Superoxide Dismutase (SOD) Assay
Cat. No. 8198, 100 tests

Introduction

Superoxide dismutase (SOD), as one of the body's most important defense mechanisms against free-radical damage, catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2). In ScienCell's SOD Assay, the superoxide anions, generated from the conversion of xanthine to uric acid and hydrogen peroxide by xanthine oxidase (XOD), reduce WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt) to water-soluble formazans, which can be measured by absorbance at 438 nm. SODs lower the rate of the reduction reaction by reducing superoxide anion concentrations. Therefore, the % Inhibition of the reduction reaction can be determined as a measurement of SOD activity.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8198a	1	SOD Assay Buffer	25 mL	2-8˚C
8198b	1	Xanthine	5 mL	-20˚C
8198c	1	EDTA	5 mL	2-8 ˚C
8198d	1	WST-1	4.9 mg	-20˚C
8198e	1	XOD	5 mL	-20˚C
8198f	1	SOD Standard (80 U/ml)	0.5 mL	-20˚C
8198g	1	Cell Lysis Buffer	10 ml	2-8ºC

Quality Control

Serially diluted SOD solutions with concentrations ranging from 0.625 to 40 units/ml are measured with the ScienCell™ SOD Assay after different time of reaction, and the resulting standard curves are shown in Figures 1. % Inhibition of reduction reaction can be calculated based on the corresponding ΔA_{438nm}/min for each SOD concentration. Positive linear relationship between % Inhibition & logarithm of SOD concentration to the base 10 (Log [SOD concentration]) can be observed within the range of 0.625 to 10 units/ml (Figure 2).

Procedures

A. Preparation of cell lysate

1. Remove culture medium from the cultured cells, wash cells twice with ice-cold PBS and remove PBS.
2. Add 100 µl of ice-cold Cell Lysis Buffer to each sample well of 24-well plate (~0.1-1×10^5 cells) and gently rock the plate side-to-side. For cells in different size wells, scale up or down the volume of Cell Lysis Buffer according to the surface area of the wells.
3. Incubate at 2-8ºC for 20 min with gentle agitation to lyse cells. Centrifuge the lysate at 14,000 × g in pre-cooled centrifuge for 3 minutes, transfer the supernatant to fresh tube and discard the pellet. Cell lysate can be stored at -70 ºC or used immediately for SOD measurement.

B. Preparation of SOD standards

1. Obtain 8 test tubes, add 150 µl of DI H_2O into each tube and label them #1 through #8.
2. Add 150 µl of the 80 U/ml SOD solution into tube #1 and mix well to get the 40 U/ml SOD standard.
3. Transfer 150 µl of the 40 U/ml SOD standard from tube #1 to tube #2 and mix well to get the 20 U/ml SOD standard.
4. Repeat step 3 for tubes #3-7 to serially dilute the SOD standards. Do not add any SOD to tube #8, which serves as the blank.

C. Preparation of the reaction mixture

1. WST-1 solution: reconstitute each vial of WST-1 (Cat #8198d) with 5 mL DI H2O. Vortex briefly and keep in the dark at -20°C until use. For longer storage, aliquot and store the reconstituted WST-1 solution at -80°C, and avoid repeated freeze/thaw cycles.
2. For each sample to be measured, mix 250 µl of SOD Assay Buffer, 50 µl of Xanthine, 50 µl of EDTA and 50 µl of WST-1 in each well of 48-well plate.
3. Add 50 µl of test sample (i.e. cell lystate) to each well of the 48-well plate containing the reaction mixture (in triplicates). For measurement of the standard curve, add SOD standard solutions according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7	#8
A	40 U/ml	20 U/ml	10 U/ml	5 U/ml	2.5 U/ml	1.25 U/ml	0.625 U/ml	Blank
B	40 U/ml	20 U/ml	10 U/ml	5 U/ml	2.5 U/ml	1.25 U/ml	0.625 U/ml	Blank
C	40 U/ml	20 U/ml	10 U/ml	5 U/ml	2.5 U/ml	1.25 U/ml	0.625 U/ml	Blank

4. Initiate the reaction by adding 50 µl of XOD solution into each well of the 48-well plate. Start recording A_{438nm} over a 20 minute interval, collecting data every 5 min.

D. Calculation

1. Average the A_{438nm} of replicate wells. Subtract the negative control (without SOD) A_{438nm} from the measured A_{438nm} to obtain the corresponding ΔA_{438nm} for each test sample and SOD standard at different reaction time.
2. Based on the ΔA_{438nm} of the SOD standard solutions, plot the standard curve of ΔA_{438nm} vs. reaction time at different SOD concentration (Figure 1). Calculate the ΔA_{438nm}/min (i.e. rate of the reduction reaction) of each SOD standard as the slope of the corresponding trend lines shown in Figure 1.
3. Determine the % Inhibition of each SOD standard as follows:

$$\%Inhibition = \frac{\left[\left(\Delta A_{438nm} / \min\right)_{Blank} - \left(\Delta A_{438nm} / \min\right)_{standard}\right]}{\left(\Delta A_{438nm} / \min\right)_{Blank}} \times 100$$

4. Based on the % Inhibition of each SOD standard (Table 1), plot the % Inhibition vs. log [SOD concentration] as the SOD Standard Inhibition Curve (Figure 2). A linear relationship can be obtained within the range of 0.625-10 U/ml. Determine the equation and R^2 value of the trend line.
5. For each test sample, plot ΔA_{438nm} vs. reaction time. Calculate the corresponding ΔA_{438nm}/min (i.e. rate of the reduction reaction) as the slope of the trend line. Determine the % Inhibition of each test sample as follows:

$$\%Inhibition = \frac{\left[\left(\Delta A_{438nm} / \min\right)_{Blank} - \left(\Delta A_{438nm} / \min\right)_{test}\right]}{\left(\Delta A_{438nm} / \min\right)_{Blank}} \times 100$$

