Superoxide Dismutase Assay Kit

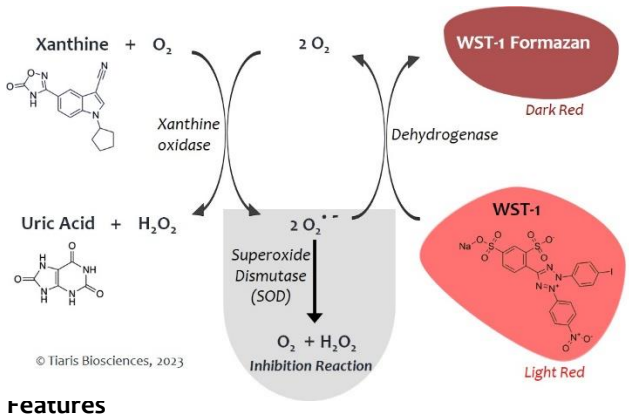
(Colorimetric Readout)

Ordering info

TBK0527, 100 assays

Description

Superoxide Dismutase Assay Kit is an excellent and efficient assay to detect superoxide dismutase (SOD) activity. SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen. **Superoxide Dismutase Assay Kit** is based on WST-1 tetrazolium salt and in the use of xanthine oxidase to generate superoxide radicals. WST-1 is converted into a water-soluble formazan dye by cellular dehydrogenases. The presence of SOD reduces the amount of WST-1 formazan produced. The rate of WST-1 reduction by superoxide anion is linearly related to the xanthine oxidase activity and the inhibition by SOD.



Features

- Accurate, simple and reproducible assay, around 3.2% inter- and intra- assay coefficient of variation.
- Wide dynamic range, linearity from 0.005 to 0.5 U SOD/ mL.
- Colorimetric readout at 450 nm.
- Highly sensitive for measure SOD activity in plasma, serum, urine, saliva, tissues, cells, etc.

Kit Components

Components	TBK0527
Radical Detector (WST-1) [a]	1 mL
SOD Reaction Buffer [a]	25 mL
SOD Dilution Buffer [b]	12 mL
XO Enzyme Solution [b]	30 µL
SOD Standard (500 U/ mL) [c]	50 µL

Order Info Kit Components: *Radical Detector (TBR0277) | SOD Reaction Buffer (TBB0568) | SOD Dilution Buffer (TBB0569) | XO Enzyme Solution (TBZ0328) | SOD Standard (TBZ0329).*

Before its use

[a] Radical Detection Solution: Dilute **1 mL Radical Detector** with **19 mL SOD Reaction Buffer**. Stable 2 months at 4 °C.

[b] Enzyme Working Solution: Spin XO Enzyme Solution tube. Add **25 µL XO Enzyme Solution** in a tube containing **2.5 mL SOD Dilution Buffer.**

Storage

Store the kit at -20 °C, excepting SOD Reaction Buffer and SOD Dilution Buffer.

Quality Control

The kit is tested in a functional assay.

Applications

- Role of SOD in physiological processes such as aging, oxidative stress, inflammation, cellular signaling, etc.
- Antioxidant therapies effectiveness.
- Analysis of environmental stressors.

PROTOCOL

I. SAMPLE PREPARATION

Serum: Collect blood without anticoagulant. Incubate it at room temperature for 30 minutes. Centrifugate at 2 000 g for 15 minutes at 4 °C. Transfer the top layer (serum) to a clean tube. The sample will be stable at -80°C for at least one month. Serum should be diluted ~ 1/5 in PBS 1x before to SOD assay.

Plasma: Collect blood using heparin, citrate, or EDTA as anticoagulant. Centrifuge it at 1 000 g for 15 minutes at 4 °C. Transfer the plasma layer (top) to a clean tube. The sample will be stable at -80 °C for at least one month. Plasma should be diluted ~ 1/3 – 1/10 in PBS 1x before to SOD assay.

Tissue: Wash the tissue with saline or PBS pH 7.2 to remove as much blood as possible. Blot the tissue with paper towels and then measure its weight. Homogenize 10 mg tissue in 5 mL ice-cold Lysis Buffer (50 mM Potassium Phosphate Buffer pH=7.2; 0.1 mM EDTA; 0.5% Triton X-100). Centrifuge the homogenate at 14 000 g for 5 minutes at 4°C. Transfer the supernatant to a clean tube. The sample will be stable at -80°C for at least one month.

Culture Cells (suspension or adherent)**:** Harvest the cells (10^6 – 10^7 cells) as usual and wash them with 1 mL PBS 1x pH=7.2. Centrifuge at 2 000 g for 10 minutes at 4°C. Break cells by sonication (60W, 0.5 sec for 15 minutes), freeze-thaw method (-20°C for 20 min, then 37°C bath 10 min, repeat twice) or resuspending in 5 mL ice-cold Lysis Buffer. Centrifuge at 10 000 g for 15 minutes at 4°C and transfer the supernatant to a clean tube.

For **other samples**, see the Manual of the kit.

II. ASSAY SETUP

All samples must run at least in duplicate. Keep the samples and standard on ice. Equilibrate at room temperature the rest of components.

The assay requires three blanks, sample dilutions and a standard curve. SOD activity can be measured either as percent inhibition activity, which does not require a standard curve, or by a direct comparison to the provided SOD Enzyme, which is used to prepare a standard curve.

- Amount of each solution for sample and blanks:

	SAMPLE	BLANK 1 *without inhibition*	BLANK 2 *sample blank*	BLANK 3 *reagent blank*
Sample	20 µL		20 µL	
Radical Detection Solution	200 µL	200 µL	200 µL	200 µL
XO Enzyme Solution	20 µL	20 µL		
Double Distilled Water		20 µL		20 µL
Dilution Buffer			20 µL	20 µL

- Standard curve: In microcentrifuges tubes, prepare the standard curve by serial dilutions of SOD Standard in SOD Dilution Buffer in a range of 100 U/mL to 0.1 U/mL. Lineal range is between 0.1 – 10 U/ mL.

- Sample Dilutions: Using SOD Dilution Buffer, make sample dilutions to fit SOD activity detected to the linear range of quantitation.

- **Plate setup**: In a flat bottom 96-well plate.

III. SOD ASSAY

1. Following the plate setup:
   - Add **20 µL** of dilution prepared for the **Standard Curve**.
   - Add **20 µL** of **Sample dilutions**.
   - Add **20 µL** of **H2O to** Blank 1 and Blank 3 wells.
   - Add **20 µL** of **SOD Dilution Buffer** to Blank 2 and Blank 3 wells.
2. Add **200 µL Radical Detection Solution** to each well.
   Ensure that the Radical Detector (WST-1) has been diluted in SOD Reaction Buffer.
3. Add **20 µL Enzyme Working Solution** to each well, except Blank 2 and Blank 3.
   Since the reaction is extremely fast (turnover 2x10^9 M^{-1}sec^{-1}), a multichannel pipette may be used to add the Enzyme Working Solution. Take care with Blank wells that not contain the enzyme.
4. Shake the plate carefully.
5. Incubate the plate at 37 °C for 20-30 minutes.
6. Read the absorbance at 450 nm.

IV. SOD ACTIVITY DETERMINATION

A. **By inhibition activity.**
Calculate SOD activity as follow

$$\text{Inhibition Rate (\%)} = \frac{(A_{\text{BLANK 1}} - A_{\text{BLANK 3}}) - (A_{\text{SAMPLE}} - A_{\text{BLANK 2}})}{A_{\text{BLANK 1}} - A_{\text{BLANK 3}}} \times 100\%$$

B. **Using the standard curve.**
See the Manual of the kit.