CREATIVE BIOMART®
Assay Kit

Catalase Activity Colorimetric/Fluorometric Assay Kit

Product Information

Cat

Kit-0178

Cat.No.

Kit-0178

Description

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□O□) to water and oxygen. 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□O□ to produce water and oxygen, the unconverted H□O□ 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 detects high pico-unit of catalase in samples.

Applications

The kit detects high pico-unit of catalase in samples.

Storage

Store kit at 4°C, 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.

Shipping

Gel Pack

Size

100 assays

Handling

OxiRed Probe: Briefly warm to completely melt the DMSO solution. Store at 4°C, protected from light. Use within two months.

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HRP: Dissolve with 220 µl Assay Buffer. Store at 4°C. Use within two months.
Positive Control Solution: Add 500 µl Assay Buffer to Positive Control. Aliquot and store at -20°C. Diluted Positive Control solution is stable for 2-3 days at 4°C & for 2 months at -20°C.
Note: Keep samples, HRP and Catalase on ice while in use.

Kit Components

- Catalase Assay Buffer
- OxiRed Probe (in DMSO)
- HRP (lyophilized)
- H□O□ (3%; 0.88M)
- Stop Solution
- Catalase Positive Control (lyophilized)

Target Species

Mammalian

Detection method Absorbance (570 nm) or Fluorescence (Ex/Em 535/587 nm)

Features & Benefits

- Simple procedure; takes ~ less than 40 minutes
- Fast and convenient

Preparation

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°C; Collect the supernatant for assay, keep on ice. Liquid samples can be tested directly. Store samples at -80°C to assay later.
Add 2 - 78 µl of 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°C 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 interfere with the assay. Keep

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DTT or β-ME below 5 µM.

Assay Protocol

2. H2O2 Standard Curve:
Dilute 5 µl of 0.88M H2O2 into 215 µl dH2O to generate 20 mM H2O2, then take 50 µl of the 20 mM H2O2 and dilute into 0.95 ml dH2O to generate 1 mM H2O2. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H2O2 solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H2O2 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 H2O2 10-fold for the standard curve (0-1 nmol range).
Note: Diluted H2O2 is unstable, prepare fresh dilution each time.
3. Catalase Reaction:
Add 12 µl fresh 1 mM H2O2 into each well (samples, positive control, and sample HC) to start the reaction, incubate at 25°C for 30 min, and then add 10 µl Stop Solution into each sample well (Sample, Positive Control; do not add Stop Solution to the HC) 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 50 µl Developer Mix containing:
46 µl Assay Buffer
2 µl OxiRed Probe
2 µl HRP solution
Add 50 µl of the Developer Mix to each test samples, controls, and standards. Mix well and incubate at 25°C for 10 min. Measure OD 570 nm in a plate reader. Note: For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution or use the fluorometric method. For the fluorometric method, decrease the 1 mM H2O2 amount to1.5 µl and OxiRed Probe to 0.3 µl in the reaction; compensate the volume with Assay Buffer.

Analysis

6. Calculation:
Signal change by catalase in sample is ΔA = AHC – Asample. AHC is the reading of sample High Control, ASample is the reading of sample in 30 min. Plot the H2O2 Standard Curve. Apply the ΔA to

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the H2O2 standard curve to get B nmol of H2O2 decomposed by catalase in 30 min reaction.
Catalase activity can be calculated:
Catalase Activity = B/(30*V)*Sample Dilution Factor = nmol/min/ml = mU/mL
Where: B is the decomposed H2O2 amount from H2O2 Standard Curve (in nmol).
V is the pretreated sample volume added into the reaction well (in ml).
30 is the reaction time 30 min.
Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 μmol of H2O2 per min at pH 4.5 at 25 °C.

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