



CheKine™ Micro Polyamine Oxidase (PAO) Activity Assay Kit

Cat #: KTB1901

Size: 48 T/48 S

96 T/96 S

	Micro Polyamine Oxidase (PAO) Activity Assay Kit		
REF	Cat #: KTB1901	LOT	Lot #: Refer to product label
	Applicable samples: Animal and Plant Tissues, Serum, Plasma or other Liquids		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Polyamine oxidase (PAO) is a key enzyme that catalyzes the oxidation of polyamines in organism. In addition, PAO can regulate the level of polyamines and the concentration of products in organism, and participate in the response to stress and the growth and development process of each plant. CheKine™ Micro Polyamine Oxidase (PAO) Activity Assay Kit can detect biological samples such as Animal and Plant Tissues, Serum or Plasma. In this kit, PAO catalyzed the oxidation of polyamines to produce hydrogen peroxide, which reacts with the substrate in the presence of peroxidase, and the product has a characteristic absorption peak at 500 nm. The activity of PAO is reflected by measuring the rate of increase in absorbance value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	70 mL	70 mL×2	4°C
Reagent II	1.5 mL	3 mL	4°C, protected from light
Reagent III	0.75 mL	1.5 mL	4°C, protected from light
Reagent IV	0.75 mL	1.5 mL	4°C, protected from light

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 light spectrophotometer capable of measuring absorbance at 500 nm
- 96-well plate or microquartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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

Reagent III : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Reagent IV : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Note: Reagent II or Reagent IV has certain irritation, so personal protection is recommended during use.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80°C for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent I, homogenize on ice. Centrifuge at 10,000 g for 20 min at 4°C. Take the supernatant and place it on the ice for testing.

2. Serum, Plasma and other Liquid Samples: Direct detection. If the solution has turbidity, centrifuge and take the supernatant for measurement.

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

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 500 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

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

Reagent	Test well (μL)
Sample	20
Reagent I	140
Reagent II	20
Reagent III	10
Reagent IV	10

3. Mix well, record the absorbance values of 0 min and 30 min at 500 nm, mark as A_1 and A_2 , and calculate $\Delta A = A_2 - A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA is less than 0.002, the sample size can be appropriately increased. If the ΔA is greater than 0.5, the sample can be appropriately diluted with Reagent I or reduce the sample quality used for extraction, the calculated result multiplied by 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.

Calculation of PAO activity

(1) Calculated by protein concentration:

Unit definition: One enzyme activity unit defines as an A500 change of 0.001 by 1 mg tissue protein per min.

$PAO (U/mg \text{ prot}) = \Delta A \times V_{\text{Total}} \div (V_{\text{Sample}} \times C_{\text{pr}}) \div 0.001 \div T = \mathbf{333.33 \times \Delta A \div C_{\text{pr}}}$

(2) Calculation according to the weight of the sample:

Unit definition: One enzyme activity unit defines as an A500 change of 0.001 by 1 g tissue per min.

$$PAO\ (U/g\ fresh\ weight) = \Delta A \times V_{Total} \div (V_{Sample} \div V_{Total\ sample}) \div 0.001 \div T = 333.33 \times \Delta A \div W$$

(3) Calculation according to the volume of liquid

Unit definition: One enzyme activity unit defines as an A500 change of 0.001 by 1 mL liquid per min.

$$PAO(U/mL) = \Delta A \times V_{Total} \div V_{Sample} \div 0.001 \div T = 333.33 \times \Delta A$$

V_{Total} : the total volume of the reaction system, 0.2 mL; V_{Sample} : the volume of the sample in the reaction system, 0.02 mL; $V_{Total\ sample}$:

The volume of Reagent I added, 1 mL; Cpr: protein concentration (mg/mL); T: reaction time, 30 min; W: sample weight, g.

Typical Data

The following data is for reference only, and experimenters need to test the samples based on their own experiments.

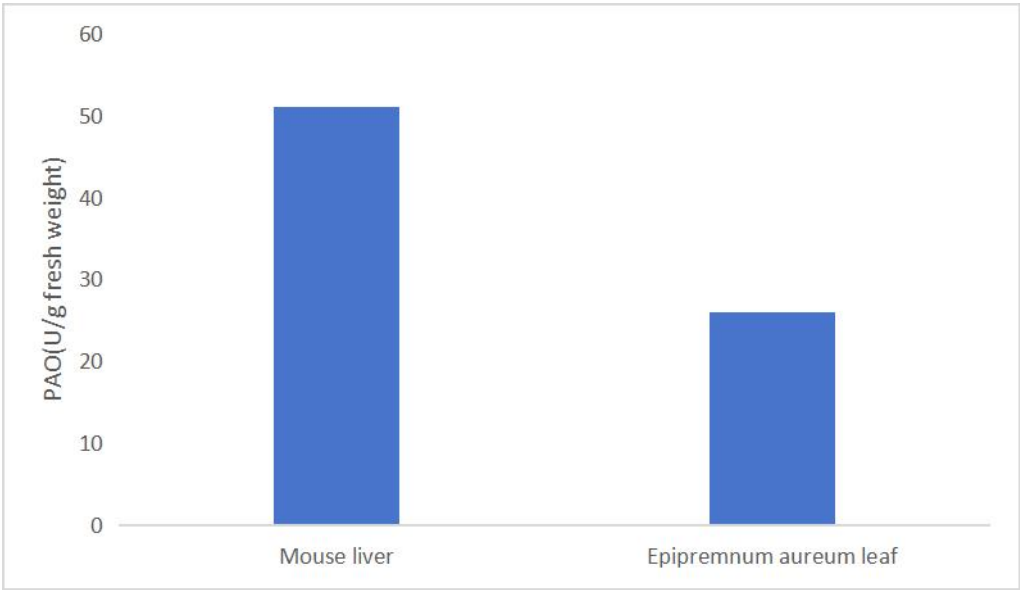


Figure 1. Determination of PAO activity in mouse liver and Epipremnum aureum leaf was detected with this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1900	CheKine™ Micro Monoamine Oxidase (MAO) Activity Assay Kit
KTB1220	CheKine™ Micro Diamine Oxidase (DAO) Activity Assay Kit
KTB1140	CheKine™ Micro Polyphenol Oxidase (PPO) 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.