CheKine™ Micro Tyrosinase (Tyr) Activity Assay Kit

Cat #: KTB1071 Size: 48 T/48 S 96 T/96 S

	Micro Tyrosinase (Tyr) Activity Assay Kit		
REF	**Cat #:** KTB1071	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Fungus, Plasma, Serum		
	Storage: Stored at 4℃ for 24 months, protected from light		

Assay Principle

Tyrosinase (EC 1.14.18.1) is a structurally complex, multi-subunit copper-containing redox enzyme widely found in microorganisms, animals, plants, and the human body. It serves as a key enzyme in melanin synthesis and is also closely involved in processes such as fruit and vegetable browning, fungal tissue protection, and insect wound healing and development. CheKine™ Micro Tyrosinase Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, cells, bacteria, fungus, plasma, serum.

The principle is based on the fact that tyrosinase catalyzes the conversion of L-Dopa to dopaquinone, which subsequently reacts with MBTH to form a pink conjugate. The absorbance value at 505 nm is measured to determine the activity of tyrosinase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	72 mL	72 mL×2	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅱ	2.4 mL	4.8 mL	4℃, protected from light
Reagent Ⅲ	0.9 mL	1.8 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; During the experiment, it was placed on the ice; Store at 4℃.

Working Reagent Ⅰ: Prepared before use; add 12 mL of Extraction Buffer to 48 T, and add 24 mL of Extraction Buffer to 96 T, ensuring complete dissolution for subsequent use; The remaining reagents can be stored at-20℃ protected from light for up to 4 weeks after aliquoting; avoid repeated freezeing and thawing.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent Ⅲ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Note: Working Reagent Ⅰ dissolves slowly, it must be thoroughly mixed using a pipette by vigorous blowing. During the mixing process, excessive air bubbles should be avoided.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, the intact cells or packaged tissue sections should be stored at -80℃ for one month. When measuring, remove the sample from-80℃ and thaw it on ice.

1. Animal and Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice(Recommended parameter:add three 3 mm steel beads, at 55 HZ, homogenize for 30s, with 10-second intervals, and repeat 4 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria:Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to homogenize or mortar on ice(Recommended parameter:add three 3 mm steel beads, at 40 HZ, homogenize for 20s, with 10-second intervals, and repeat 3 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Fungus: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice or grind manually until no obvious tissue fragments remain. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma and Serum: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in 1.5 mL EP tube.

Reagent	Test Tube (μL)	Blank Tube (μL)
Working Reagent Ⅰ	200	200
Reagent Ⅱ	40	40
Sample	27	0
Deionized Water	0	27
Mix well, Incubate at 37℃ for 20 min		
Reagent Ⅲ	13	13

3. Mix well, Centrifuge at 12,000 g for 3 min at 4℃ take 200 μL into 96-well microplate or microglass cuvette, record the absorbance at 505 nm. The Test Well is marked as A_{Test}.,the Blank Well is recorded as A_{Blank}.Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$.

Note: (1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. (3) If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1, the sample can be appropriately diluted with Extraction Buffer, or decrease the sample quantity appropriately.

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.

Calculation of the tyrosinase activity

1. Calculated by protein concentration

Active unit definition: A unit of enzyme activity is defined as the amount that causes an absorbance change of 0.005 per minute at 505 nm per milligram of tissue protein in the reaction system.

Tyrosinase (U/mg prot)=$\Delta A_{Test} \times V_{Total} \div (V_{Sample} \times Cpr) \div 0.005 \div T$=**$103.7 \times \Delta A_{Test} \div Cpr$**

2. Calculated by sample fresh weight

Active unit definition:A unit of enzyme activity is defined as the amount that causes an absorbance change of 0.005 per minute at 505 nm per g tissue in the reaction system.

Tyrosinase (U/g)=$\Delta A_{Test} \times V_{Total} \div (V_{Sample} \div V_{Total\ sample} \times W) \div 0.005 \div T$=**$103.7 \times \Delta A_{Test} \div W$**

3. Calculated by bacteria or cell number

Active unit definition:A unit of enzyme activity is defined as the amount that causes an absorbance change of 0.005 per minute at 505 nm per 10^6 bacteria or cells in the reaction system.

Tyrosinase (U/10^6)=$\Delta A_{Test} \times V_{Total} \div (V_{Sample} \div V_{Total\ sample} \times N) \div 0.005 \div T$=**$103.7 \times \Delta A_{Test} \div N$**

4. Calculated by volume of serum (plasma)

Active unit definition: A unit of enzyme activity is defined as the amount that causes an absorbance change of 0.005 per minute at 505 nm per mL of serum (plasma) in the reaction system.

Tyrosinase (U/mL)=$\Delta A_{Test} \times V_{Total} \div V_{Sample} \div 0.005 \div T$=**$103.7 \times \Delta A_{Test}$**

V_{Total}: Total volume of reaction system, 0.28 mL; V_{Sample}: Added the sample volume, 0.027 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL;T: Reaction time, 20 min; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; N: total number of cells, use 10^6 as the unit (e.g., total number of cells: 5×10^6, N=5).

Typical Data

Example:

1. Test 0.103 g mouse kidney, prepared the sample following the above protocol and measured with the 96-well plate. The measured values were: A_{Test}=0.143, A_{blank}=0.048, and $\Delta A_{Test} = A_{Test} - A_{blank}$=0.143–0.048=0.095. Calculated based on sample mass: Tyrosinase (U/g) =$103.7 \times \Delta A_{Test} \div W$=95.646 U/g.

2. Test 0.100 g spinach, prepared the sample following the above protocol and measured with the 96-well plate. The measured values were: A_{Test} =0.293, A_{blank}=0.046, and $\Delta A_{Test} = A_{Test} - A_{blank}$=0.293-0.046=0.247. Calculated based on sample mass: Tyrosinase (U/g) =$103.7 \times \Delta A_{Test} \div W$ =256.139 U/g.

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1551	CheKine™ Micro Non-protein Sulfhydryl Content Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit-20260205
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.