CheKine™ Micro Diamine Oxidase (DAO) Activity Assay Kit

Cat #: KTB1220 Size: 48 T/48 S 96 T/96 S

	Micro Diamine Oxidase (DAO) Activity Assay Kit		
REF	**Cat #:** KTB1220	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria, Urine		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

DAO (EC1.4.3.6) is widely found in animals (intestinal mucosa, lung, liver, kidney, etc.), plants and microorganisms. It catalyzes the oxidation of diamines to aldehydes. Its activity is closely related to nucleic acid and protein synthesis, and can reflect the integrity and injury degree of intestinal mechanical barrier. CheKine™ Micro Diamine Oxidase (DAO) Activity Assay Kit provides a convenient tool for detection of DAO Activity. The principle is that DAO catalyzes 1,5-pentanediamine to produce aldehyde and hydrogen peroxide. The oxidation of o-Dianisidine(3,3′-Dimethoxybenzidine) with hydrogen peroxide to produce colored substances was catalyzed by exogenous Horseradish peroxidase, the colored substances have a maximum absorption peak detected at about 460 nm. The enzyme activity of DAO was calculated by detecting the rate of increase in absorption at 460 nm. The kit can detect serum (plasma), animal and plant tissues, cells, bacteria, cell supernatants, urine and other samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	75 mL	75 mL×2	4℃
Reagent Ⅰ	0.24 mL	0.48 mL	4℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, 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 spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, ice maker, incubator
- Deionized water

• Homogenizer (for tissue samples)

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℃, protected from light.

Note: Reagent Ⅰ is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Working Reagent Ⅰ: Prepared before use. According to the dosage mix Extraction Buffer and Reagent Ⅰ in a ratio of 108:2; Working Reagent Ⅰ is freshly prepared.

Working Reagent Ⅱ: Prepared before use. Add 4.8 mL deionized water for 96 T or 2.4 mL deionized water for 48 T to dissolve. The remaining reagent can be stored at 4℃, protected from light for 1 week.

Working Reagent Ⅲ: Prepared before use. Add 2.4 mL deionized water for 96 T or 1.2 mL deionized water for 48 T to dissolve at 37℃ water bath. The remaining reagent can be stored at -20℃ and protected from light for 1 week after aliquoting to avoid repeated freezing and thawing. It is normal for Working Reagent Ⅲ to have a little precipitation. If it affects the results, please filter it.

Sample Preparation

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

1. Animal Tissues: Weigh 0.1 g tissues, add 1 mL 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. Plant Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 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. 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 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.
4. Serum, Plasma, Cell Supernatant, Urine or other liquid samples: Tested directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 460 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheat the incubator to 37℃.
3. Add the following reagents respectively into the 96-well microplate or microglass cuvette:

Reagent	Control Well（μL）	Test Well（μL）
Sample	50	50
Extraction Buffer	20	0
Working Reagent Ⅰ	110	110
Working Reagent Ⅱ	20	20
Working Reagent Ⅲ	0	20
Mix well, incubate in 37°C for 5 min. Then reading the values at 460 nm. Finally, calculate $\Delta A=A_{Test}-A_{Control}$.		

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.005, increase the sample quantity appropriately. If ΔA is greater than 0.8, the sample can be appropriately diluted, 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 plates calculation formula

1. Calculation the activity of DAO in animal and plant tissues

(1) Calculated by protein concentration

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 1 mg tissues protein reaction system is defined as a unit of enzyme activity.

DAO (U/mg prot)=ΔA÷d÷ε×$V_{Reaction\ Total}$÷(Cpr×V_{Sample})÷T×n**=213×ΔA÷Cpr×n**

(2) Calculated by fresh weight of samples

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 1 g tissues reaction system is defined as a unit of enzyme activity.

DAO (U/g)=ΔA÷d÷ε×$V_{Reaction\ Total}$÷(W÷$V_{Extraction}$×V_{Sample})÷T×n**=213×ΔA÷W×n**

2. Calculate the activity of DAO in liquid sample

Unit definition: 1μmol Oxidized o-Dianisidine produced per min in 1mL liquid sample reaction system is defined as a unit of enzyme activity.

DAO (U/mL)=ΔA÷d÷ε×$V_{Reaction\ Total}$÷V_{Sapmle}÷T×n**=213×ΔA×n**

3. Calculated the activity of DAO by cells or bacteria number

Unit definition: 1 μmol Oxidized o-Dianisidine produced per min in 10^4 cells or bacteria reaction system is defined as a unit of enzyme activity.

DAO (U/10^4)=ΔA÷d÷ε×$V_{Reaction\ Total}$÷(500×V_{Sample}÷$V_{Extraction}$)÷T×n**=0.427×ΔA×n**

Where: ΔA=A_{Test}-$A_{Control}$; d: 96-well plate diameter, 0.5 cm; ε: Oxidized o-Dianisidine molar extinction coefficient, 7.5×10^{-3} mL/μmol/cm; $V_{Reaction\ Total}$: total reaction volume, 0.2 mL; Cpr: sample protein concentration, mg/mL; V_{Sample}: sample volume added, 0.05 mL; T: reaction time, 5 min; n: dilution factor; W: sample weight, g; $V_{Extraction}$: Extraction Buffer volume added, 1 mL; 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

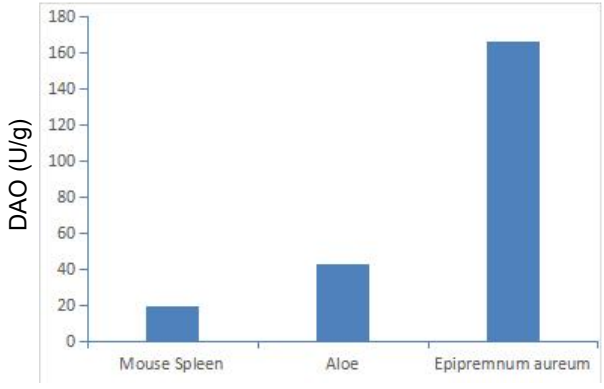

Figure1. DAO activity in mouse spleen, aloe and epipremnum aureum respectively. Assays were performed following kit protocol

Recommended Products

Catalog No.	Product Name
KTB1310	CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit
KTB1140	CheKine™ Micro Polyphenol Oxidase (PPO) Activity Assay Kit
KTB1070	CheKine™ Micro Xanthine Oxidase (XO) Assay Kit
KTB1210	CheKine™ Micro Superoxide Anion Assay Kit
KTB1200	CheKine™ Micro Protein Carbonyl 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.