（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Glutamate Synthase (GOGAT) Activity Assay Kit

Catalog No：AMS.E-BC-K659-M-96T

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader（330-350 nm）

Detection range：8.84–321.05 U/L

This manual must be read attentively and completely before using this product.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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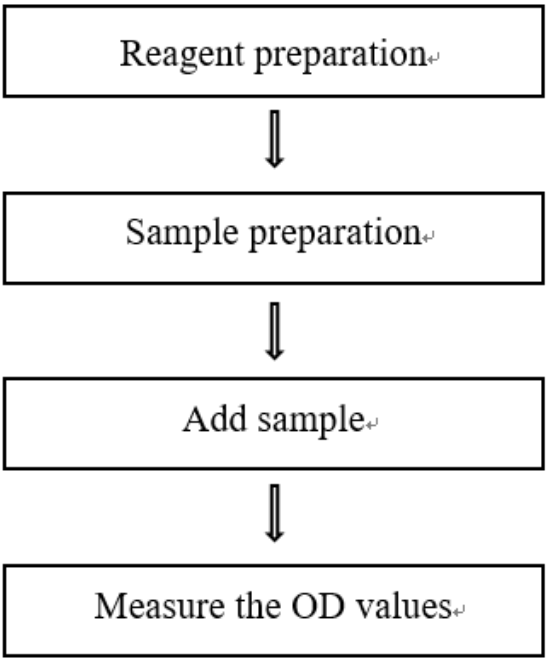
Assay summary



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Intended use

This kit can be used to measure glutamate synthase (GOGAT) activity in serum, animal and plant tissue samples.

Detection principle

GOGAT mainly exists in prokaryotes, saccharomyces yeasts and proplasts of non-green tissues of higher plants. GOGAT and glutamine synthetase (GS) constitute the GS/GOGAT cycle and participate in the regulation of ammonia assimilation.

GOGAT catalysis the reaction that transfer the amino from glutamine to a-KG to form two molecules of glutamic acid using NADH as the electron donor. The decreasing rate of NADH that can be measured at 340 nm can reflect the activity of GOGAT.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL ×1 vial	50 mL ×2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	16 mL ×1 vial	26 mL ×1 vial	-20℃, 12 months
Reagent 3	Substrate A	Powder ×2 vials	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate B	Powder ×2 vials	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent	Powder ×2 vials	Powder ×2 vials	-20℃, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagent preparation

Size 1(48 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of reaction working solution:

Dissolve one vial of substrate A, one vial of substrate B and one vial of chromogenic agent with 7.5 mL of buffer solution, mix well to dissolve. The reaction working solution should be prepared on spot. Store at 2-8℃ for 12 h protected from light.

Size 2(96 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of reaction working solution:

Dissolve one vial of substrate A, one vial of substrate B and one vial of chromogenic agent with 12.5 mL of buffer solution, mix well to dissolve. The reaction working solution should be prepared on spot. Store at 2-8℃ for 12 h protected from light.

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Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat kidney tissue homogenate	2-4
10% Rat liver tissue homogenate	2-4
10% Rat heart tissue homogenate	2-4
10% Mouse liver tissue homogenate	2-4
Bovine serum	1
10% pleurotus cornucopiae tissue homogenate	1
10% beech mushroom tissue homogenate	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① It should be ensured that the powder in the prepared reaction working solution is completely dissolved.

② It's better to measure no more than 4 samples at same time.

Operating steps

① Sample well: Add 20 μL of sample to the wells.

② Add 200 μL of reaction working solution into each well.

③ Mix fully with microplate reader for 5 s. Measure the OD value of each well at 0 min recorded as A_1. Incubate at 25℃ for 4 min and measure the OD value of each well recorded as A_2.

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Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: The amount of GOGAT in 1 L serum or plasma that catalyze and decompose 1 μmol NADH in 1 minute at 25°C is defined as 1 unit

$$\text{GOGAT activity (U/L)} = \frac{\Delta A_{340}}{\varepsilon \times d} \times V_{总} \div V_{样} \div T \times f \times 10\text{^}6$$

2. Tissue sample:

Unit definition: The amount of GOGAT in 1 g tissue protein that catalyze and decompose 1 μmol NADH in 1 minute at 25°C is defined as 1 unit.

$$\text{GOGAT activity (U/gprot)} = \frac{\Delta A_{340}}{\varepsilon \times d} \times V_{总} \div V_{样} \div C_{pr} \div T \times f \times 10\text{^}6$$

[Note]

ΔA_{340}：$A_1 - A_2$.

ε：The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm.

d：Optical path, 0.65 cm.

V_{total}：The total volume of the reaction system, 0.22 mL.

V_{sample}：The volume of the sample, 0.02 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

10^6：1 mol/L=1×10^6 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	13.50	84.50	167.00
%CV	2.9	2.4	2.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	13.50	84.50	167.00
%CV	5.7	5.4	6.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （U/L）	56.7	148	252
Observed Conc. （U/L）	56.1	155.4	272.2
recovery rate(%)	99	105	108

Sensitivity

The analytical sensitivity of the assay is 8.84 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis:

For rat liver tissue, dilute the sample of 10% rat liver tissue homogenate for 4 times with extraction solution, take 20 μL of the diluted sample, and carry the assay according to the operation table. The results are as follows:

The A_1 of the sample is 0.875, after 4 minutes of reaction, the A_2 of the sample is 0.842, the concentration of protein in sample is 12.96 gprot/L, and the calculation result is:

GOGAT activity (U/gprot) = (0.875 – 0.842) ÷(6220 ×0.65) ×0.22 ÷0.02 ÷4 ×4 ×10^6

= 6.93 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 12.96 gprot/L, dilute for 4 times), 10% rat kidney tissue homogenate (the concentration of protein is 7.16 gprot/L, dilute for 4 times) , 10% beech mushroom tissue homogenate (the concentration of protein is 1.30 gprot/L) and bovine serum according to the protocol, the result is as follows:

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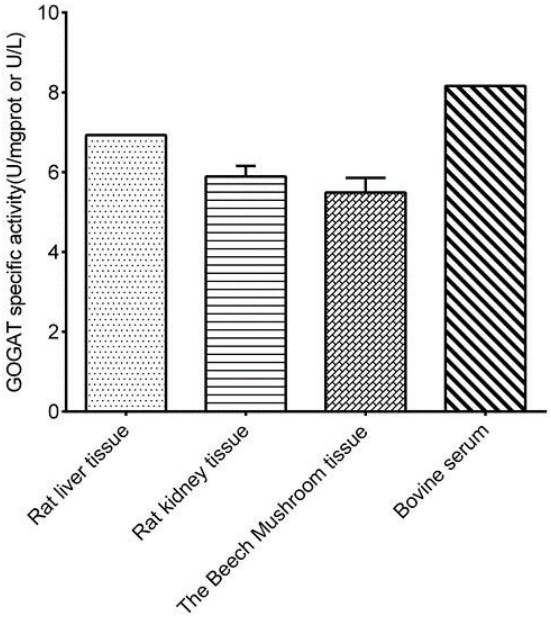

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Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. AMSBIO will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

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