughly.	
100 U/㎖ horseradish-peroxidase (HRP)	Add 200 ㎕ of 1X Reaction Buffer to the HRP vial and mix well.	Store the remaining solution in small aliquots at -20°C.
20 mM Hydrogen -peroxide (H_2O_2)	Mix 23 ㎕ of 3.0% H_2O_2 with 977 ㎕ of dH2O.	The mixed solution has very low stability, so it is difficult to store. Only prepare the required amount for each experiment.
1,000 U/㎖ catalase	Mix 100 ㎕ of dH_2O with the Catalase vial.	Store the remaining solution in small aliquots at -20°C.

▪ Interference

1. The reaction product, Resorufin, generated by Oxi-Probe is unstable in the presence of thiol compounds such as dithiothreitol (DTT) and 2-mercaptoethanol.
2. During the experiment, the final concentration of DTT and 2-mercaptoethanol in the sample should not exceed 10 μM.
3. Pay attention to the pH during the experiment (optimal pH = 7~8).
4. The absorbance or fluorescence of the final product, Resorufin, is affected by pH. Below pKa = 6.0, the absorption or fluorescence wavelength of Resorufin changes, and sensitivity significantly decreases. Additionally, Oxi-Probe is unstable at pH above 8.5, making accurate measurements difficult. Therefore, ensure that the experiment is conducted at a pH of 7~8 and use the included Reaction Buffer (pH 7.5) for the experiment.

▪ General Protocol

1. **Catalase standard preparation**

   : Mix 5 ㎕ of 1,000 U/㎖ catalase solution with 995 ㎕ of 1X Reaction Buffer to create a 5.0 U/㎖ catalase standard solution.

   Prepare the catalase standard by placing the solution in a 96-well plate as follows.

   ① For both the standard and sample, it is recommended to prepare at least two replicates for each to increase accuracy in your experiment.

   ② Always measure the standard during each experiment.

   When diluting the Catalase standard, it is recommended to sufficiently dilute each concentration and use the required amount.

   Ex) When preparing standard #2, mix 5.0U/ml catalase standard 3.75 ㎕ + 1X reaction buffer 71.25 ㎕ to make a total of 75 ㎕ and divide them into the well 25 ㎕ at a time of experiment.

No	5.0U/㎖ catalase standard	1X Reaction Buffer	Total volume	Final catalase concentration
1	-	25 ㎕	25 ㎕	0 mU/㎖
2	1.25 ㎕	23.75 ㎕	25 ㎕	62.5 mU/㎖
3	2.5 ㎕	22.5 ㎕	25 ㎕	125 mU/㎖
4	5 ㎕	20 ㎕	25 ㎕	250 mU/㎖
5	10 ㎕	15 ㎕	25 ㎕	500 mU/㎖
6	20 ㎕	5 ㎕	25 ㎕	1000 mU/㎖

Table. protocol for catalase standard curve.

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2. Prepare the sample and add 25 ㎕ of the prepared sample to each well of the 96-well plate.

   ➢ If you need to dilute the sample, please use 1X Reaction Buffer for dilution.

3. **40 μM H_2O_2 preparation**

   : Prepare a mixture of 10 ㎕ of 20 mM H_2O_2 solution and 4.99 ㎖ of 1X Reaction Buffer.

4. Add 25 ㎕ of 40 μM H_2O_2 solution to each well containing the sample and standard.

5. Incubate the plate in the dark at room temperature for 30 minutes.

6. **Oxi-Probe/HRP Working Solution preparation:** (100assay)

Components	Volume
Oxi-Probe	50㎕
100U/㎖ horseradish-peroxidase (HRP)	20㎕
1X Reaction Buffer	5㎖

Table. protocol for Oxi-Probe/HRP Working Solution.

7. After the reaction in step 5 is complete, add 50 ㎕ of Oxi-Probe/HRP Working Solution to each well.

8. Incubate the plate at 37°C in the dark for 30 minutes.

9. After the reaction is complete, measure the reaction values using a plate reader.

   ① Using Fluorescence plate reader – Excitation: 530 ~ 560nm

   Emission: 580 ~ 590nm

   (optimal Ex/Em = 540/590)

   ② Using Absorbance plate reader – 560nm

▪ Calculation

1. Subtract each well measurement from the negative control measurement.
   * Negative control = catalase standard #1 (0 mU/㎖ catalase)

2. Activity of HRP in HRP 1 Unit: This refers to the amount of production of 1.0 mg of purpurogallin from pyrogallol for 20 seconds at pH 6.0, 20℃.

3. Catalase 1 unit: It refers to the amount of decomposing 1 mole of H2O2 for 1 minute at pH 7.0, 25℃.

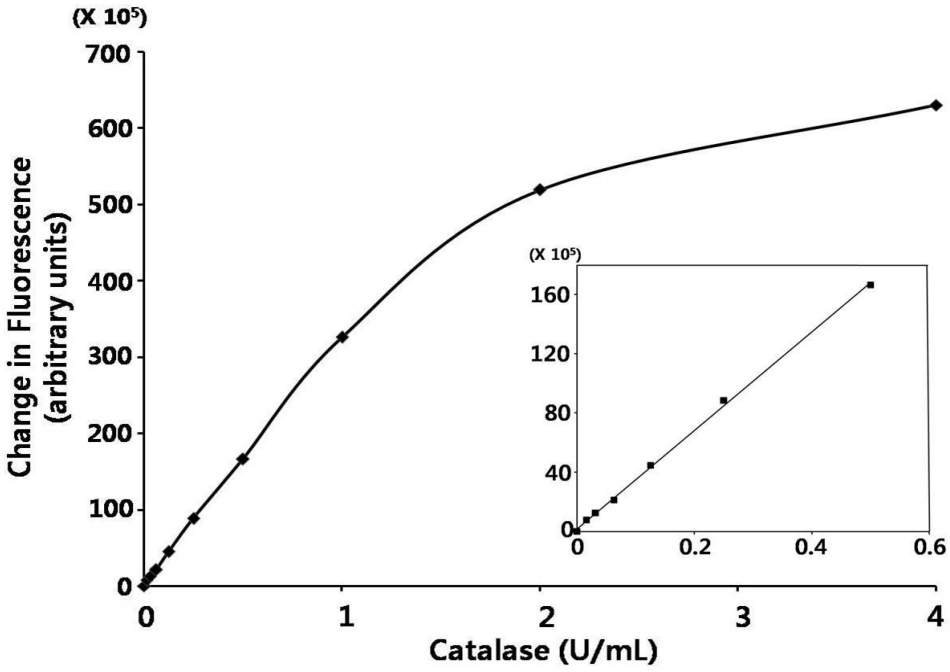

Fig. Catalase standard curve. Assay was performed following the kit protocol.

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▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione peroxidase (GPx) Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Fluorometric/Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay Kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay
	EZ-NAD/NADH Assay Kit (Colorimetric)	DG-NAD100	100 Assay