# Superoxide Dismutase Microplate Assay Kit User Manual

**Catalog # FTA0010**

(Version 1.2F)

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 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 |
| Substrate Diluent | 12 ml x 1 | 4 °C |
| Substrate | Powder x 1 | 4 °C |
| Enzyme | 30 µl x 1 | 4 °C |
| Dye Reagent | Powder x 1 | 4 °C |
| Standard | Powder x 1 | -20 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

**Note:**

**Substrate**: add 1 ml Substrate Diluent into the tube before use, mix; then transfer all substrate to the Substrate Diluent bottle; if any insoluble, please heat at 50 °C to dissolve.

**Dye Reagent**: add 5 ml distilled water to dissolve before use.

**Enzyme**: add 1 ml Reaction Buffer to dissolve before use.

**Standard**: add 1 ml distilled water to dissolve before use, mix. Then add 0.01 ml diluent standard into 0.99 ml distilled water, mix. It will be 30 U/ml.

## 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. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for $5\times10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, 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 into the microplate:

| Reagent | Sample | Standard | Blank |
|---|---|---|---|
| Substrate | 120 µl | 120 µl | 120 µl |
| Enzyme | 10 µl | 10 µl | 10 µl |
| Sample | 20 µl | -- | -- |
| Standard | -- | 20 µl | -- |
| Distilled water | -- | -- | 20 µl |
| Dye Reagent | 50 µl | 50 µl | 50 µl |
| Mix, incubate at 37 °C for 30 minutes, record absorbance measured at 560 nm. | | | |

**Note:**

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

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

## VI. CALCULATION

1. According to the protein concentration of sample

$$SOD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / (V_{Sample} \times C_{Protein})$$

$$= 30 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / C_{Protein}$$

2. According to the weight of sample

$$SOD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / (V_{Sample} \times W / V_{Assay})$$

$$= 30 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 30 \times (OD_{Blank} - OD_{Sample}) / (OD_{Blank} - OD_{Standard}) / N$$

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

$C_{Standard}$: the standard activity, 30 U/ml;

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.02 ml;

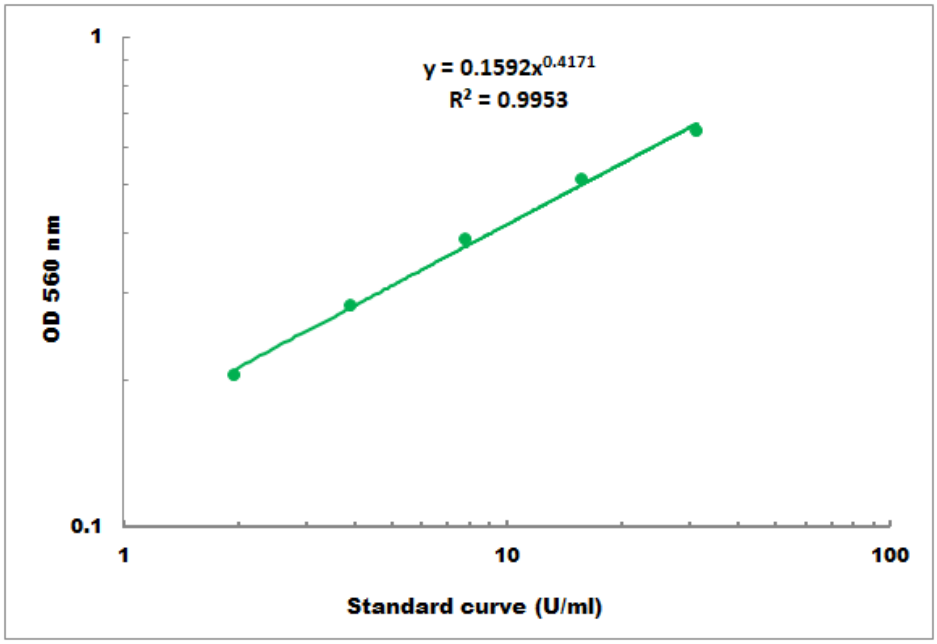
$V_{Sample}$: the volume of sample, 0.02 ml;

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

N: the quantity of cell or bacteria, $N \times 10^4$.

## VII. TYPICAL DATA

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




Detection Range: 1 U/ml - 30 U/ml

## VIII. TECHNICAL SUPPORT

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

## IX. NOTES