Glutamate Synthase (GOGAT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC0075

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Store at 2-8℃.

Reagent I: Liquid 20 mL×1. Store at 2-8℃.

Reagent II: Powder×2. Store at 2-8℃.

Reagent III: Powder×2. Store at 2-8℃.

Reagent IV: Powder×2. Store at -20℃.

Working solution: Take one tube of Reagent II, Reagent III and Reagent IV, mix them together and dissolve in 10 mL of Reagent I. Prepare when the solution will be used. It can be stored at -20℃ 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 α-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:

Microplate reader/spectrophotometer, desk centrifuge, adjustable transferpettor, water bath, micro quartz cuvette/96 well UV flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice, 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 suggested to add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 10000 ×g for 10 minutes at 4℃ 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

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

II. Determination procedure:

1. Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set spectrophotometer 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	180
Sample	20

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℃ water bath for 5 minutes (If the microplate reader has temperature control function, adjust the temperature to 25℃). 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:

A. micro quartz cuvette

1. Protein concentration:

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

GOGAT activity (U/mg prot)=$[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (Vs \times Cpr) \div T = 321.5 \times \Delta A \div Cpr$

2. Sample weight:

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

GOGAT activity (U/g weight)=$[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (W \div Ve \times Vs) \div T = 321.5 \times \Delta A \div W$

3. Bacteria or cultured cells number:

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

GOGAT activity (U/10^4 cell) =$[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (Vs \div Ve \times 500) \div T = 0.643 \times \Delta A$

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,2×10^{-4} L;

Vs: Supernate volume, 0.02 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 5 minutes;

W: Sample weight(g);

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

500: 5 million cells or bacteria;

10^9: Unit conversion coefficient, 1 mol $=10^9$ nmol.

B. 96 well UV flat-botton plate:

Change the d-1cm in the above formula to d-0.6cm (96 UV plate light path).

Note:

1. Samples are placed on ice during the measurement to prevent enzyme deactivation.
2. It is better for two people to do this experiment at the same time, one for colorimetric and the other for timing to ensure the accuracy of the experimental results.
3. If A>1.5 or ΔA>0.6(the microplate reader detects ΔA>0.4), the sample can be measured after being appropriately diluted with distilled water. If ΔA is too small, the sample can be measured after extending the enzymatic reaction time (10 minutes or 15 minutes) or increasing the sample volume.
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 ΔA=A1-A2= 1.3015-1.0895=0.212 with micro quartz plate. Calculate the enzyme activity according to the sample mass:

GOGAT activity (U/g weight) = 321.5 × ΔA÷W = 321.5 × 0.212÷0.1 = 681.58 U/g weight.

2. Take 0.1 g of Chlorophytum and add 1 mL of extract solution for homogenate grinding. After taking the supernatant, operate according to the determination steps. Measure and calculate ΔA=A1-A2= 0.9753-0.966=0.0093 with micro quartz cuvette. Calculate the enzyme activity according to sample mass:

GOGAT activity (U/g weight) = 321.5×ΔA÷W= 321.5× 0.0093÷0.1 = 29.9 U/g weight.

Recent Product Citations:

[1] Huang Y, Qin M, Lai J, Liang J, Luo X, Li C. Assessing OBT formation and enrichment: ROS signaling is involved in the radiation hormesis induced by tritium exposure in algae. J Hazard Mater. 2023 Feb 5;443(Pt A):130159. doi: 10.1016/j.jhazmat.2022.130159. Epub 2022 Oct 12. PMID: 36283218.

[2] Chen T, Zhao MX, Tang XY, Wei WX, Wen X, Zhou SZ, Ma BH, Zou YD, Zhang N, Mi JD, Wang Y, Liao XD, Wu YB. The tigecycline resistance gene tetX has an expensive fitness cost based on increased outer membrane permeability and metabolic burden in Escherichia coli. J Hazard Mater. 2023 Sep 15; 458:131889. doi: 10.1016/j.jhazmat.2023.131889. Epub 2023 Jun 19. PMID: 37348375.

For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

[3] Chu G, Wang Q, Song C, Liu J, Zhao Y, Lu S, Zhang Z, Jin C, Gao M. Platymonas helgolandica- driven nitrogen removal from mariculture wastewater under different photoperiods: Performance

evaluation, enzyme activity and transcriptional response. Bioresour Technol. 2023 Mar; 372:128700. doi: 10.1016/j.biortech.2023.128700. Epub 2023 Feb 2. PMID: 36738978.

[4] Chen Z, Qiu S, Li M, Zhou D, Ge S. Instant Inhibition and Subsequent Self-Adaptation of Chlorella sp. Toward Free Ammonia Shock in Wastewater: Physiological and Genetic Responses. Environ Sci Technol. 2022 Jul 5;56(13):9641-9650. doi: 10.1021/acs.est.1c08001. Epub 2022 Jun 23. PMID: 35737736.

[5] Chen C, Chu Y, Huang Q, Zhang W, Ding C, Zhang J, Li B, Zhang T, Li Z, Su X. Morphological, physiological, and transcriptional responses to low nitrogen stress in Populus deltoides Marsh. clones with contrasting nitrogen use efficiency. BMC Genomics. 2021 Sep 27;22(1):697. doi: 10.1186/s12864-021-07991-7. PMID: 34579659; PMCID: PMC8474845.

References：

[1] del Pilar Cordovilla M, Pérez J, Ligero 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.

[2] Singh R P, Srivastava H S. Increase in glutamate synthase (NADH) activity in maize seedlings in response to nitrate and ammonium nitrogen [J]. Physiologia Plantarum, 1986, 66: 413-416.

[3] Meng S, Zhang CX, Su L. et al. Nitrogen uptake and metabolism of Populus simonii in response to PEG-induced drought stress [J]. Environmental and Experimental Botany, 2016, 123: 78-87

Related Products:

BC0080/BC0085	Nitrate Reductase (NR) Activity Assay Kit
BC1500/BC1505	Nitrate Content In Plants Assay Kit
BC1520/BC1525	Plant Ammonia Nitrogen Content Assay Kit
BC1480/BC1485	Nitrite Content In Soil And Water Assay Kit
BC1490/BC1495	Nitrite Content In Food Assay Kit