Data sheet

Catalase Activity Assay kit
(Colorimetric/Fluorometric)

Cat. No: CA063 (100 assays)

Introduction

Catalase is a heme enzyme that is present in nearly all living organisms. Catalase converts the reactive oxygen species hydrogen peroxide to water and oxygen and thereby mitigates the toxic effects of hydrogen peroxide. Oxidative stress is hypothesized to play a role in the development of many chronic or late-onset diseases such as diabetes, asthma, Alzheimer's disease, systemic lupus erythematosus, rheumatoid arthritis, and cancers.

Catalase Activity Assay Kit provides a highly sensitive and simple assay for measuring Catalase activity in biological samples. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, In the presence of horseradish peroxidase (HRP), the unconverted H_2O_2 reacts 1:1 with the fluorogenic substrate 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP Known as Amplex Red® probe) to produce a product highly fluorescent (resorufin), which can be measured at Ex/Em=535/587nm (fluorometric method) or at 570 nm (Colorimetric method).

Features

- Easy to use
- Just a few minutes procedure (~30-40 minutes)
- Sensitive assays for measuring Catalase in various biological samples such as: **Cell lysate, cell culture media, tissue extracts, plasma, serum, urine and others.**

Shipping Conditions

Gel pack

Storage

1. Store the kit at 4°C, protect from light.

Kit Contents

	Amount
Probe (in DMSO)	200 µl
CAT Assay buffer	20 ml
H_2O_2 (0.88M)	50 µl
HRP solution	250 µl
Stop solution	1.5 ml
Catalase Positive Control	5 µl

Sample Preparation

Homogenize 0.1 gram tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold **CAT Assay Buffer**; Centrifuge at 10,000 x g for 15 min at 4°C; Collect the supernatant for assay, keep on ice.
Liquid samples can be tested directly.
Store samples at -80°C to assay later.

Preparation of working solutions

1. Prepare a **20 mM H_2O_2 working solution** by diluting the H_2O_2 (0.88M) stock solution into the appropriate volume of $_dH_2O$. For instance, dilute 5 µl of H_2O_2 (0.88M) into 215 µl $_dH_2O$ to generate 20 mM. Please note that although the H_2O_2 (0.88M) stock solution has been stabilized to slow degradation, the 20 mM H_2O_2 working solution will be less stable and must be used within 4hours.

Diluted H_2O_2 is unstable, prepare fresh dilution each time.

2. **Catalase Positive Control**: Dilute the Catalase Positive Control with 500 µL of CAT Assay Buffer. **Store at -20°C for 2 months** or 2-3 days at 4°C. Keep on ice while in use.

N-Acetyl-3,7-Dihydroxyphenoxazine (Amplex Red) → Resorufin Highly fluorescent

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canvax

of the product.

Assay procedure

1. Assay

Equilibrate all materials and prepared reagents to room temperature prior to use.
It is recommended to assay all standards, controls and samples in duplicate.

1.1 Prepare a H_2O_2 standard curve: Prepare a 1mM H_2O_2 solution by adding 50µl of the 20mM H_2O_2 solution to 0.95 ml CAT Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H_2O_2 solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 standard. Bring the final volume to 90 µl with CAT Assay Buffer. Add 10 µl Stop Solution into each well (See table).

For the fluorometric assay, dilute the standard H_2O_2 10-fold for the standard curve (0-1 nmol range).

	nmol/well					
	0	**2**	**4**	**6**	**8**	**10**
H_2O_2 1mM	0 µl	2 µl	4 µl	6 µl	8 µl	10 µl
CAT assay buffer	90 µl	88 µl	86 µl	84 µl	82 µl	80 µl
Stop Solution	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl

1.2 Dilute samples in CAT assay Buffer: Add 5-78 µl samples into each well, and adjust volume to total **78 µl** with CAT Assay Buffer. (Catalase Positive Control: Use 5 µl and adjust volume to total **78 µl** with CAT Assay Buffer)
In separate wells, prepare **sample High Control (HC)** with the same amount of sample then bring total volume to 78 µl with CAT Assay Buffer. Add 10 µl of Stop Solution into the sample HC, mix and incubate at 25°C for 5 min to completely inhibit the catalase activity in samples as High Control.

Reducing agents in samples may interfere with the assay. Keep DTT or β-ME below 5 µM.

1.3 Catalase Reaction: Add **12 µl** fresh 1 mM H_2O_2 into each well (samples, Controls and **samples HC**) to start the reaction, incubate at 25°C for 30 min.
Add **10 µl** Stop solution into each sample well to **stop the reaction** (Note: High Control and standard curve wells already contain Stop Solution).

1.4. Develop Mix: Prepare a develop Mix containing (for each well):

- 46 µl CAT Assay Buffer
- 2 µl Probe
- 2 µl HRP solution

Add 50 µl of the Develop Mix to each test samples, samples HC, controls, and standards. Mix well and incubate at 25°C for 10 min.

1.5 Read: Measure the fluorescence or absorbance in a microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm or absorbance at ~570 nm.

2. Calculation

Signal change by catalase in sample is **ΔA = AHC – AS**
AHC is the reading of sample High Control.
AS is the reading of sample in 30 min.

Plot the corrected absorbance values for each standard as a function of the final concentration of catalase. Apply the **ΔA** to the H_2O_2 standard curve to get **B nmol of H_2O_2** decomposed by catalase in 30 min reaction.

$$\text{Catalase Activity} = \frac{B}{30xV} \times D$$

B is the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol).
V is the pretreated sample volume added into the reaction well (in ml).
30 is the reaction time 30 min.
D is the Sample Dilution Factor