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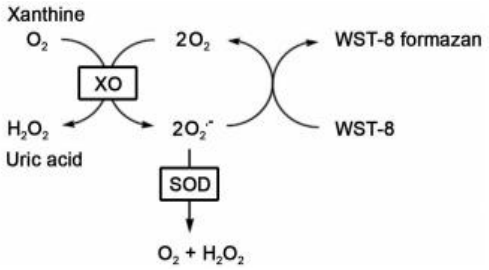
CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit

Cat #: KTB1030 Size: 48 T/96 T

	Micro Superoxide Dismutases (SOD) Activity Assay Kit		
REF	**Cat #:** KTB1030	LOT	**Lot #:** Refer to product label
	Applicable samples: Serem, Plasma, Animal and Plant Tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

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. CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit provides a simple and easy assay for the quantitative determination of SOD enzyme activity in serum, plasma, animal and plant tissues, cells 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. The activity of SOD was negatively correlated with the amount of formazan.



Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	10 mL	20 mL	4℃
Sample Diluent	10 mL	20 mL	4℃

Lysis Buffer (5×)	10 mL	20 mL	4℃
WST-8	300 μL	600 μL	-20℃, protected from light
Enhancer	60 μL	120 μL	-20℃, protected from light
Xanthine Oxidase	60 μL	120 μL	-20℃
Xanthine	300 μL	600 μL	-20℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, centrifuge
- Deionized water
- Dounce homogenizer (for tissue samples)

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℃.

Sample Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

1×Lysis Buffer: Equilibrate to room temperature before use. Dilute Lysis Buffer (5×) with deionized water to 1×Lysis Buffer. Store at 4℃.

WST-8: Ready to use as supplied. Keep on ice protected from light during the assay. Store aliquots at -20℃, protected from light.

Enhancer: Ready to use as supplied. Keep on ice protected from light during the assay. Store aliquots at -20℃, protected from light.

Note: Enhancer is normally a magenta solution, please contact Abbkine staff in case of yellowing.

Xanthine: Ready to use as supplied. Keep on ice during the assay. Store aliquots at -20℃. The Xanthine reagent may appear to be turbid. Briefly vortex this tube before pipetting.

Working Xanthine Oxidase: Store aliquots at -20℃. Take an appropriate amount and dilute 20 times with Sample Diluent before use. Keep on ice during the assay, protected from light.

Working Reagent: Prepare before use. For each well, mix 74 μL Assay Buffer, 5 μL Xanthine, 5 μL WST-8 and 1 μL Enhancer.

Sample Preparation

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL 1×Lysis Buffer and homogenize on ice. Centrifuge at 12,000 g at 4℃ for 5 min, use supernatant for detection.

2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, centrifuge at 800 g for 2 min, discard the supernatant. Resuspend cells in 1 mL cold 1×Lysis Buffer. After 10 min on ice, centrifuge at 12,000 g at 4℃ for 5 min, use supernatant for detection.

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. Serum, Plasma: Serum is collected according to conventional methods, and diluted by Sample Diluent for detection; Blood is collected with anticoagulants and mix upside down. Centrifuge at 4℃, 600 g for 10 min, transfer the supernatant to another new tube and diluted by Sample Diluent for detection.

4. Plant Tissues: Weigh 0.1 g tissue, add 1 mL of cold 1×Lysis Buffer, and ultrasonic break on ice for 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s). Centrifuge at 12,000g for 5 min at 4℃, use supernatant for detection.

Note: If not assayed immediately, samples can be stored at -80℃ for one month. For additional measurement, it is

recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Incubate the Working Reagent at 37℃ (for mammals) or 25℃ (for other species) for more than 5 min before measurement.
3. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Sample well (μL)	Sample Control well (μL)	Blank well (μL)	Blank Control well (μL)
Sample	20	20	0	0
Working Reagent	80	0	80	0
Working Xanthine Oxidase	20	0	20	0
Sample Diluent	0	100	20	120

4. Mix well, incubate at 37℃ for 30 min, read optical density at 450 nm. Finally, calculate $\Delta A_{Sample}=A_{Sample}-A_{Sample\ Control}$, $\Delta A_{Blank}=A_{Blank}-A_{Blank\ Control}$.

Note: The Blank well and Blank Control only needs to measure 1 time. Each different color sample should set a corresponding control well, to eliminate the background OD value of the sample itself. If the measured value of the sample is larger than that of the blank well, it may be because the influence of impurities in the sample is too great. In order to reduce the influence of impurities, the supernatant of the sample is extracted and then tested with Sample Diluent diluted 10 times, which can normally make the test normal. Multiply by the corresponding dilution in the calculation formula.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of Inhibition percentage

Inhibition percentage=$(\Delta A_{Blank}-\Delta A_{Sample})\div\Delta A_{Blank}\times100\%$

Note: Try to keep the sample's inhibition percentage in the range of 10-90%. If the calculated inhibition percentage is less than 10% or greater than 90%, it is usually necessary to adjust the sample amount. If the determined inhibition percentage is too high, the sample needs to be appropriately diluted with Sample Diluent; if the determined inhibition percentage is low, it is necessary to increase sample concentration.

2. SOD active unit definition: When the inhibition percentage in the above-mentioned xanthine oxidase coupling reaction system is 50%, the SOD enzyme activity in the reaction system is defined as an enzyme activity unit (U/mL).

3. Calculation of SOD activity

(1) Calculation of serum (plasma) SOD activity

SOD activity (U/mL)=[Inhibition percentage÷(1-Inhibition percentage)×V_{Total}]÷V_{Sample}×n

=6×Inhibition percentage÷(1-Inhibition percentage)×n

(2) Calculation of tissues/cells SOD activity

a. Calculated by protein concentration

SOD activity (U/mg prot)=[Inhibition percentage÷(1-Inhibition percentage)×V_{Total}]÷(V_{Sample}×Cpr)×n

=6×Inhibition percentage÷(1-Inhibition percentage)÷Cpr×n

b. Calculated by fresh weight of samples

SOD activity (U/g fresh weight)=[Inhibition percentage÷(1-Inhibition percentage)×V_{Total}]÷(W×V_{Sample}÷$V_{Sample\ Total}$)×n

=6×Inhibition percentage÷(1-Inhibition percentage)÷W×n

c. Calculated by number of cells

SOD activity (U/10^4 cells)=[Inhibition percentage÷(1-Inhibition percentage) ×V_{Total}]÷(500×V_{Sample}÷$V_{Sample\ Total}$)×n

=0.012×Inhibition percentage÷(1-Inhibition percentage)×n

Where: V_{Total}: Total reaction volume, 0.12 mL; V_{Sample}: sample volume added, 0.02 mL; $V_{Sample\ Total}$: 1×Lysis Buffer added to samples,1 mL; n: dilution factor; Cpr: sample protein concentration, mg/mL; 500: Total number of cells, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide (H_2O_2) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.