

# Product Information & Manual

Glutamate Synthase/GOGAT Activity  
Assay Kit (Colorimetric)

NBP3-52330

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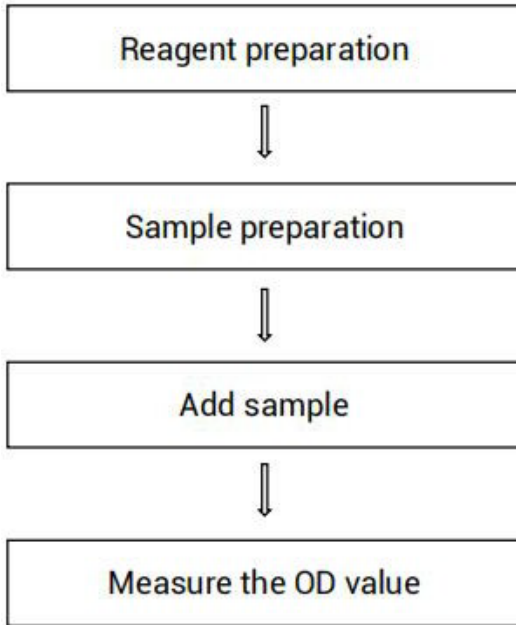
Novus kits are  
guaranteed for 6 months  
from date of receipt.

**For research use only.  
Not for diagnostic or  
therapeutic procedures.**

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## Assay summary



## 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 °C, 12 months                |
| Reagent 2 | Buffer Solution     | 16 mL × 1 vial   | 26 mL × 1 vial   | -20 °C, 12 months                |
| Reagent 3 | Substrate A         | Powder × 2 vials | Powder × 2 vials | -20 °C, 12 months, shading light |
| Reagent 4 | Substrate B         | Powder × 2 vials | Powder × 2 vials | -20 °C, 12 months, shading light |
| Reagent 5 | Chromogenic Agent   | Powder × 2 vials | Powder × 2 vials | -20 °C, 12 months, shading light |
|           | UV Microplate       | 96 wells         |                  | No requirement                   |
|           | Plate Sealer        | 2 pieces         |                  |                                  |
|           | Sample Layout Sheet | 1 piece          |                  |                                  |

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 °C 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 °C for 12 h protected from light.

## 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  $-80^{\circ}\text{C}$  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  $\mu\text{L}$  extraction solution with a dounce homogenizer at  $4^{\circ}\text{C}$ .
- ④ Centrifuge at  $10000\times g$  for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant.

### 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  $\mu\text{L}$  of sample to the wells.
- ② Add 200  $\mu\text{L}$  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  $25^\circ\text{C}$  for 4 min and measure the OD value of each well recorded as  $A_2$ .

## Calculation

The sample:

### 1. Serum (plasma) sample:

**Unit definition:** The amount of GOGAT in 1 L serum or plasma that catalyze and decompose 1  $\mu\text{mol}$  NADH in 1 minute at 25°C is defined as 1 unit

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

### 2. Tissue sample:

**Unit definition:** The amount of GOGAT in 1 g tissue protein that catalyze and decompose 1  $\mu\text{mol}$  NADH in 1 minute at 25°C is defined as 1 unit.

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

### [Note]

$\Delta A_{340}$  :  $A_1 - A_2$ .

$\varepsilon$  : The molar extinction coefficient of NADH,  $6.22 \times 10^3 \text{ L/mol/cm}$ .  $d$  : Optical path, 0.65 cm.

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

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

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

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

$10^6$  :  $1 \text{ mol/L} = 1 \times 10^6 \mu\text{mol}$ .

## 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.

**Detection range : 8.84-321.05 U/L**

## Appendix II Example Analysis

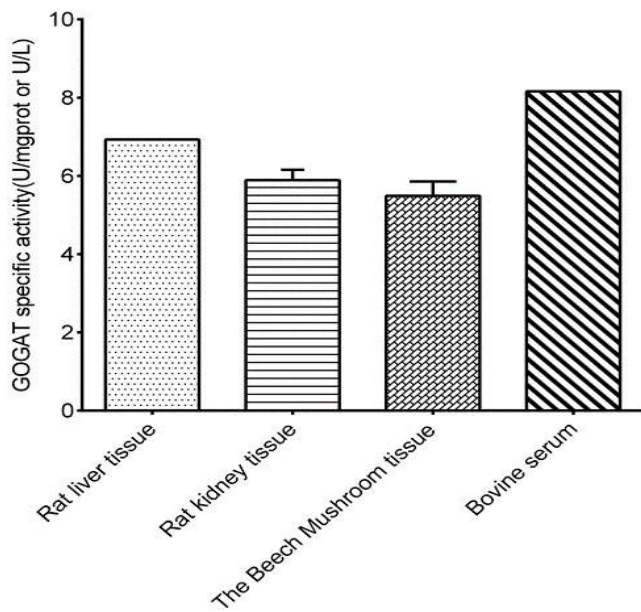
### Example analysis :

For rat liver tissue, dilute the sample of 10% rat liver tissue homogenate for 4 times with extraction solution, take 20  $\mu\text{L}$  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  $\text{gprot/L}$ , and the calculation result is:

$$\begin{aligned}\text{GOGAT activity (U/gprot)} &= (0.875 - 0.842) \div (6220 \times 0.65) \times 0.22 \div 0.02 \div 4 \times 4 \times 10^6 \\ &= 6.93 \text{ U/gprot}\end{aligned}$$

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



## 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. Novus Biologicals 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.