Amplite® Colorimetric Superoxide Dismutase (SOD) Assay Kit *Enhanced Sensitivity*

Catalog number: 11308
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 11308)
Component A: WST-1	Freeze (< -15 °C), Minimize light exposure	2 bottles
Component B: SOD Enzyme solution (50X)	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Component C: NADH	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)

OVERVIEW

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide is one of the main reactive oxygen species in cells. It is a substantial contributor of pathology associated with neurodegenerative diseases, ischemia reperfusion injury, atherosclerosis and aging. SODs are an important antioxidant defense in nearly all cells exposed to superoxide radicals. In fact, mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation. Amplite® Colorimetric Superoxide Dismutase (SOD) Assay Kit provides a rapid and sensitive method for the measurement of SOD activity. It is well-known that NADH and SOD enzyme system generates superoxide radicals that reduce WST-1 into a yellow color formazan dye that has maximum absorption around 440 nm. SOD inhibits the reduction of WST-1 by catalyzing the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, thus reduces the 440 nm absorption of the formazan product. The reduction of 440 nm absorption is proportional to SOD activity. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format.

AT A GLANCE

Protocol Summary

1. Prepare and add SOD standards or test samples (50 µL)
2. Add SOD working solution 1 (25 µL)
3. Add SOD working solution 2 (25 µL)
4. Incubate at room temperature for 30 - 60 minutes
5. Monitor absorbance at 440 nm

Important Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	440 nm
Recommended plate	Clear bottom

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

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 SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11308

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 10U/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 WST-1 (Component A) and mix well. Then add 50 µL of 50X SOD Enzyme solution (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 NADH (Component C) and mix well. Then, transfer 50 µL of NADH stock solution into 2.5 mL Assay Buffer (Component E) to make SOD working solution 2.

SAMPLE EXPERIMENTAL PROTOCOL

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

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3	...	...
SD4	SD4	...	...
SD5	SD5	...	...
SD6	SD6	...	...
SD7	SD7	...	...

Table 2. Reagent composition for each well.

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Well	Volume	Reagent
SD1-SD7	50 µL	Serial Dilution (100 to 0.041 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 440 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

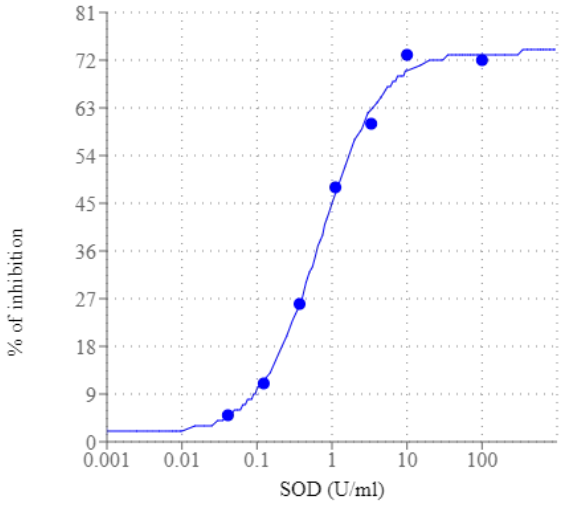


Figure 1. OD dose response was measured with Amplite® Colorimetric Superoxide Dismutase Assay Kit in a 96-well white wall/clear bottom plate with a Spectrum Max microplate reader (Molecular Devices).

DISCLAIMER

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