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.

\[
2 \text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{O}_2 + 2 \text{H}_2\text{O}
\]

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 (λ_{max} = 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.

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 three 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 Assay Buffer. 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 40µL 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.

<table>
<thead>
<tr>
<th>No</th>
<th>400 µM H₂O₂ + H₂O</th>
<th>Vol (µL)</th>
<th>H₂O₂ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100µL + 0µL</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>60µL + 40µL</td>
<td>100</td>
<td>240</td>
</tr>
<tr>
<td>3</td>
<td>30µL + 70µL</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>0µL + 100µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

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 λ_{max} = 585/530nm.

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.

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

R_{\text{SAMPLE Blank}} and R_{\text{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, centrifuge tubes, uncoated 96-well plates, optical density plate reader, fluorescence plate reader, homogenizer etc.

LITERATURE