Technical Manual

ColorFluor Catalase Activity Assay Kit

Catalogue Code: BA0083

Pack Size: 100 assays

Research Use Only

DESCRIPTION

CATALASE (EC 1.11.1.6), is an 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. The Assay Genie ColorFluor Catalase Activity Assay Kit directly measures catalase degradation of H_2O_2 using a redox dye. The change in color intensity at 570nm or fluorescence intensity ($\lambda_{em/ex}$ = 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.

KIT CONTENTS:

Assay Buffer: 25 mL **HRP Enzyme:** 120 µL **Dye Reagent**: 120 µL
H2O2 Solution: 100 µL 3% H_2O_2 **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 (10^6) 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% H_2O_2 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 with 95 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.

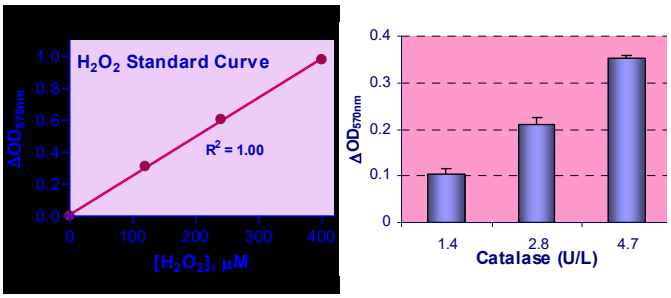
5. Read optical density at 570nm (550 to 585nm) or fluorescence intensity at $\lambda_{em/ex}$ = 585/530nm.

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,

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

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

MATERIALS REQUIRED, BUT NOT PROVIDED
Pipetting devices, flat-bottom 96-well plates, plate reader.



PUBLICATIONS

1. Chiu CC et al (2012). Beneficial Effects of Ocimum gratissimum Aqueous Extract on Rats with CCl(4)-Induced Acute Liver Injury. Evid Based Complement Alternat Med 2012:736752.

2. Hadzi-Petrushev N et al (2012). L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J Therm Biol 37(5):361-365.

3. Zhu T et al (2012) Effects of the iron-chelating agent deferoxamine on triethylene glycol dimethacrylate, 2-hydroxylethyl methacrylate, hydrogen peroxide-induced cytotoxicity. J Biomed Mater Res B Appl Biomater 100(1):197-205.

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