Monoamine Oxidase (MAO) Activity Assay Kit

Cat. No. KITG-046 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Monoamine oxidase (MAO) is mainly present in various organs of vertebrates, especially secretory glands, brain, and liver. It is also found in invertebrates and plant sprouts such as legumes, where it catalyzes the metabolic process of monoamine substances. MAO has important physiological functions, and its activity can reflect the degree of liver fibrosis. Abnormal metabolism can easily lead to disorders in intracellular monoamine neurotransmitter transport, thereby causing various diseases. Monoamine oxidase can catalyze the deamination of monoamine substrates to generate corresponding aldehydes, which are further oxidized to acids. The substrate has a characteristic absorption peak at 360 nm. The activity of monoamine oxidase can be characterized by the rate of change in absorbance.
Description	This Monoamine Oxidase (MAO) Activity Assay Kit provides a convenient and sensitive ultraviolet spectrophotometric method for measuring MAO activity. The assay is based on the catalytic deamination of monoamine substrates by MAO, producing aldehydes that are further oxidized to acids. The substrate has a characteristic absorption peak at 360 nm, and the enzyme activity is determined by measuring the rate of change in absorbance. The kit is suitable for tissue samples, bacterial/cell samples, and liquid samples. The kit includes all necessary components for performing the assay and can accommodate 50 tests/48 samples.
Form	Liquid
Applications	Measurement of monoamine oxidase activity in tissue homogenates; Detection of MAO activity in bacterial and cell lysates; Determination of MAO activity in serum and

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	plasma samples; Quality control of MAO preparations; Research on monoamine oxidase-related physiological and pathological processes including liver fibrosis and neurotransmitter metabolism disorders
Notes	This kit is for research use only and not for use in diagnostic procedures. ΔA should be controlled within the range of 0.02-0.8. It is recommended to appropriately adjust the sample volume or dilute the sample before measurement, and modify the calculation accordingly. To ensure accurate results and avoid reagent loss, please carefully read the instructions before measurement to confirm whether reagent storage and preparation are adequate and whether the operation steps are clear. It is essential to select 2-3 samples with expected large differences for pre-testing. Store the kit at 2-8°C. For research use only. Not for use in diagnostic procedures.
Storage	Store at 2-8°C.
Shipping	Shipped with ice packs
Reaction conditions	Reaction temperature: 37°C Reaction time: 60 min Measurement wavelength: 360 nm Cuvette: 1 mL quartz cuvette (path length 10 mm) Preheating time: 30 min
Unit definition	Unit definition at 37°C, pH=7.6: The amount of enzyme that catalyzes the conversion of 1 nmol of substrate per minute per mg of tissue protein is defined as one enzyme activity unit (U/mg prot). The amount of enzyme that catalyzes the conversion of 1 nmol of substrate per minute per gram of tissue is defined as one enzyme activity unit (U/g). The amount of enzyme that catalyzes the conversion of 1 nmol of substrate per minute per 10^4 cells is defined as one enzyme activity unit (U/10^4 cell). The amount of enzyme that catalyzes the conversion of 1 nmol of substrate per minute per mL of liquid sample is defined as one enzyme activity unit (U/mL).
Usage	Kit Components (50 Tests/48 Samples): Extraction Buffer A: Liquid, 50 mL × 1 bottle (

