Superoxide Anion Microplate Assay Kit User Manual

Catalog # CAK1171

(Version 1.3A)

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.

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 Anion 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
Dye Reagent II	Powderx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate:add 2 mldistilled waterto dissolve before use.

Dye Reagent I: add 8 ml distilled waterto dissolve before use.

Dye Reagent II: add 8 ml absolute alcoholto dissolve before use.

Standard:add 1 ml distilled water to dissolve before use; then add 10 µl into 990 µl distilled water. The concentration will be 1 mmol/L.

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. 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.

V. 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the protein concentration of sample

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

3. According to the weight of sample

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

4. According to the quantity of cell or bacteria

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

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

$C_{Standard}$: the standard concentration, 1mmol/L = 1 μmol/ml;

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

W: the weight of sample, g;

$V_{Standard}$: the volume of standard,40 μl = 0.04 ml;

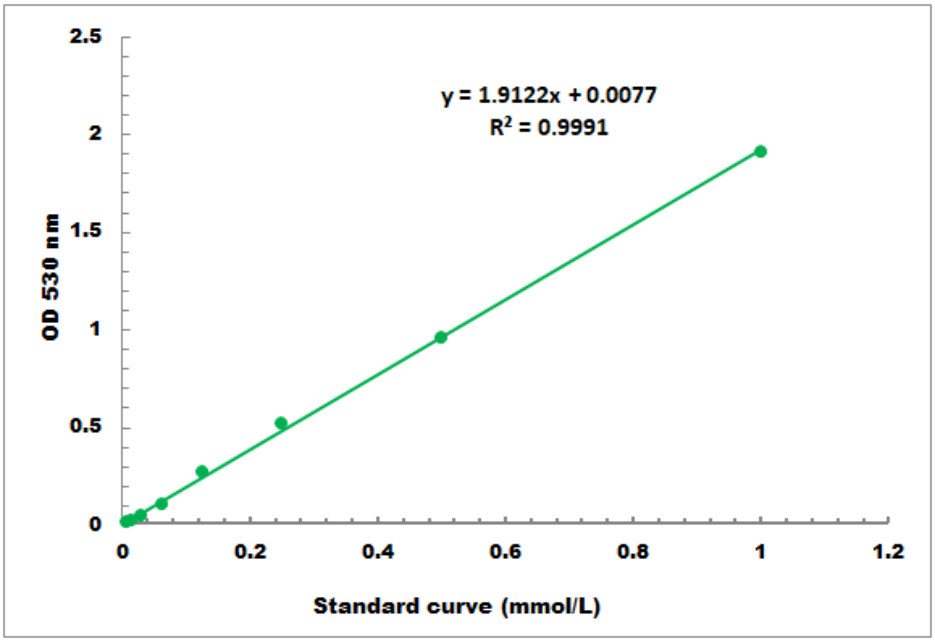
V_{Sample}: the volume of sample,20 μl = 0.02 ml;

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

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES