CREATIVE BIOMART®
Assay Kit

Catalase Assay Kit

Product Information

Cat.No. Kit-2435

Product Overview

The Fluorimetric Catalase Assay Kit provides a quick and sensitive method for the measurement of catalase activity. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Catalase reacts with H2O2 to produce water and oxygen (O2). CM Red also reacts with H2O2 to generate a red fluorescent product. Therefore the reduction in fluorescence intensity is proportional to catalase activity. The CM Red substrate used in the assay enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader or an absorbance microplate reader. With the Fluorimetric Catalase Assay Kit, we have detected as little as 30 mU/mL catalase in a 100 µL reaction volume.

Warning

Thaw all the kit components at room temperature before starting the experiment. The component A is unstable in the presence of thiols such as DTT and β-mercaptoethanol. The final concentration of the thiols higher than 10 µM would significantly decrease the assay dynamic range. NADH and glutathione (reduced form: GSH) may interfere with the assay.

Size

200 Tests

Kit Components

Component A: CM Red Freeze (< -15 °C), Minimize light exposure 1 vial
Component B: H2O2, Freeze (< -15 °C), Minimize light exposure, 1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer, Refrigerated (2-8 °C), 1 bottle (50 mL)
Component D: Horseradish Peroxidase, Freeze (< -15 °C), Minimize light exposure, 1 vial (20 units)
Component E: Catalase Standard, Freeze (< -15 °C), Minimize light exposure, 1 vial (1000 U/mL, 50 µL)
Component F: DMSO, Freeze (< -15 °C), 1 vial (200 µL)

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Preparation

KEY PARAMETERS
Fluorescence microplate reader
Excitation: 540 nm
Emission: 590 nm
Cutoff: 570 nm
Recommended plate: Solid black

PREPARATION OF STOCK SOLUTIONS
Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.
1. CM Red Substrate stock solution (200X)
Add 65 µL of DMSO (Component F) into the vial of CM Red (Component A) to make 200X Substrate stock solution. The stock solution should be used promptly.
2. HRP stock solution (100 U/mL)
Add 200 µL of Assay Buffer (Component C) into the vial of Horseradish Peroxidase (Component D) to make 100 U/mL HRP stock solution.
3. H2 O 2 stock solution (10 mM)
Add 10 µL of 3% H 2 O 2 (0.88 M, Component B) into 870 µL of Assay Buffer (Component C) to make 10 mM H 2 O 2 stock solution.
Note: The diluted H 2 O 2 stock solution is not stable. The unused portion should be discarded.
4. H2 O 2 assay buffer (1X)
Add 5 µL of 10 mM H 2 O 2 stock solution into 5 mL of Assay Buffer (Component C) to make 1X H 2 O 2 assay buffer.
5. Catalase standard solution (2 U/mL)
Add 2 µL of 1000 U/mL Catalase Standard (Component E) into 1000 µL of Assay Buffer (Component C) to make 2 U/mL Catalase standard solution.

PREPARATION OF STANDARD SOLUTION
Catalase standard

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Take 2 U/mL Catalase standard solution (CS7) and perform 1:2 serial dilutions to get serially diluted Catalase standard (CS6 - CS1) with Assay Buffer (Component C).

PREPARATION OF WORKING SOLUTION
Add 25 µL of 200X CM Red substrate stock solution and 15 µL of 100 U/mL HRP stock solution into 5.0 mL of Assay Buffer (Component C) and mix well to prepare CM Red working solution.
Note: Keep from light.

Assay Protocol

Table 1. Layout of Catalase standards and test samples in a solid black 96-well microplate. CS= Catalase Standards (CS1 - CS7, 0.031 to 2 U/mL), BL=Blank Control, TS=Test Samples
BL BL TS TS
CS1 CS1 ... ...
CS2 CS2 ... ...
CS3 CS3
CS4 CS4
CS5 CS5
CS6 CS6
CS7 CS7

Table 2. Reagent composition for each well
Well Volume Reagents
CS1 - CS7 50 µL Serial Dilution (0.031 to 2 U/mL)
BL 50 µL Assay Buffer (Component C)
TS 50 µL test sample

1. Prepare Catalase standards (CS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of H 2 O 2 assay buffer to each well of Catalase standard, blank control, and test samples to make the total Catalase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of H 2 O 2 assay buffer into each well instead, for a total volume of 50 µL/well.

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3. Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
4. Add 50 µL of CM Red working solution into each well of Catalase standard, blank control, and test samples to make the total assay volume of 150 µL/well. For a 384-well plate, add 25 µL of CM Red working solution into each well instead, for a total volume of 75 µL/well.
5. Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
6. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 540 ± 10, Emission = 590 ± 10 nm (Cutoff = 570 nm) (optimal Ex/Em = 540/590 nm).

Note: The contents of the plate can also be transferred into a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.