CheKine™ Micro Monoamine Oxidase (MAO) Activity Assay Kit

Cat #: KTB1900 Size: 48 T/96 T

	Micro Monoamine Oxidase (MAO) Activity Assay Kit		
REF	**Cat #:** KTB1900	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Monoamine Oxidase (MAO, EC1.4.3.4) mainly exists in various organs of vertebrates, especially secretory glands, brain and liver. It also exists in plants such as invertebrates and legumes. Although the content of MAO is low in vivo, it has important physiological functions and its activity can reflect the degree of liver fibrosis. In addition, the abnormal activity of MAO leads to the disorder of the monoamine neurotransmitter in the cell, which leads to a variety of diseases. CheKine™ Micro Monoamine Oxidase (MAO) Activity Assay Kit provides a simple method for detecting MAO activity in a variety of biological samples such as serum, plasma, animal and plants tissues, cells, bacteria. In the assay, MAO catalyzes the deamination of monoamine substrates to the corresponding aldehydes, which are further oxidized to acid. The substrate has a characteristic absorption peak at 360 nm. The rate of monoamine substrates decrease at 360 nm can reflect MAO activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	50 mL	100 mL	4℃
Extraction Buffer Ⅱ	50 mL	100 mL	4℃
Assay Buffer	60 mL	100 mL+20 mL	4℃
Substrate	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 360 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, water bath
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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

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

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

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal and plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize. Centrifuge at 1,000 g for 10 min at 4℃, discard the precipitate. Transfer the supernatant to another pre-cooled centrifuge tube, and 4℃, 10,000 g centrifuge for 30 min, discard supernatant. Add 1 mL pre-cooled Extraction Buffer II to the precipitation, shake to mix well, and 4℃, 16,000 g centrifuge for 40 min, discard supernatant. Add 1 mL pre-cooled Assay Buffer, shake to mix well. Keep it on ice to be tested.

2. Cells or bacteria: Collect 5 x 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 Ⅰ 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 1,000 g for 10 min at 4℃, discard the precipitate. Transfer the supernatant to another pre-cooled centrifuge tube, and 4℃, 10,000 g centrifuge for 30 min, discard supernatant. Add 1 mL pre-cooled Extraction Buffer Ⅱ to the precipitation, shake to mix well, and 4℃, 16,000 g centrifuge for 40 min, discard supernatant. Add 1 mL pre-cooled Assay Buffer, shake to mix well. Keep it on ice to be tested.

3. Serum (plasma) sample: Tested directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 360 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. For blank well, add 180 μL Assay Buffer and 20 μL Substrate. Tap the plate gently to mix the components thoroughly. The absorbance values were measured at 360 nm is A_1. 37℃ water bath for 60 min, and measure optical density at 360 nm again as A_2.

3. For test well, add 20 μL sample, 160 μL Assay Buffer and 20 μL Substrate. Tap the plate gently to mix the components thoroughly. The absorbance values were measured at 360 nm is A_3. 37℃ water bath for 60 min, and measure optical density at 360 nm again as A_4.

4. Calculate: $\Delta A=(A_3-A_4)-(A_1-A_2)$.

Note: It is need to use UV microplate. Blank well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with Assay Buffer, the calculated result multiplied by the dilution factor, 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.

A. 96-well UV plate calculation formula

1. Calculated by protein concentration

Active unit definition: The enzyme that converts 1 nmol of substrate in 1 min from 1 mg protein at 37℃, pH 7.6 is defined as a unit of enzyme activity.

MAO (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^6]÷(V_{Sample}×Cpr)÷T**=228×ΔA÷Cpr**

2. Calculated by fresh weight of samples

Active unit definition: The enzyme that converts 1 nmol of substrate in 1 min from 1 g sample at 37℃, pH 7.6 is defined as a unit of enzyme activity.

MAO (U/g)=[ΔA×V_{Total}÷(ε×d)×10^6]÷(V_{Sample}÷$V_{Sample\ Total}$×W)÷T**=228×ΔA÷W**

3. Calculated by bacteria, cells numbers

Active unit definition: The enzyme that converts 1 nmol of substrate in 1 min from 1×10^4 bacteria or cells at 37℃, pH 7.6 is defined as a unit of enzyme activity.

MAO (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^6]÷(500×V_{Sample}÷$V_{Sample\ Total}$)÷T=228×ΔA÷500**=0.456×ΔA**

4. Calculation by liquid volume

Active unit definition: The enzyme that converts 1 nmol of substrate in 1 min from 1 mL serum or plasma at 37℃, pH 7.6 is defined as a unit of enzyme activity.

MAO (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^6]÷(V_{Sample}×10^{-3})**=228×ΔA**

Where: V_{Total}: Total reaction volume, 0.2 mL; ε: Substrate molar extinction coefficient, 1460 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm ;10^6: Unit conversion factor, 1 mL/nmol/cm=1×10^6 L/mol/cm; V_{Sample}: Sample volume added, 0.02 mL; Cpr: Sample protein concentration, mg/mL; T: Reaction time, 60 min; $V_{Sample\ Total}$: Extraction Buffer Ⅰ volume added, 1 mL; W: Sample weight, g; 500: Total number of cells, bacterium, 5×10^6; 10^{-3}: 1mL=1×10^{-3} L.

B. Microquartz cuvette calculation formula

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

Recommended Products

Catalog No.	Product Name
KTB1700	CheKine™ Micro Tissue and Blood Alkaline Phosphatase (AKP/ALP) Assay Kit
KTB1070	CheKine™ Micro Xanthine Oxidase (XO) Assay Kit

Disclaimer

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