CheKine™ Micro Ornithine Aminotransferase (δ-OAT) Activity Assay Kit

Cat #: KTB1421 Size:48 T/96 T

	Micro Ornithine Aminotransferase (δ-OAT) Activity Assay Kit		
REF	**Cat #:** KTB1421	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Ornithine aminotransferase (δ-OAT) is one of the key enzymes for the synthesis of proline by ornithine as a precursor, which plays an important role in adapting plants to stress. CheKine™ Micro Ornithine Aminotransferase (δ-OAT) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria, serum or plasma. In the kit, ornithine and α-ketoglutarate can undergo acyl transfer reaction under the action of δ-OAT and NADH to produce NAD and pyrrolaldehyde 5-carboxylic acid (P5C). NADH has a special absorption peak at 340 nm. By measuring the change in absorbance at 340 nm, the level of δ-OAT activity can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	55 mL	110 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1×2	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath pot, cryogenic centrifuge machine
- 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℃.

Reagent Ⅱ: Prepared before use. 48 T add 2.5 mL Reagent Ⅰ , 96 T add 5mL Reagent Ⅰ, fully dissolve, and store the inexhaustible reagents at 4℃, protected from light.

Reagent Ⅲ: Prepared before use. 48 T add 2.5 mL Reagent Ⅰ , 96 T add 5mL Reagent Ⅰ, fully dissolve, and store the inexhaustible reagents at 4℃, protected from light.

Reagent Ⅳ: Add 10mL Reagent I to each bottle before use, fully dissolve, pack and store at -20℃. Unused reagent can store at -20℃ for one week, protected from light.

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: Weigh 0.1 g tissue, 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. Bacteria: Collect 5×10^6 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 3 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s). 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: Direct detection.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat 5 min with Reagents Ⅱ, Ⅲ, Ⅳ at 37℃.
3. Sample measurement. (The following operations are operated in the the 96-well UV plate or microglass cuvette)

Reagent	Test Well (μL)
Sample supernatant	20
Reagent Ⅱ	60
Reagent Ⅲ	60
Reagent Ⅳ	60

4. Mix well, detect the absorbance at 340 nm at the time of 10s record as A1. Quickly incubate 10 min at 37℃ without light, immediately measure the absorbance at the time of 6 min 10 s which record as A2, Calculate ΔA =A1-A2.

Note: Start the timing when you join the sample.After adding the reagent in turn, the reagent should be mixed as quickly as possible and the OD value should be determined to reduce the error time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is greater than 0.5, the sample can be appropriately diluted with deionized water, 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.

Calculation of the δ-OAT activity

(1) Calculated by sample protein concentration

Unit definition: One unit of δ-OAT activity is defined as the amount of enzyme that per milligram of protein oxidation 1 nmoL of NADH per minute in the reaction system.

δ-OAT(nmol/min/mg prot)=ΔA÷(ε×d)×$V_{Reaction}$÷(V_{sample}×Cpr) ÷T=**321.54×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: One unit of δ-OAT activity is defined as the amount of enzyme that per gram of tissue oxidation 1 nmoL of NADH per minute in the reaction system.

δ-OAT(nmol/min/g fresh weight)=ΔA÷(ε×d)×$V_{Reaction}$÷(W ×V_{sample}÷$V_{Total\ sample}$) ÷T=**321.54×ΔA÷W**

(3) Calculated by cells

Unit definition: One unit of δ-OAT activity is defined as the amount of enzyme that per 10^4 cells oxidation 1 nmoL of NADH per minute in the reaction system.

δ-OAT(nmol/min/10^4 cells)= ΔA÷(ε×d)×$V_{Reaction}$÷(V_{sample}×n÷$V_{Total\ sample}$) ÷T**=321.54×ΔA÷n**

(4) Calculated by volume of liquid samples

Unit definition: One unit of δ-OAT activity is defined as the amount of enzyme that per milliliter of liquid oxidation 1 nmoL of NADH per minute in the reaction system.

δ-OAT(nmol/min/mL) = ΔA÷(ε×d)×$V_{Reaction}$÷V_{sample}÷T=**321.54×ΔA**

$V_{Reaction}$: Total reaction volume, 0.2 mL; ε: Molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate light path, 0.5 cm; Vs_{ample}: Added sample volume, 0.02 mL; $V_{Total\ sample}$: Added Extract Buffer volume, 1 mL; T: Reaction time, 10 min; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

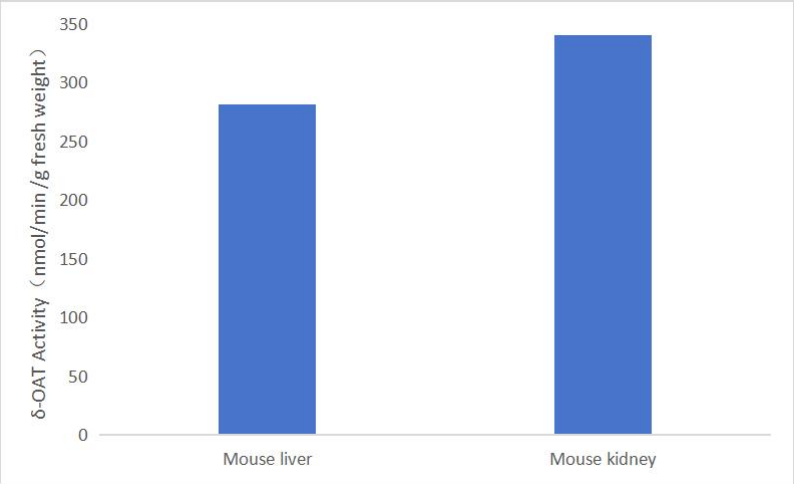

Figure 1. Determination of δ-OAT activity in mouse stomach and kidney by this kit

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB4021	CheKine™ Micro Leucine Arylamidase(LAP) Activity Assay Kit

Disclaimer

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