# Catalase Assay Kit (Colorimetric/Fluorometric)

LS-K245-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

## Introduction

CATALASE (EC 1.11.1.6), is a ubiquitous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen.

$$2\ H_2O_2 \xrightarrow{catalase} O_2 + 2\ H_2O$$

By preventing excessive $H_2O_2$ build up, catalase allows important cellular processes which produce $H_2O_2$ 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. LSBio's' improved assay directly measures catalase degradation of $H_2O_2$ using a redox dye. The change in color intensity at 570nm or fluorescence intensity (λem/ex = 585/530nm) is directly proportional to the catalase activity in the sample.

## Key Features

- Sensitive and accurate. Use 10 µL sample. Linear detection range 0.2 to 5 U/L 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.

## Components

| Component | K245-100 |
|---|---|
| | 100 Tests |
| Assay Buffer | 25 mL |
| HRP Enzyme | 120 µL |
| Dye Reagent | 120 µL |
| $H_2O_2$ Solution | 100 µL 3% $H_2O_2$ |
| Positive Control | 8 µL Catalase |

## Materials Not Supplied

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

## Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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## 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 fluorometric 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 $dH_2O$ (final 4.8 mM). Prepare enough 50 µM $H_2O_2$ Substrate for sample, positive control and sample blank by mixing, for each well, 1 µL of the 4.8 mM $H_2O_2$ with95 µL Assay Buffer. Note: diluted $H_2O_2$ 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. $H_2O_2$ Standard Curve. Mix 40µL of the 4.8 mM $H_2O_2$ with 440 µL $dH_2O$ to yield 400 µM $H_2O_2$. 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 $H_2O_2$ + $H_2O$ | Vol (µL) | $H_2O_2$ (µM) |
|---|---|---|---|
| 1 | 100µL + 0µL | 100 | 400 |
| 2 | 60µL + 40µL | 100 | 240 |
| 3 | 30µL + 70µL | 100 | 120 |
| 4 | 0µL + 100µL | 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) or fluorescence intensity at λem/ex = 585/530nm.

**CalculationS**

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,

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

$R_{SAMPLE\ Blank}$ 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 µ*mole of $H_2O_2$ per min at pH 7.0 and room temperature.

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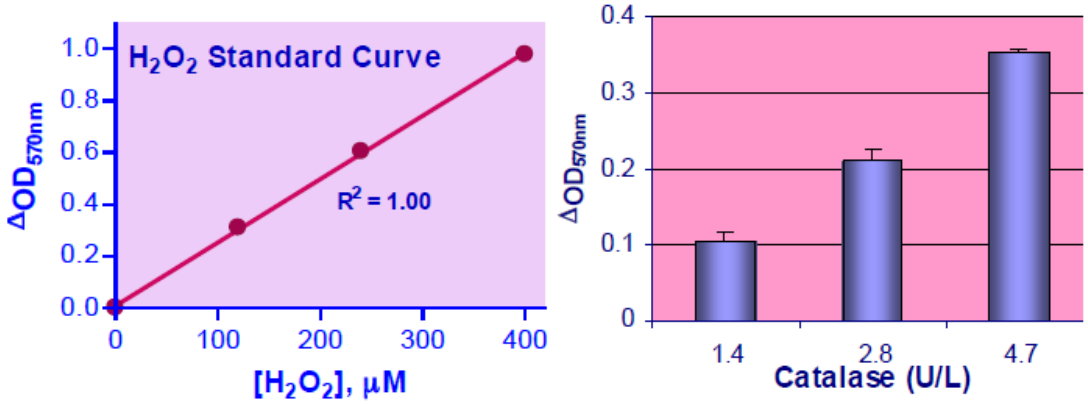
**Sample Data**

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