CheKine™ Micro Xanthine Oxidase Activity Assay Kit

Cat #: KTB1070 Size: 48 T/96 T

	Micro Xanthine Oxidase Activity Assay Kit		
REF	**Cat #:** KTB1070	LOT	**Lot #:** Refer to product label
	Detection range: 15.6-500 mU/mL		**Sensitivity:** 15.6 mU/mL
	Applicable samples: Animal and Plant Tissues, Cells, Serum or Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Xanthine oxidase (EC 1.17.3.2), or xanthine oxidoreductase is recognized as the terminal enzyme of purine catabolism in humans, catalyzing the hydroxylation of hypoxanthine to xanthine and then to uric acid. When acting as an NADH oxidase, XO is a generator of superoxide (O^{2-}), a powerful reactive oxygen species (ROS). XO has also been noted to produce hydrogen peroxide (H_2O_2) and superoxide (O^{2-}) during ischemia-reperfusion injury. Due to their highly reactive nature, these ROS affect various molecular components of the cell, with excess amounts leading to cell degeneration and death. XO is present in appreciable amounts in the liver and jejunum in healthy individuals. However, in various liver disorders, levels of circulating XO have been seen to increase dramatically. Therefore, determination of serum XO level serves as a sensitive indicator of acute liver damage such as jaundice. CheKine™ Micro Xanthine Oxidase Activity Assay Kit provides a simple method for detecting XO concentration in a variety of samples, including Animal and Plant Tissues, Cells, Serum or Plasma. The principle is XO oxidizes xanthine to superoxide (O^{2-}). Superoxide (O^{2-}) reacts with a tetrazolium salt WST-8 dye to form a water-soluble colored formazan product, which can be easily quantified at 450 nm. Therefore, the Xanthine Oxidase present in the sample is proportional to the signal obtained.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	10 mL	20 mL	4℃
Sample Diluent	5 mL	10 mL	4℃
WST-8	300 μL	600 μL	-20℃, protected from light
Enhancer	60 μL	120 μL	-20℃, protected from light
Xanthine	300 μL	600 μL	-20℃

Materials Required but Not Supplied

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- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette
- Refrigerated centrifuge, water bath
- Precision pipettes, disposable pipette tips
- Deionized water
- 50 mM phosphate buffer solution (ratio: 0.05 mol/L K_2HPO_4 : 0.05 mol/L KH_2PO_4=80.2: 19.8; pH 7.4), EDTA, Triton X-100
- 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℃.

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

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

Xanthine: Ready to use as supplied. Keep on ice before use. Store in aliquots at -20℃.

Working Reagent: For 96-well plates, 85 μL of Working Reagent per well, prepare before use and use immediately. Working Reagent ratio: 74 μL Assay Buffer, 5 μL Xanthine, 5 μL WST-8, 1 μL Enhancer. Working Reagent is freshly prepared.

Sample Preparation

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL lysate (50 mM phosphate buffer, 0.1 mM EDTA, 0.5%Triton X-100), and homogenize on ice. 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 lysate (50 mM phosphate buffer, 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 into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL lysate (50 mM phosphate buffer, 0.1 mM EDTA, 0.5%Triton X-100) to ultrasonically disrupt the cell 5 min (power 20%, 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.
4. Plasma and Serum: Direct detection.

Note:

1. If not assayed immediately, fresh samples can be stored at -80℃ for one month. All samples after lysis can be diluted in 50 mM phosphate buffer (pH 7.4).

2. The Xanthine reagent may appear to be turbid. Briefly vortex this tube before pipetting.

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. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Test Well (μL)
Supernatant	0	20
Sample Diluent	20	0
Working Reagent	80	80

3. Mix well and kept at room temperature (25℃) and protect from light for 30 min. The absorbance value (OD value) is measured at 450 nm. The Blank Well is marked as A_{Blank} and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$.

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Note: Blank only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the A_{Test} values are higher than 1.0, dilute the supernatant with Sample Diluent to an appropriate multiple. Multiply the results with the dilution factor.

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. Standard curve formula: y=574.46x+8.2497, R^2=0.9995, where: x is $\Delta A_{Standard}$; y is the concentration of the standard solution (mU/mL).

2. Calculation of the concentration of XO in the sample:

Bring the ΔA_{Test} of the sample into the equation to get the y value (mU/mL).

(1) Calculated by fresh weight of samples

XO activity (mU/g fresh weight)=$y\times V_{Sample}\div(W\times V_{Sample}\div V_{Sample\ total})\times n$**=y÷W×n**

(2) Calculated by volume of liquid samples

XO activity (mU/mL)=$y\times V_{Sample}\div V_{Sample}\times n$**=y×n**

(3) Calculated by number of cells

XO activity (mU/10^4)=$y\times V_{Sample}\div(\text{number of Cell}\times V_{Sample}\div V_{Sample\ total})\times n$ **=y÷500×n**

Where: V_{Sample}: add sample volume, 0.05 mL; W: weight of sample, g; $V_{Sample\ total}$: add Assay Buffer volume to sample, 1 mL; n: the sample dilution factor; 500: Total number of cells, 5×10^6.

Note: If the sample is further diluted, it needs to be multiplied by the dilution factor n.

Recommended Products

Catalog No.	Product Name
KTB1140	CheKine™ Micro Polyphenol Oxidase (PPO) Activity Assay Kit
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) 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.

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