CREATIVE BIOMART®
Assay Kit

Superoxide Dismutase (SOD) Activity Colorimetric Assay Kit

Product Information

Cat.No. Kit-2300

Product Overview

The Colorimetric Superoxide Dismutase (SOD) Assay Kit provides a quick and sensitive method for the measurement of SOD activity in solutions. In the assay, xanthine is converted to superoxide radical ions, uric acid and hydrogen peroxide by xanthine oxidase (XO). Superoxide reacts with SOD560 to generate a product that absorbs around 560 nm. SOD inhibits the reaction of SOD560 with superoxide, thus reduces the absorption at 560 nm. The reduction in the absorption of SOD560 at 560 nm is proportional to SOD activity. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format.

Warning

Thaw all the kit components at room temperature before starting the experiment.

Size

200 Tests

Kit Components

Component A: SOD560, Freeze (< -15 °C), Minimize light exposure, 2 bottles
Component B: 50X Xanthine, Freeze (< -15 °C), Minimize light exposure, 1 vial (100 µL)
Component C: Xanthine Oxidase, Freeze (< -15 °C), Minimize light exposure, 2 vials
Component D: SOD Standard, Freeze (< -15 °C), Minimize light exposure, 1 vial (500 Units)
Component E: Assay Buffer, Freeze (< -15 °C), 1 bottle (20 mL)

Preparation

KEY PARAMETERS
Absorbance microplate reader
Absorbance: 560 nm
Recommended plate: Clear bottom

PREPARATION OF STOCK SOLUTIONS

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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.
SOD standard solution (10 kU/mL): Add 50 µL of Assay Buffer (Component E) into the vial of SOD Standard (Component D) to make 10 kU/mL standard solution.

PREPARATION OF STANDARD SOLUTION
SOD standard: Add 10 µL of 10 kU/mL SOD standard solution into 990 µL of Assay Buffer (Component E) to get 100 U/mL SOD standard solution (SD7). Take 100 U/mL SOD standard solution (SD7) and perform 1:10 in Assay Buffer (Component E) to get 10 U/mL SOD standard solution (SD6). Take 10 U/mL standard solution (SD6) and perform 1:3 serial dilutions to get serially diluted SOD standards (SD5 - SD1) with Assay Buffer (Component E).

PREPARATION OF WORKING SOLUTION
1. SOD working solution 1 Add 2.5 mL of Assay Buffer (Component E) into the bottle of SOD560 (Component A) and mix well. Then add 50 µL of 50X Xanthine (Component B) into this bottle to make SOD working solution 1.
Note: This SOD working solution 1 should be prepared before the experiment, and kept from light. SOD working solution 1 is not stable and the unused portion should be discarded.
2. SOD working solution 2 Add 50 µL Assay Buffer (Component E) into the vial of Xanthine Oxidase (Component C) and mix well. Then, transfer 50 µL of Xanthine Oxidase stock solution into 2.5 mL Assay Buffer (Component E) to make SOD working solution 2.

Assay Protocol

Table 1. Layout of SOD standards and test samples in a clear bottom 96-well microplate. SD=SOD Standards (SD1 - SD7, 0.041 to 100 U/mL); BL=Blank Control; TS=Test Samples.
BL BL TS TS
SD1 SD1 ... ...
SD2 SD2 ... ...
SD3 SD3
SD4 SD4
SD5 SD5

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SD6 SD6
SD7 SD7

Table 2. Reagent composition for each well.
Well Volume Reagent
SD1 - SD7 50 µL Serial Dilution (0.041 to 100 U/mL)
BL 50 µL Assay Buffer (Component E)
TS 50 µL test sample

1. Prepare SOD standards (SD), 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 25 µL of SOD working solution 1 to each well of SOD standard, blank control, and test samples to make the total assay volume of 75 µL/well. For a 384-well plate, add 12.5 µL of SOD working solution 1 into each well instead, for a total volume of 37.5 µL/well.
3. Add 25 µL of SOD working solution 2 to each well of SOD standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 12.5 µL of SOD working solution 2 into each well instead, for a total volume of 50 µL/well.
4. Incubate the reaction at room temperature for 30 to 60 minutes, protected from light.
5. Monitor the absorbance with an absorbance plate reader at 550 to 560 nm.