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CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit

Cat #: KTB3040 Size: 48 T/96 T

	Micro Glutamate Synthase (GOGAT) Assay Kit		
REF	**Cat #:** KTB3040	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissue, Cell, Bacteria, Serum, Plasma		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Glutamate synthase (GOGAT) mainly exists in prokaryotes, yeasts and non-green tissues of higher plants. Together with glutamine synthase (GS), glutamate synthase (GS) forms the GS/GOGAT cycle and participates in the regulation of ammonia assimilation. CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit provides a simple, convenient and rapid GOGAT activity detection method, which is suitable for the detection of animal and plant tissue, cell, bacteria, serum, plasma and other samples. The detection principle is that GOGAT uses NADH as the electron donor to catalyze the amino transfer of glutamine to α-ketoglutaric acid to form two-molecule glutamate. The decreasing rate of NADH absorbance in 340 nm can reflect the GOGAT activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃
Reagent Ⅱ	10 mL	20 mL	4℃
Reagent Ⅲ	1	2	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated 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℃.

Working Solution: Before use, Add 9 mL Reagent Ⅱ to Reagent Ⅲ, mix it well and prepare for use. Store at 4℃, protected from light.

Sample Preparation

Note: Fresh samples are recommended. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Cell (bacteria): Collect 5×10^6 cell or bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum (Plasma): Direct detection.

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 ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Working Solution was incubated at 37℃ (mammals) or 25℃ (other species) for 30 min.

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

Reagent	Test Well（μL）
Sample	20
Working Solution	180

4. After mixing quickly, record the absorbance values of 20 s and 5 min 20 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A_{Test}=A_{Test1}-A_{Test2}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

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 plates calculation formula

1. Calculation of GOGAT activity in serum

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum per min.

$GOGAT\ (U/mL)=[\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\div T=\mathbf{643\times\Delta A_{Test}}$

2. Calculation of GOGAT activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

$GOGAT\ (U/mg\ prot)=[\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times Cpr)\div T=\mathbf{643\times\Delta A_{Test}\div Cpr}$

(2) Calculation according to the weight of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min.

GOGAT (U/g fresh weight)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]÷($W \div V_{Reagent\ I} \times V_{Sample}$)÷T=**643×$\Delta A_{Test} \div W$**

3. Calculation of GOGAT activity in cells or bacteria

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 cells or bacteria per min.

GOGAT (U/10^4)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]÷($V_{Sample} \div V_{Reagent\ I} \times 500$)÷T=**1.286×$\Delta A_{Test}$**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; $V_{Reagent\ I}$: the volume of the Reagent Ⅰ, 1 mL; V_{Sample}: the volume of the supernatant in the reaction system, 0.02 mL; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 5 min; W: sample weight, g; 500: total number of cells or bacteria, 5 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

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
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase（GDH）Assay Kit
KTB3050	CheKine™ Micro Water and Soil Nitrite Content Assay Kit

Disclaimer

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