6. Suppose the equation of the trend line of the Standard Inhibition Curve is $y = Ax + B$, calculate the SOD concentration of test sample as follows:

$$[SOD] = 10^{\frac{\%Inhibition - B}{A}}$$

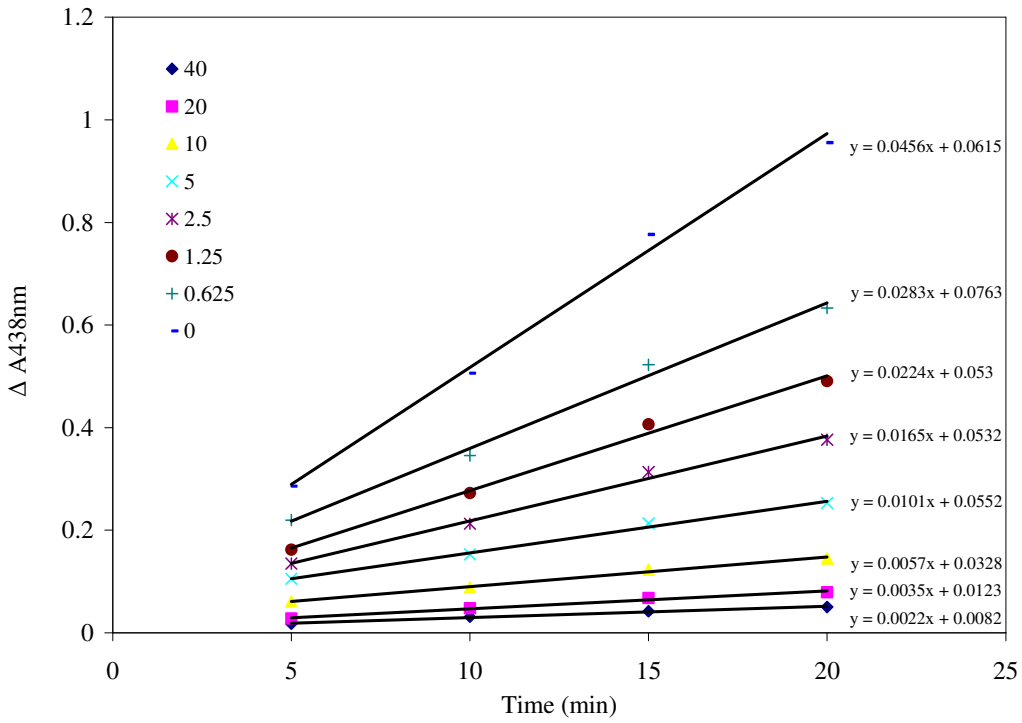

Figure 1. Standard curves of ΔA_{438nm} vs. reaction time for SOD standard solution with different concentrations.

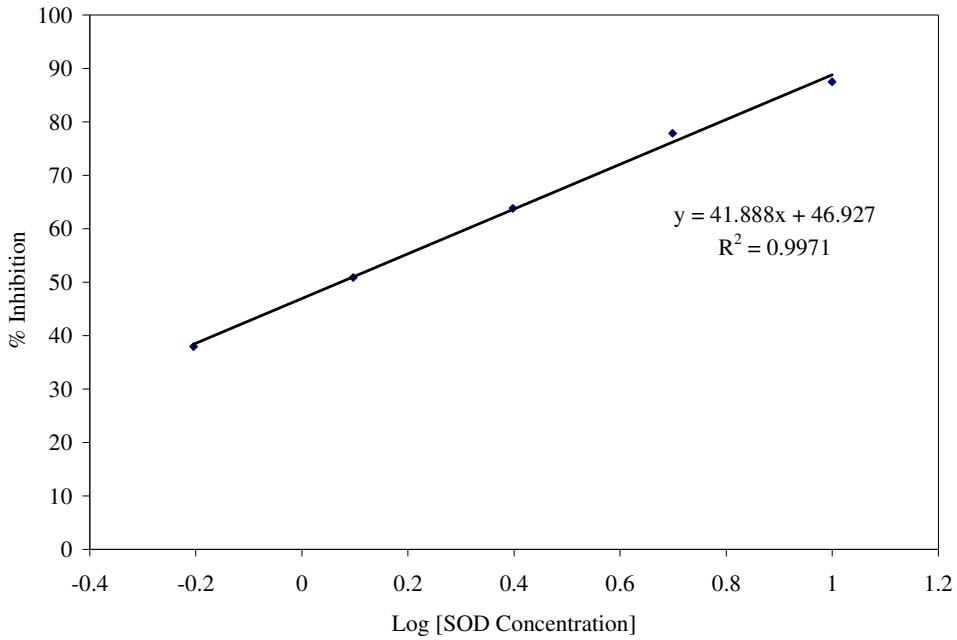

Figure 2. SOD Standard Inhibition Curve of % Inhibition vs. Log [SOD Concentration].

Table 1. Measurement of SOD Standard Inhibition Curve.

SOD concentration	**Log [SOD concentration]**	**ΔA_{438nm}/min**	**% Inhibition**
40	1.60	0.0022	95.2
20	1.30	0.0035	92.3
10	1.00	0.0057	87.5
5	0.70	0.0101	77.9
2.5	0.40	0.0165	63.8
1.25	0.097	0.0224	50.9
0.625	-0.20	0.0283	37.9
0 (Blank)		0.0456	0