CheKine™ Micro Superoxide Anion Scavenging Capacity Assay Kit

Cat #: KTB1080 Size: 48 T/96 T

	Micro Superoxide Anion Scavenging Capacity Assay Kit		
REF	**Cat #**: KTB1080	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Blood, Drug		
	Storage: Storage at -20℃ for 6 months, protected from light		

Assay Principle

The superoxide anion (O_2^-) is a short-lived radical production by the addition of an electron to oxygen. It is formed in response to environmental factors such as UV light, cigarette smoke, environmental pollutants, and -radiation, or derived from oxidases like xanthine oxidase or NADPH oxidase. Once formed, O_2^- attacks cellular components and causes damage to lipids, proteins and DNA. This can initiate numerous diseases, including cancer, atherosclerosis, rheumatoid arthritis, diabetes, liver damage, and central nervous system disorders. The study of scavenging superoxide anion radicals has been gained more and more attention. CheKine™ Micro Superoxide Anion Scavenging Capacity Assay Kit provides a simple and easy colorimetric assay for the study of Superoxide anion Scavenging ability in animal and plant tissues, cell and other biological fluids. In this 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, which can be easily quantified. Sample scavenges the O_2^- thus less O_2^- is available for the chromogenic reaction. This scavenging ability of sample is measured by colorimetric method at OD 450 nm.

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℃
WST-8	300 μL	600 μL	-20℃, protect from light
Enhancer	60 μL	120 μL	-20℃, protect 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 OD450 nm

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• 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
• Ice maker, refrigerated centrifuge
• Deionized water
• Dounce homogenizer (for tissue samples)

Reagent Preparation

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℃.
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℃, protect from light.
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℃, protect from light.
Working Xanthine Oxidase: Mix well prior to making dilutions. Make a 1:20 dilution of the concentrated Xanthine Oxidase solution with Sample Diluent in a clean plastic tube as needed according to the Positive control and samples. Keep on ice during the assay. Store aliquots at -20℃.
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 Reagent: For 96-well plates, prepare 85 μL Working Reagent for each well, mix 74 μL Assay Buffer, 5 μL Xanthine, 5 μL WST-8 and 1 μL Enhancer. Working Reagent is freshly prepared.

Sample Preparation

1. Animal Tissues: Perfuse tissue with ice-cold PBS to remove any red blood cells. Homogenize tissue at 1 mL/0.1 g in ice-cold Lysis Buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissue, add 1 mL ice-cold Lysis Buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100) and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells: Collect 5×10^6 cells, wash cells with ice-cold PBS, centrifuge, and discard the supernatant. Resuspend cells in 1 mL of ice-cold Lysis Buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). Incubate on ice for 10 min, and then centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Blood Samples: Collect serum, or plasma (heparin, citrate or EDTA) using standard protocols. The erythrocyte pellet can be lysed in 5×volume of ice-cold deionized water; centrifuge at 12,000 g for 5 min to pellet the erythrocyte membranes. Dilute serum/plasma (1:5), red cell lysate (1:100) with Sample Diluent prior to assay.
5. Drug: Diluted into a certain concentration. For example, 1 mg/mL.

Note: The following substances should be avoided in sample preparation. Ascorbic acid, Sodium Azide, >0.2% SDS, >1% NP-40 and >1% Tween-20. If not assayed immediately, samples can be stored at -80℃ for 1 month. All samples can be diluted in 50 mM potassium phosphate, pH 7.4.

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. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Control Well (μL)	Test Well (μL)
Sample	0	0	20

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Sample Diluent	40	20	0
Working Reagent	80	80	80
Working Xanthine Oxidase	0	20	20

3. Mix well. The absorbance values at 450 nm were measured as A_0, incubate for 60 min at room temperature in the dark, and then record the absorbance values at 60 min as A_{60}. calculate $\Delta A = A_{60} - A_0$, recorded as ΔA_{Blank}, $\Delta A_{Control}$, ΔA_{Test}, respectively, and $\Delta\Delta A_{Control} = \Delta A_{Control} - \Delta A_{Blank}$, $\Delta\Delta A_{Test} = \Delta A_{Test} - \Delta A_{Blank}$.

Data Analysis

Superoxide anion scavenging rate calculation formula:

$D\% = (\Delta\Delta A_{Control} - \Delta\Delta A_{Test}) \div \Delta\Delta A_{Control} \times 100\%$

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1210	CheKine™ Micro Superoxide Anion Assay Kit

Disclaimer

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

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