Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit

Catalog number: 11305
Unit size: 200 Tests

Component	Storage	Amount
Component A: ReadiView™ 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)

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. The Amplite™ 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 ReadiView™ SOD560 to generate a product that absorbs around 560 nm. SOD inhibits the reaction of ReadiView™ SOD560 with superoxide, thus reduces the absorption at 560 nm. The reduction in the absorption of ReadiView™ 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.

AT A GLANCE

Protocol Summary

1. Prepare 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 560 nm

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	560 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 SOLUTION

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/11305

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 ReadiView ™ 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.

SAMPLE EXPERIMENTAL 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		
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.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (% of inhibition) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate SOD samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

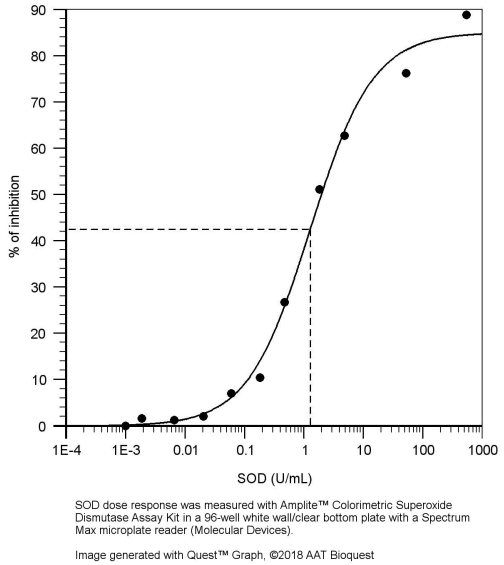

Figure 1. SOD dose response was measured with Amplite™ Colorimetric Superoxide Dismutase Assay Kit in a 96-well white wall/clear bottom plate with a Spectrum Max microplate reader (Molecular Devices).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.