CheKine™ Superoxide Dismutases (SOD) Activity Assay Kit

Item NO.	Product Name
KTB1030	CheKine™ Superoxide Dismutases (SOD) Activity Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201805

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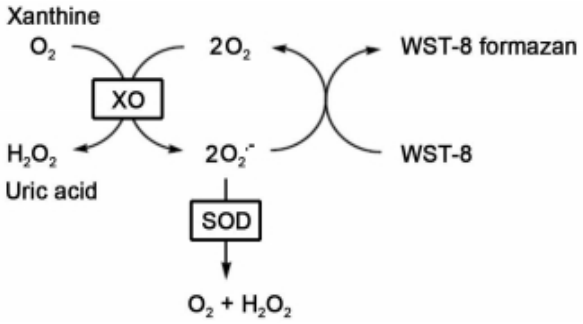
INTRODUCTION

Background

Superoxide dismutases (SOD, EC1.15.1.1) are enzymes that catalyze the dismutation of superoxide anion into O_2 and H_2O_2. They are an important antioxidant defense against the toxicity of superoxide radical in all cells exposed to O_2. Aberrant SOD activities have been linked to diseases such as amyotrophic lateral sclerosis, perinatal lethality, neural disorders and cancer. There are three major families of superoxide dismutase: Cu/Zn, Fe/Mn, and the Ni type. Three forms of superoxide dismutase are present in humans. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular.

Assay principle

The Superoxide dismutase (SOD) Activity Assay Kit provides a simple and easy colorimetric assay for the quantitative determination of SOD enzyme activity in serum, plasma, tissue/cell lysates and other biological fluids. In the assay, superoxide anion (O_2^-) is provided by xanthine oxidase (XO) catalyzed reaction. O_2^- reacts with a tetrazolium salt WST-8 dye to form a water-soluble colored formazan product. SOD scavenges the O_2^- thus less O_2^- is available for the chromogenic reaction. This inhibition activity of SOD is measured by colorimetric method at OD 450 nm.



Storage/Stability

Storage at -20°C and keep from light. The kit has a storage time of 6 months from receipt.

Assay Restrictions

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	48T	96T	480T	
Assay Buffer	10 mL	20 mL	100 mL	4ºC
Sample Diluent	10 mL	20 mL	100 mL	4ºC
WST-8	300 μL	600 μL	3 mL	-20ºC, protect from light
Enhancer	60 μL	120 μL	600 μL	-20ºC, protect from light
SOD Standard (200 U/mL)	200 μL	200 μL	1 mL	-20ºC
Xanthine Oxidase	60 μL	120 μL	600 μL	-20ºC
Xanthine	300 μL	600 μL	3 mL	-20ºC

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PREPARATION

Reagent preparation

Note: Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
Sample Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
WST-8: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
Enhancer: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
SOD Standard: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
Xanthine Oxidase: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
Xanthine: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.

ASSAY PREPARATION

Sample Preparation

Note: If not assayed immediately, samples can be stored at -80°C for one month. All samples can be diluted in 50 mM potassium phosphate, pH 7.4.

1. Tissue samples. Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 5 mL/g in cold lysis buffer (50mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). Centrifuge at 12,000g for 5 minutes at 4°C. Use supernatant for total SOD assay.

2. Cell samples.
1) Suspension cells: Centrifuge 1-2 x 10^6 cells at 800g for 2 minutes and discard supernatant. Wash cells with cold PBS, centrifuge, and discard the supernatant. Resuspend cells in 0.5 mL of cold lysis Buffer (50mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). After 10 min on ice, centrifuge at 12,000g for 5 min. Use supernatant for total SOD assay.
2) Adherent cells: Wash 1-2 x 10^6 cells with cold PBS. Place dish on ice. Add 0.5 mL of cold lysis buffer. After 10 min on ice, collect cells/debris with a rubber policeman. Centrifuge the cell extract at 12,000g for 5 min. Use supernatant for total SOD assay.
Note: if it is desired to determine cytosolic and mitochondrial SOD activities separately, tissue/cell samples can be prepared according to Mattiazzi et al (2002). JBC 277: 29626-33.

3. Blood samples: Collect serum, or plasma (heparin, citrate or EDTA) using standard protocols. The erythrocyte pellet can be lysed in 5x volume of cold dH_2O; centrifuge at 12,000g for 5 min to pellet the erythrocyte membranes. Dilute serum/plasma 1:5, red cell lysate 1:100 prior to SOD assay.

Prior to assay, bring all reagents to room temperature (25°C). The Xanthine reagent may appear to be turbid. Briefly vortext this tube before pipetting.

Assay procedure

1. Standards. Dilute standards as below.

	Volume of Standard	Sample Diluent	Concentration
Std.1	50 µL 200 U/mL SOD	-	200 U/mL
Std.2	50 µL of Std.1 (200 U/mL)	50 µL	100 U/mL
Std.3	50 µL of Std.2 (100 U/mL)	50 µL	50 U/mL
Std.4	50 µL of Std.3 (50 U/mL)	50 µL	25 U/mL
Std.5	50 µL of Std.4 (25 U/mL)	50 µL	12.5 U/mL
Std.6	50 µL of Std.5 (12.5 U/mL)	50 µL	6.25 U/mL
Std.7	50 µL of Std.6 (6.25 U/mL)	50 µL	3.13 U/mL
Blank	-	50 µL	0 U/mL

Transfer 20 µL SOD standards to separate wells of a clear flat-bottom 96-well plate. Transfer 20 µL samples to separate wells.

2. Prepare enough Working Reagent for the standard and sample wells.
1) For each well, mix 74 µL Assay Buffer, 5 µL Xanthine, 5 µL WST-8 and 1µL Enhancer. Transfer 80 µL Working Reagent to each well and tap plate to mix.
2) For each well, dilute the Xanthine Oxidase 1:20 in Sample Diluent. Quickly add 20 µL diluted Xanthine Oxidase to each assay well (use of a multi-channel pipettor is recommended). Tap plate to mix.

3. Immediately read optical density at 450nm (OD_0). Incubate for 60 min at room temperature (25°C) in the dark. Read optical density at 450nm again (OD_{60}).

DATA ANALYSIS

Calculation of results

For each standard and sample well, calculate $\Delta OD_{60} = OD_{60} – OD_0$.
Calculate $\Delta\Delta OD_{60} = \Delta OD_{60Blank} - \Delta OD_{60}$ for each standard and sample where $\Delta OD_{60Blank}$ is the ΔOD_{60} for the Blank with no SOD activity and highest possible absorbance.
Plot the Standard Curve $\Delta\Delta OD_{60}$ vs [SOD] (U/mL). Use the $\Delta\Delta OD_{60}$ for sample to determine SOD activity of sample from the standard curve.