

## Glutamate Synthase(GOGAT) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Cat No:** AK0600

**Size:**50T/48S

### Components:

Extract solution: Liquid 60 mL×1. Store at 4°C .

Reagent I: Liquid 60 mL×1. Store at 4°C .

Reagent II: Powder×2. Store at 4°C .

Reagent III: Powder×2. Store at 4°C .

Reagent IV: Powder×2. Store at -20°C .

Working solution: Take one tube of Reagent II, Reagent III and Reagent IV, mix them together and dissolve in 30 mL of Reagent I. Prepare when the solution will be used. It can be stored at -20°C after dispensing to avoid repeated freezing and thawing.

### Product Description:

Glutamine oxoglutarate aminotransferase (also known as Glutamate synthase) is an enzyme and frequently abbreviated as GOGAT. GOGAT is mainly found in the protoplasts of prokaryotes, yeasts and non-green tissues of higher plants. Together with glutamine synthetase (GS), it constitutes the GS/GOGAT cycle and participates in the regulation of ammonia assimilation.

GOGAT uses NADH as an electron donor to catalyze the amino transfer of glutamine to  $\alpha$ -ketoglutarate to form two molecules of glutamic acid. The activity of GOGAT can be determined by the decrease rate of NADH at 340 nm.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, adjustable transferpettor, water bath, 1 mL quartz cuvette, mortar/homogenizer, ice and distilled water.

### Procedure:

#### I. Sample preparation:

1. Bacteria or cells: Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to bacteria or cells ( $10^4$ ): Extract solution (mL) is 500~1000:1 to extract. It is suggest to add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 20% or 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 10000  $\times$ g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue: according to tissue weight (g): Extract solution (mL) is 1:5~10 to extract. Add 1 mL of Extract

solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice before testing.

## II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Working solution should be prepared some time ahead and balance to room temperature before use.
3. Add the following reagents

| Reagent (μL)     | Test tube (T) |
|------------------|---------------|
| Working solution | 900           |
| Sample           | 100           |

Mix thoroughly and timing after add sample, detect the absorbance at 340 nm at the time of 20 seconds record as A1. Then place dishes with the reaction solution in a 25°C water bath for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(5 min 20s).

$$\Delta A = A1 - A2.$$

## III. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADH in the reaction system per minute every milligram protein.

$$\text{GOGAT (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \times C_{pr}) \div T = 321.5 \times \Delta A \div C_{pr}$$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADH in the reaction system per minute every gram tissue.

$$\text{GOGAT (U/g)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (W \div V_e \times V_s) \div T = 321.5 \times \Delta A \div W$$

3. Bacteria or cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADH in the reaction system per minute every 1 0000 cells or bacteria.

$$\text{GOGAT (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \div V_e \times 500) \div T = 0.643 \times \Delta A$$

$\epsilon$ : NADH molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{rv}$ : Total reaction volume,  $1 \times 10^{-3}$  L;

$V_s$ : Supernate volume, 0.1 mL;

$V_e$ : Extract solution volume, 1 mL;

$C_{pr}$ : Sample protein concentration (mg/mL);

T: Reaction time , 5 minutes;

W: Sample weight(g);

500: 5 million cells or bacteria.

### Note:

1. During the determination, the samples should be placed on ice to avoid denaturation and deactivation.
2. It's better to do this experiment with two people at the same time, one for color comparison and one for timing, so as to ensure the accuracy of the experimental results.
3. When A1 is greater than 1.5 or  $\Delta A$  is greater than 0.6, it is recommended to dilute the sample with distilled water for determination. If  $\Delta A$  is too small, the enzymatic reaction time can be prolonged (10minutes or 15 minutes) or the added sample volume can be increased for measurement.
4. As the Extract solution contains a certain concentration of protein (about 1 mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

### Experimental Examples:

1. Take 0.1 g of red bean sprouts and add 1 mL of extract solution for homogenate grinding. After taking the supernatant, operate according to the determination steps. Measure and calculate  $\Delta A = A1 - A2 = 1.23 - 1.067 = 0.163$  with micro quartz plate. Calculate the enzyme activity according to the sample mass.

$$\text{GOGAT (U/g mass)} = 321.5 \times \Delta A \div W = 321.5 \times 0.163 \div 0.1 = 524 \text{ U/g mass.}$$

2. Take 0.1 g of Barnyard grass and add 1 mL of extract solution for homogenate grinding. After taking the supernatant, operate according to the determination steps. Measure and calculate  $\Delta A = A1 - A2 = 1.416 - 1.404 = 0.012$  with a micro quartz cuvette.

$$\text{GOGAT (U/g mass)} = 321.5 \times \Delta A \div W = 321.5 \times 0.012 \div 0.1 = 38.58 \text{ U/g mass.}$$

### Recent Product Citations:

[1] Fei Ding, Qiannan Hu, Meiling Wang, et al. Knockout of SISBPASE Suppresses Carbon Assimilation and Alters Nitrogen Metabolism in Tomato Plants. International Journal of Molecular Sciences. December 2018;(IF4. 183)

[2] Jie Wang, Wei Zhou, Hui Chen, et al. Ammonium Nitrogen Tolerant Chlorella Strain Screening and Its Damaging Effects on Photosynthesis. Frontier in Immunology. January 2019;(IF4.259)

[3] Meng L, Li W, Zhang S, et al. Effects of sucrose amendment on ammonia assimilation during sewage sludge composting[J]. Bioresource technology, 2016, 210: 160- 166.

### References:

[1] del Pilar Cordovilla M, Pérez J, Ligeró F, et al. Partial purification and characterization of NADH-glutamate synthase from faba bean (*Vicia faba*) root nodules[J]. Plant science, 2000, 150(2): 121- 128.

### Related Products:

- |               |   |
|---------------|---|
| AK0301/AK0300 | Nitrate Reductase (NR) Activity Assay Kit   |
| AK0428/AK0427 | Nitrate Content In Plants Assay Kit         |
| AK0426/AK0425 | Plant Ammonia Nitrogen Content Assay Kit    |
| AK0432/AK0431 | Nitrite Content In Soil And Water Assay Kit |
| AK0430/AK0429 | Nitrite Content In Food Assay Kit           |