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Store at 4°C) Extraction Buffer B: Liquid, 50 mL × 1 bottle (Store at 4°C) Reagent I: Liquid, 100 mL × 1 bottle (Store at 4°C) Reagent II: Liquid, 6 mL × 1 bottle (Store at 4°C, protected from light) Materials Required but Not Provided: UV spectrophotometer 1 mL quartz cuvette (path length 10 mm) Mortar/homogenizer Adjustable pipettes Benchtop centrifuge Constant temperature water bath/incubator Distilled water Crude Enzyme Extract Preparation: 1. Tissue Samples: Process samples according to the ratio of tissue mass (g): Extraction Buffer A volume (mL) = 1:(5-10). It is recommended to weigh approximately 0.1 g of tissue, add 1 mL of Extraction Buffer A, and homogenize on ice. Centrifuge at 4°C, 10000 g for 10 min. Transfer the supernatant to another pre-cooled centrifuge tube and centrifuge at 4°C, 10000 g for 30 min. Discard the supernatant and retain the precipitate. Add 1 mL of pre-cooled Extraction Buffer B to the precipitate, mix thoroughly by vortexing, and centrifuge at 4°C, 16000 g for 40 min. Discard the supernatant and retain the precipitate. Add 1 mL of pre-cooled Reagent I to the precipitate, mix thoroughly by vortexing. This is the crude enzyme extract. Keep on ice for testing. 2. Bacterial or Cell Samples: Collect bacteria or cells by centrifugation into a centrifuge tube. Process samples according to the ratio of bacterial/cell count (10^4): Extraction Buffer A volume (mL) = (500-1000):1. It is recommended to add 1 mL of Extraction Buffer A to 5 million bacteria or cells. Sonicate on ice (power 300W, ultrasound 3s, interval 7s, total time 3 min). Centrifuge at 4°C, 10000 g for 10 min. Transfer the supernatant to another pre-cooled centrifuge tube and centrifuge at 4°C, 10000 g for 30 min. Discard the supernatant and retain the precipitate. Add 1 mL of pre-cooled Extraction Buffer B to the precipitate, mix thoroughly by vortexing, and centrifuge at 4°C, 16000 g for 40 min. Discard the supernatant and retain the precipitate. Add 1 mL of pre-cooled Reagent I to the precipitate, mix thoroughly by vortexing. This is the crude enzyme extract. Keep on ice for testing. 3. Serum (Plasma), Culture Medium and Other Liquid Samples: Test directly or dilute appropriately before testing. Assay Procedure: 1. Preheat the UV spectrophotometer for more than 30 min, set the wavelength to 360 nm, and zero with distilled water. 2. Add the following reagents sequentially into centrifuge tubes (μL): Test Group: - Crude enzyme extract: 100 μL - Reagent I: 800 μ

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	L - Reagent II: 100 μL Blank Group: - Reagent I: 900 μL - Reagent II: 100 μL 3. Absorbance Measurement: Mix thoroughly and immediately start timing. Measure the initial absorbance at 360 nm, recorded as A1_test and A1_blank. After accurate reaction at 37°C for 60 min, measure the absorbance at 360 nm, recorded as A2_test and A2_blank. Calculate ΔA1_test = A1_test - A1_blank, ΔA2_test = A2_test - A2_blank, ΔA = ΔA1_test - ΔA2_test. Note: Only 1-2 groups need to be measured for the blank group. Data Analysis: 1. Calculation by Tissue Protein Concentration: Unit definition: At 37°C, pH=7.6, the amount of enzyme that converts 1 nmol of substrate per minute per mg of tissue protein is defined as one enzyme activity unit. MAO (U/mg prot) = (ΔA × V_total × 10^6) / (ε × d × V_sample × Cpr × T) = 114.16 × ΔA / Cpr 2. Calculation by Tissue Sample Mass: Unit definition: At 37°C, pH=7.6, the amount of enzyme that converts 1 nmol of substrate per minute per gram of tissue is defined as one enzyme activity unit. MAO (U/g) = (ΔA × V_total × 10^6) / (ε × d × V_sample × W × T) = 114.16 × ΔA / W 3. Calculation by Bacterial or Cell Count: Unit definition: At 37°C, pH=7.6, the amount of enzyme that converts 1 nmol of substrate per minute per 10^4 bacteria or cells is defined as one enzyme activity unit. MAO (U/10^4 cell) = (ΔA × V_total × 10^6) / (ε × d × V_sample × cell_count × T) = 114.16 × ΔA / cell_count 4. Calculation by Liquid Sample Volume: Unit definition: At 37°C, pH=7.6, the amount of enzyme that converts 1 nmol of substrate per minute per mL of liquid sample is defined as one enzyme activity unit. MAO (U/mL) = (ΔA × V_total × 10^6) / (ε × d × V_sample × T) = 114.16 × ΔA Where: V_sample: Volume of crude enzyme extract added to the reaction, 0.1 mL V_total: Total reaction volume, 1 mL ε: Molar extinction coefficient of substrate, 1460 L/mol/cm d: Path length of 1 mL quartz cuvette, 1 cm W: Sample mass, g Cpr: Sample protein concentration, mg/mL T: Reaction time, 60 min 10^6: Unit conversion factor, 1 mmol = 10^6 nmol cell_count: Number of cells, 10^4
Extinction Coefficient	1460 L/mol/cm

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