Catalase Assay Kit

Catalog Number KA0884

100 assays

Version: 04

Intended for research use only

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Introduction

Background

Catalase (EC 1.11.1.6) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It functions to catalyze the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. The Catalase Assay Kit provides a highly sensitive, simple, direct and HTS-ready assay for measuring Catalase activity in biological samples. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, the unconverted H_2O_2 reacts with OxiRed probe to produce a product, which can be measured at 570 nm (Colorimetric method) or at Ex/Em=535/587nm (fluorometric method). Catalase activity is reversely proportional to the signal. The kit can detects 1 µU or less of catalase activity in samples.

General Information

Materials Supplied

List of component

Component	Amount
Catalase Assay Buffer	25 ml
OxiRed Probe (in DMSO)	200 µl
HRP (lyophilized)	1 vial
H_2O_2 (0.88M)	25 µl
Stop Solution	1 ml
Catalase Positive Control	2 µl

Storage Instruction

Store kit at 4℃, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

Assay Protocol

Reagent Preparation

- ✓ OxiRed Probe: Briefly warm to completely melt the DMSO solution. Store at 4℃, protected from light. Use within two months.
- ✓ HRP Solution: Dissolve with 220 µl Assay Buffer. Store at 4℃. Use within two months.
- ✓ Positive Control Solution: Add 500 µl Assay Buffer to Positive Control. Aliquot and store at -20℃. Diluted Positive Control solution is stable for 2-3 days at 4℃ & for 2 months at -20℃.

  Note: Keep samples, HRP and Catalase on ice while in use.

Assay Procedure

1. Sample and Positive Control Preparations

   Homogenize 0.1 gram tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 min at 4℃; Collect the supernatant for assay, keep on ice. Liquid samples can be tested directly. Store samples at -80℃ to assay later.

   Add 2-78 µl samples or 1-5 µl Positive Control Solution into each well, and adjust volume to total 78 µl with Assay Buffer. Prepare sample High Control (HC) with the same amount of sample in separate wells then bring total volume to 78 µl with Assay Buffer. Add 10 µl of Stop Solution into the sample HC, mix and incubate at 25℃ for 5 min to completely inhibit the catalase activity in samples as High Control. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the linear range.

   Reducing agents in samples may interfere with the assay. Keep DTT or β-ME below 5 µM.
2. H_2O_2 Standard Curve

   Dilute 5 µl of 0.88M H_2O_2 into 215 µl dH_2O to generate 20 mM H_2O_2, then take 50 µl of the 20 mM H_2O_2 and dilute into 0.95 ml dH_2O to generate 1 mM H_2O_2. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H_2O_2 solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 standard. Bring the final volume to 90 µl with Assay Buffer. Add 10 µl Stop Solution into each well. For the fluorometric assay, dilute the standard H_2O_2 10-fold for the standard curve (0-1 nmol range).

   Note: Diluted H_2O_2 is unstable, prepare fresh dilution each time.
3. Catalase Reaction

   Add 12 µl fresh 1 mM H_2O_2 into each well of both samples and sample HC to start the reaction, incubate at 25℃ for 30 min, and then add 10 µl Stop solution into each sample well to stop the reaction *(Note: High Control and standard curve wells already contain Stop Solution).*
4. Develop Mix

   Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Develop Mix containing:

46 µl Assay Buffer

2 µl OxiRed Probe

2 µl HRP solution

Add 50 µl of the Develop Mix to each test samples, controls, and standards. Mix well and incubate at 25℃ for 10 min. Measure O.D. 570 nm in a plate reader.

Note: For low amounts of catalase, you can either increase the incubate time prior to adding the Stop Solution or use the fluorometric method. For the fluorimetric method, decrease the 1 mM H_2O_2 amount to 1.5 µl and OxiRed Probe to 0.3 µl in the reaction; compensate the volume with Assay Buffer.

Data Analysis

Calculation of Results

Signal changes by catalase in sample is $\Delta A = A_{HC} - A_{Sample}$. A_{HC} is the reading of sample High Control, A_{Sample} is the reading of sample in 30 min. Plot the H_2O_2 Standard Curve. Apply the ΔA to the H_2O_2 standard curve to get B nmol of H_2O_2 decomposed by catalase in 30 min reaction. Catalase activity can be calculated:

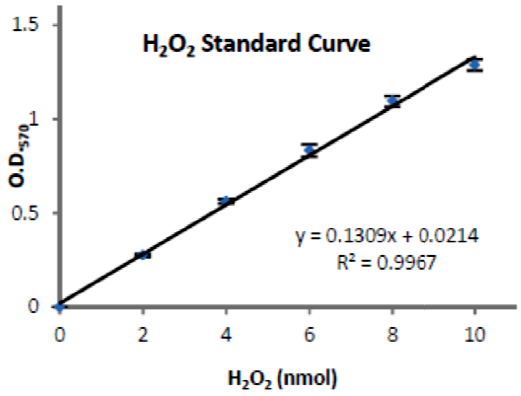
$$\text{Catalase Activity} = \frac{B}{30 \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$$

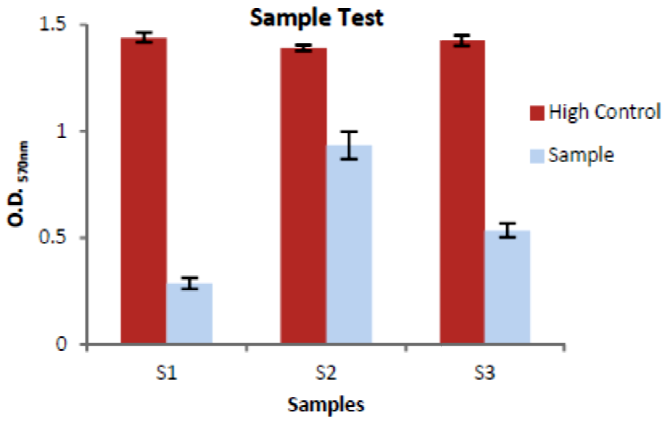
Where: B is the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol).

V is the pretreated sample volume added into the reaction well (in ml).

30 is reaction time 30 min.

Unit definition: One unit of catalase is the amount of catalase decomposes 1.0 μmol of H_2O_2 per min at pH 4.5 at 25℃.





Resources

Troubleshooting

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.