CheKine™ Micro Manganese Peroxidase (MnP) Activity Assay Kit

Cat #: KTB1151 Size: 48 T/48 S 96 T/96 S

	Micro Manganese Peroxidase (MnP) Activity Assay Kit		
REF	**Cat #:** KTB1151	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Manganese peroxidase (MnP, EC1.11.1.13) is a type of peroxidase containing heme, mainly found in basidiomycetes. It belongs to the lignin degrading enzyme system and can effectively degrade lignin, as well as difficult to degrade chlorides, azides, DTT, polycyclic aromatic hydrocarbons, etc. in wastewater and soil. Under the presence of Mn^{2+}, MnP oxidizes guaiacol to tetra methoxyphenol, with a characteristic absorption peak at 465 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70×2 mL	4℃
Reagent Ⅰ	1.2 mL	2.4 mL	4℃
Reagent Ⅱ	2.5 mL	5 mL	4℃, protected from light
Reagent Ⅲ	1.2 mL	2.4 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 465 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, incubator
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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

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℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Add Extraction Buffer in a ratio of 1:5-10 based on sample mass (g) and Extraction Buffer volume (mL) (it is recommended to weigh approximately 0.1 g of tissue and add 1 mL of Extraction Buffer) and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or Cells: Collect bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation, and add the Extraction Buffer in a ratio of 500-1000:1 based on the number of bacteria or cells (10^4): Extraction Buffer volume (mL) (it is recommended to add 1 mL of Extraction Buffer to 5 million bacteria or cells) to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 465 nm, visible spectrophotometer was returned to zero with deionized water.
2. Before measurement, take out a portion of the Extraction Buffer, Reagent I, Reagent II, and Reagent III according to the experimental dosage, and preheat them at 37℃ (for mammals) or 25℃ (for other animals) for more than 10 min.
3. Enzymatic reaction (The following operations are operated in the microglass cuvette or 96-well plate):

Reagent	Test Well (μL)
Sample supernatant	20
Extraction Buffer	100
Reagent I	20
Reagent II	40
Reagent III	20

Mix well and measure the absorbance value A_1 at 465 nm for 30 s and the absorbance value A_2 after 2 min and 30 s. Calculate $\Delta A=A_2-A_1$.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If there are too many samples to be tested at once, Extraction Buffer, Reagent I, Reagent II, and Reagent III can be mixed into a working solution in a ratio of 5:1:2:1 according to the amount used, and then preheated. During the measurement, follow a ratio of 20 μL sample + 180 μL working solution to a microglass cuvette or 96 well plate for measurement. If the ΔA is greater than 0.6, it is recommended to dilute the sample with Extraction Buffer and measure it; If the ΔA is less than 0.001, the sample size can be increased appropriately before retesting. Pay attention to synchronously modifying calculation formulas

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.

A. 96-well plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: The amount of enzyme required per mg of tissue protein to oxidize 1 nmol of guaiacol per min in the reaction system is one enzyme activity unit.

MnP (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T**=826×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: The amount of enzyme required to oxidize 1 nmol of guaiacol per min in the reaction system is one enzyme activity unit per g of tissue.

MnP (U/g fresh weight)=ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷T**=826×ΔA÷W**

3. Calculated by number of bacteria or cells

Active unit definition: The amount of enzyme required for every 10^4 bacteria or cells to oxidize 1 nmol of guaiacol per min in the reaction system is one enzyme activity unit.

MnP (U/g 10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(500×V_{Sample}÷$V_{Total\ Sample}$)÷T**=1.652×ΔA**

4. Calculated by sample volume

Active unit definition: The amount of enzyme required per mL of serum (plasma) to oxidize 1 nmol of guaiacol per min in the reaction system is one enzyme activity unit.

MnP (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T**=826×ΔA**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: molar extinction coefficient of guaiacol: 12,100 L/mol/cm; d: 96-well plate diameter, 0.5 cm; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: Sample mass, g; T: reaction time, 2 min; 500: total number of bacteria or cells, 5×10^6.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

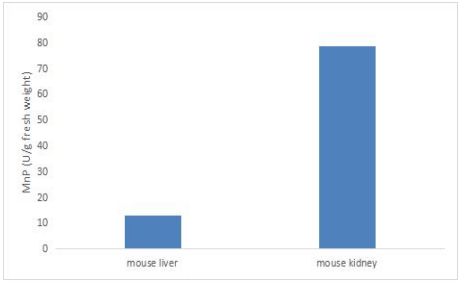

Figure 1. Determination MnP activity in mouse liver and mouse kidney by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric 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.