Superoxide Anion

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1171

(Version 1.4A)

Detection and Quantification of Superoxide Anion(SOA)

ContentinSerum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

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

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I. INTRODUCTION

Superoxide anion (O2-) is a short-lived radical of molecular oxygen that plays key roles in the immune system and intracellular functions. The enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-acytochrome-b-558-containing, plasma-membrane-bound enzyme complex-synthesizes superoxide anion by transferring an electron to molecular oxygen. Superoxide anion is a potent oxidant, which is released by leukocytes to damage infectious organisms.Superoxide anion is also implicated in oxidative stress damage, tumor promotion, and cell growth and DNA synthesis, which superoxide anion affects through the cell signaling pathway of Ras.

Superoxide AnionColorimetric Microplate Assay Kit is a sensitive assay for determining Superoxide Anioncontent in various samples. The color intensity at 530 nm is linear to the Superoxide Anioncontent in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30ml x 4	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent I	Powderx 1	4 °C, keep in dark
Dye Reagent II	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 530 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Absolute alcohol

IV. REAGENT PREPARATION

Substrate:Briefly centrifuge prior to opening. Add 2 ml distilled waterto dissolve before use.Store at 4 °C for 1 monthor -20 °C for 6 months after reconstitution.

Dye Reagent I: Add 8 ml distilled waterto dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks.

Dye Reagent II: Add 8 ml absolute alcoholto dissolve before use.Keep in dark and use within 48 hours.

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 100 mmol/Lstandardsolution.Then dilute to1mmol/Lstandard top solutionby adding10 µl stock solution into 990µl distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156mmol/L. The standards should be kept in dark at 4 °Cand use within 48 hours.

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

Weigh 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 in the microplate:

Reagent*	Sample**	Standard	Blank
Sample	20 µl	--	--
Standard	--	40 µl	--
Distilled water	--	--	40 µl
Substrate	20 µl	--	--
Mix, cover the plate and incubate at 37°C for 20minutes.			
Dye Reagent I	80 µl	80 µl	80 µl
Dye Reagent II	80 µl	80 µl	80 µl
Mix, cover the plate andincubate at37°C for 20minutes, record absorbance measured at 530 nm.			

Note:

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

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**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

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

$C_{Protein}$: the sample protein concentration, mg/ml

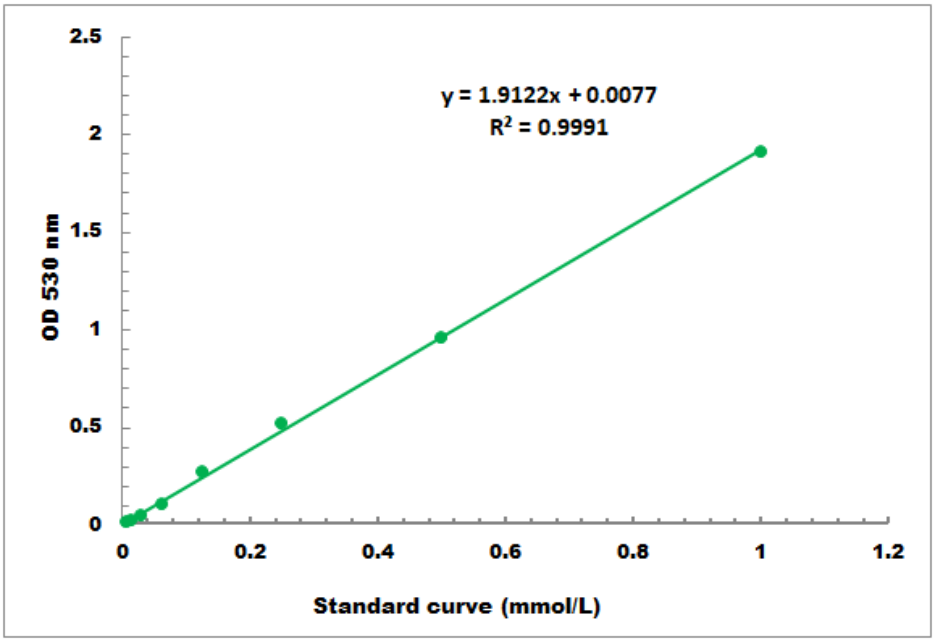
W: the weight of sample, g

V: the volume of sample in sample preparation, ml

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L