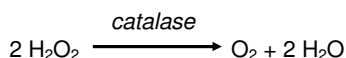


EnzyChrom™ Catalase Assay Kit (ECAT-100)

Quantitative Colorimetric/Fluorimetric Catalase Determination

DESCRIPTION

CATALASE (EC 1.11.1.6), is an ubiquitous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H₂O₂) to water and oxygen.



By preventing excessive H₂O₂ build up, catalase allows important cellular processes which produce H₂O₂ as a byproduct to occur safely. Deficiency in catalase activity has been associated with grey hair and peroxisomal disorder acatalasia. Simple, direct and high-throughput assays for catalase activity find wide applications. BioAssay Systems' improved assay directly measures catalase degradation of H₂O₂ using a redox dye. The change in color intensity at 570nm or fluorescence intensity ($\lambda_{em/ex} = 585/530\text{nm}$) is directly proportional to the catalase activity in the sample.

KEY FEATURES

Sensitive and accurate. Use 10 μL sample. Linear detection range 0.2 - 5 U/L (OD) or 0.02 - 0.5 U/L (Fluorescence) catalase activity.

Simple and Convenient. The procedure involves adding a Substrate to sample, incubation for 30 min, followed by a Detection Reagent and reading the optical density or fluorescence intensity.

APPLICATIONS:

Direct Assays: catalase activity in biological samples e.g. serum, plasma, urine, saliva, cell culture etc.

Drug Discovery/Pharmacology: effects of drugs on catalase activity.

KIT CONTENTS:

Assay Buffer: 25 mL **HRP Enzyme:** 120 μL **Dye Reagent:** 120 μL
H₂O₂ Solution: 100 μL 3% H₂O₂ **Positive Control:** 8 μL Catalase

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Tissue (10 mg) and cells (10⁶) are homogenized in 200 μL cold PBS. Centrifuge 10 min at 14,000 rpm to pellet any debris. Use clear supernatant for assay.

Note: SH-containing reagents (e.g. β -mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be kept below 10 μM in the sample.

ASSAY PROCEDURE

1. **Reagent Preparation.** Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening. Keep thawed HRP Enzyme on ice.

For colorimetric assays, use a clear flat-bottom 96-well plate. For fluorimetric assays, use a solid black flat-bottom 96-well plate.

Samples and Controls: transfer 10 μL sample into wells of the 96-well plate. In addition, for each assay run, prepare one sample blank well that contains only 10 μL Assay Buffer.

Add 400 μL Assay Buffer to Positive Control tube and mix well. Transfer 10 μL of the reconstituted Positive Control into separate wells.

Note: (1). For unknown samples, perform several dilutions to ensure that catalase activity is within the linear range 0.2 to 5 U/L. (2) The provided catalase serves as a positive control to ensure assay is working and should not be used to calculate the Sample catalase activity.

2. **Enzyme Reaction.** Mix 5 μL 3% H₂O₂ and 914 μL dH₂O (final 4.8 mM). Prepare enough 50 μM H₂O₂ Substrate for sample, positive control and sample blank by mixing, for each well, 1 μL of the 4.8 mM H₂O₂ with

95 μL Assay Buffer. *Note: diluted H₂O₂ is not stable. Prepare fresh dilutions for each experiment.*

Add 90 μL of the 50 μM Substrate to these wells to initiate the catalase reaction. Tap plate quick to mix. Incubate 30 min at room temperature. During the incubation time, proceed with Steps 3 and 4 below.

3. **H₂O₂ Standard Curve.** Mix 40 μL of the 4.8 mM H₂O₂ with 440 μL dH₂O to yield 400 μM H₂O₂. Prepare standards as shown in the Table below. Transfer 10 μL standards into separate wells of the 96-well plate. Add 90 μL Assay Buffer to the standards.

No	400 μM H ₂ O ₂ + H ₂ O	Vol (μL)	H ₂ O ₂ (μM)
1	100 μL + 0 μL	100	400
2	60 μL + 40 μL	100	240
3	30 μL + 70 μL	100	120
4	0 μL + 100 μL	100	0

4. **Detection.** Prepare enough Detection Reagent by mixing, for each reaction well (Sample, Control and Standard wells), 102 μL Assay Buffer, 1 μL Dye Reagent and 1 μL HRP Enzyme.

At the end of the 30 min incubation (Step 2), add 100 μL Detection Reagent per well. Tap plate to mix. Incubate for 10 min.

5. Read optical density at 570nm (550 to 585nm).

The fluorescence assay (585/530nm) is more sensitive than the OD assay. Use 20, 12, 6 and 0 μM H₂O₂ for the standard curve.

CALCULATION

Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the catalase activity of Sample,

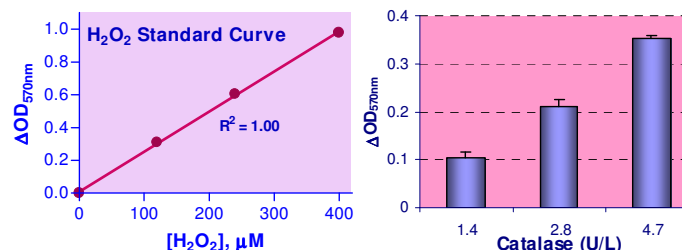
$$\text{Catalase (U/L)} = \frac{R_{\text{Sample Blank}} - R_{\text{Sample}}}{\text{Slope } (\mu\text{M}^{-1}) \times 30 \text{ min}} \times n$$

$R_{\text{SAMPLE Blank}}$ and R_{SAMPLE} are optical density or fluorescence intensity readings of the Sample Blank and Sample, respectively. Slope is determined from the standard curve. 30 min is the catalase reaction time. n is the sample dilution factor.

Unit definition: one unit is the amount of catalase that decomposes 1 μmole of H₂O₂ per min at pH 7.0 and room temperature.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, flat-bottom 96-well plates, plate reader.



PUBLICATIONS

- Pradhan, Arnab, et al (2017). Elevated catalase expression in a fungal pathogen is a double-edged sword of iron. PLoS pathogens 13.5: e1006405.
- AlAsmari, A. F., et al (2020). Elucidation of the molecular mechanisms underlying sorafenib-induced Hepatotoxicity. Oxidative Medicine and Cellular Longevity, 2020, 1-10
- Sahin, T. D., et al. (2020). Infliximab prevents dysfunction of the vas deferens by suppressing inflammation and oxidative stress in rats with chronic stress. Life Sciences, 250, 117545.

