Superoxide Dismutase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1010

(Version 1.8I)

Detection and Quantification of Superoxide Dismutase (SOD) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Superoxide Dismutases (SOD) catalyze the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2), and as such, provides an important defense against the toxicity of superoxide radical. SOD1 and SOD2-deficient mice spontaneously develop liver cancer and are prone to develop tumors, whereas over expression of SOD protects tumor cells from apoptosis.

Superoxide Dismutase Activity Colorimetric Microplate Assay Kit, ions generated from the conversion of xanthine to uric acid, and hydrogen peroxide by xanthine oxidase (XOD), convert NBT to NBT-diformazan. NBT-diformazan absorbs light at 560 nm. SODs reduce superoxide ion concentrations and thereby lower the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in experimental samples.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	14 mlx 1	4 °C
Substrate	2ml x 1	-20 °C, keep in dark
Substrate Diluent	6 ml x 1	4 °C
Enzyme	200µlx 2	-20 °C, keep in dark
Enzyme Diluent	2 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Positive Control Diluent	2 ml x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 560 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Substrate Working Solution: Briefly centrifuge Substrate prior to opening. Prepareready-to-use Substrate Working Solution by mixing Substrate and Substrate Diluent at a ratio of 1:3 (v/v). Keep Working Solution in dark and use within 1 hour.

Dye Reagent: Add 10 ml distilled water to dissolve before use.

Enzyme: Briefly centrifuge prior to opening. Add1 ml Enzyme Diluent to each vial before use.Store at -20 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Dissolvein2 ml Positive Control Diluent.Store at -20 °C for 1 month.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Blank 1	Blank 2	Positive Control
Reaction Buffer	70 µl	70 µl	70 µl	70 µl	70 µl
SubstrateWorking Solution	40 µl	40 µl	40 µl	40 µl	40 µl
Dye Reagent	50 µl	50 µl	50 µl	50 µl	50 µl
Sample	20µl	--	20µl	--	--
Distilled water	--	20µl	20µl	40µl	--
Positive Control	--	--	--	--	20µl
Enzyme	20µl	20µl	--	--	20µl
Mix, incubate at room temperature for 2 minutes,record absorbance measured at 560 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of SOD is defined as theamount of SODthat inhibits the XOD activity by 50% under the assay conditions.

Inhibition rate %: Calculate the SOD activity (Inhibition rate %) using the following equation

$$\text{SOD activity (Inhibition rate \%)} = \frac{(OD_{Control}-OD_{Blank2}) - (OD_{Sample}-OD_{Blank1})}{OD_{Control}- OD_{Blank2}} \times 100\%$$

1. According to the weight of sample

$$\text{Activity} = \frac{\text{Inhibition rate \%} \times V_{Total}}{50\% \times V_{Sample} \times W} (U/g)$$

2. According to the volume of sample

$$\text{Activity} = \frac{\text{Inhibition rate \%} \times V_{Total} \times d}{50\% \times V_{Sample}} (U/mL)$$

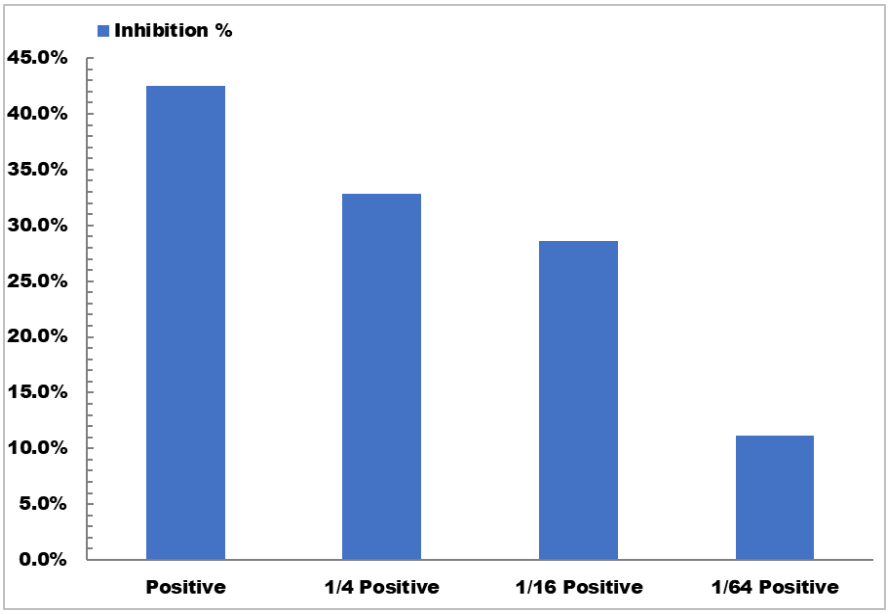
W: theweight of sample, g;

V_{Total}: the volume of reaction assay, 0.2 ml;

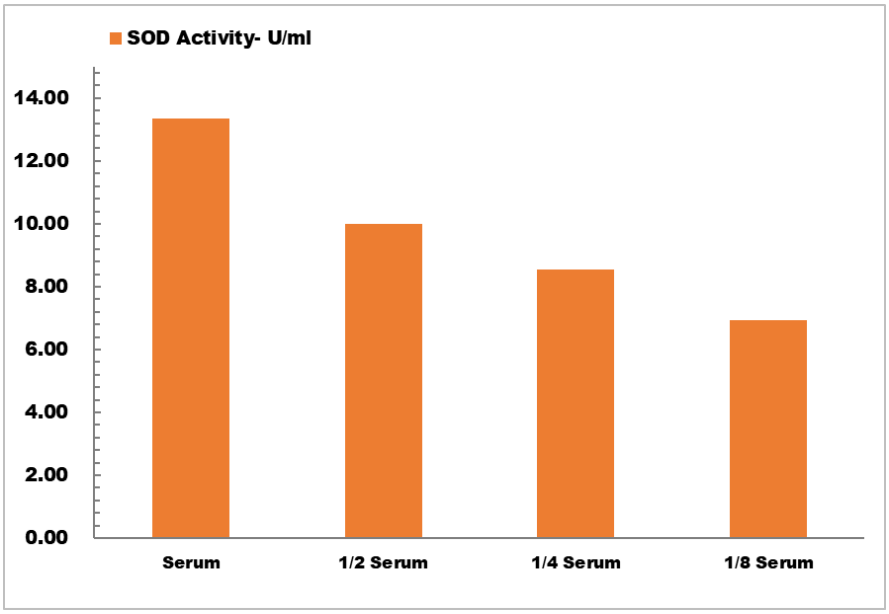
V_{Sample}: the volume of sample, 0.02 ml;

d:Dilution factor of the Sample

VIII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration



Determination of Superoxide Dismutasesactivity in serum