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	5 mL	-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 (~$0.1\text{-}1\times10^5$ 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. 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.
2. 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

3. 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:

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

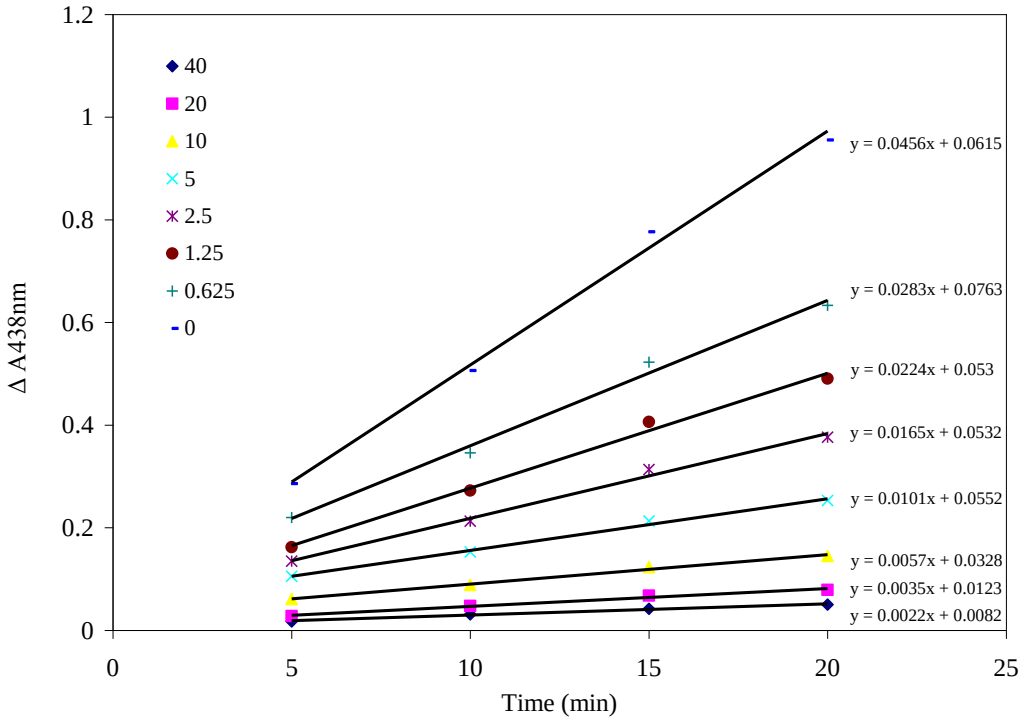

Figure 1. Standard curves of ΔA_{438nm} vs. reaction time for SOD standard solution with different concentrations.

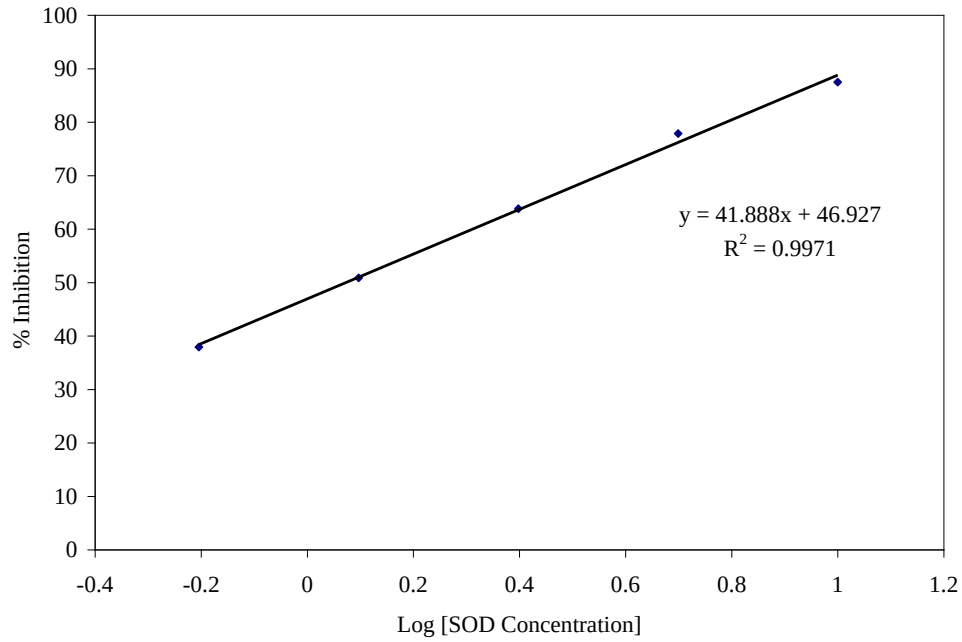

Figure 2. SOD Standard Inhibition Curve of % Inhibition vs. Log [SOD Concentration].

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