Technical Bulletin

Catalase Assay Kit

Catalogue number MAK531

Product Description

Catalase is a ubiquitous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen.

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\ H_2O$$

By preventing excessive H_2O_2 build up, catalase allows important cellular processes which produce H_2O_2 as a byproduct to occur safely. Simple, direct and high-throughput assays for catalase activity find wide applications. The catalase assay kit directly measures catalase degradation of H_2O_2 using a redox dye. The change in color intensity at 570_{nm} or fluorescence intensity ($\lambda_{em/ex}$ = $585/530_{nm}$) is directly proportional to the catalase activity in the sample.

The linear detection range of the kit is 0.2 to 5 U/L catalase activity. The kit is suitable for catalase activity determination in biological samples such as serum, plasma, urine, saliva and cell culture, as well as for studying the effects of drugs on catalase activity.

Components

The kit is sufficient for 100 colorimetric/fluorometric assays in 96-well plates.

- Assay Buffer 25 mL
  Catalogue Number MAK531A
- HRP Enzyme 120 µL
  Catalogue Number MAK531B
- Dye Reagent 120 µL
  Catalogue Number MAK531C
- 3% H_2O_2 Solution 100 µL
  Catalogue Number MAK531D
- Positive Control 8 µL
  Catalogue Number MAK531E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric or fluorescence multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assays. Black 96-well plates with clear bottoms are recommended for fluorescence assays. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL Centrifuge tubes.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge all tubes prior to opening. Equilibrate all components to room temperature prior to use.

HRP Enzyme: Keep the thawed enzyme on ice.

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

Procedure

All Samples and Standards should be run in duplicate.

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

Sample Preparation

1. Homogenize 10 mg of tissue and 10^6 cells in 200 µL cold PBS.
2. Centrifuge 10 min at 14,000 rpm to pellet any debris. Use clear supernatant for assay.
3. 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

Note: For unknown Samples, perform several dilutions to ensure that catalase activity is within the linear range 0.2 to 5 U/L.

Positive Control

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

Note: The provided catalase serves as a positive control to ensure assay is working and should not be used to calculate the Sample catalase activity.

Assay Reaction

1. Prepare 4.8 mM H_2O_2 by mixing 5 µL 3% H_2O_2 and 914 µL purified water.
2. 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 H_2O_2 with 95 µL Assay Buffer.
3. Transfer 90 µL of the 50 µM Substrate to these wells to initiate the catalase reaction.
4. Tap plate quick to mix. Incubate for 30 minutes at room temperature.

During the incubation time proceed with Standard Curve and Detection Reagent preparation.

Note: Diluted H_2O_2 is not stable. Prepare fresh dilutions for each experiment.

Standard Curve

1. Prepare 400 µM H_2O_2 by mixing 40 µL of 4.8 mM H_2O_2 with 440 µL purified water. Dilute standard in purified water as mentioned in Table 1.

Table 1.
Dilution of Standards

No.	400 µM H_2O_2+ H_2O	H_2O_2 (µM)
1	100 µL + 0 µL	400
2	60 µL + 40 µL	240
3	30 µL + 70 µL	120
4	0 µL + 100 µL	0

Fluorometric Assay: Further dilute the Standards 1:20 to achieve 20, 12, 6, and 0 µM H_2O_2.

2. Transfer 10 µL Standards into separate wells of a clear flat-bottom 96-well plate. Add 90 µL Assay Buffer to the standards.

Detection Reagent Preparation

Prepare enough detection reagents for each Sample, Control and Standard wells. For each well, prepare 104 µL of Detection Reagent according to Table 2.

Table 2.
Preparation of Detection Reagents

Reagent	Volume
Assay Buffer	102 µL
Dye reagent	1 µL
HRP Enzyme	1 µL

Measurement

1. Upon completion of the 30-minute incubation in the Assay Reaction step, add 100 µL Detection Reagent per well. Tap plate to mix. Incubate for 10 minutes.
2. Read optical density at 570nm (550 to 585nm) or fluorescence intensity at λem/ex = 585/ 530nm.

Results

1. Subtract the blank value (standard #4) from all the standard values.
2. Plot the ΔOD or ΔF against the standard concentrations.
3. Determine the slope and calculate catalase concentration of samples using the below given equation:

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

Where:

$R_{SAMPLE\ blank}$ 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 = Catalase reaction time

n = Sample dilution factor.

Unit Definition: One unit is the amount of catalase that decomposes 1 µmole of H_2O_2 per min at pH 7.0 and room temperature.

Figure 1.
Example of typical H_2O_2 standard curves by colorimetric assay.

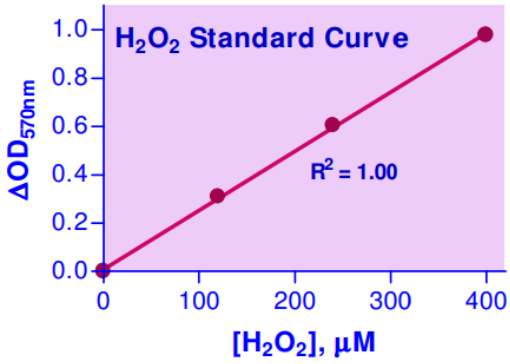
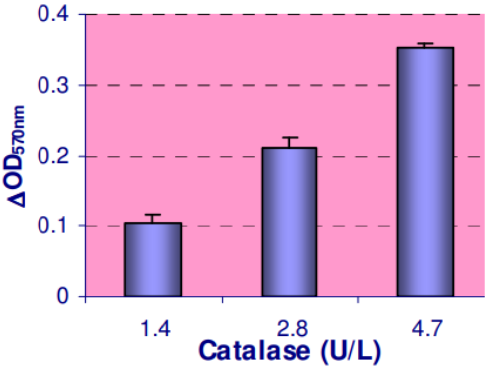


Figure 2.
Example of Catalase activity in a colorimetric assay.



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