Glutamate Synthase (GOGAT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0070

Size:50T/48S

Components:

Extract solution: Liquid 60 mL×1. Store at 2-8℃.

Reagent I: Liquid 60 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 30 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:

Spectrophotometer, desk centrifuge, adjustable transferpettor, water bath, mortar/homogenizer/cell ultrasonic crusher, 1 mL quartz cuvette, 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 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 for 10

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minutes at 4℃ 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℃ 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). ΔA=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.

GOGAT activity (U/mg prot) =[ΔA÷(ε×d)×10^9×Vrv]÷(Vs×Cpr)÷T=321.5×ΔA÷Cpr

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.

GOGAT activity (U/g weight)=[ΔA÷(ε×d)×10^9×Vrv]÷(W÷Ve×Vs)÷T=321.5×ΔA÷W

3. Bacteria or cells number:

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 10000 cells or bacteria.

GOGAT activity (U/10^4 cell) =[ΔA÷(ε×d)×10^9×Vrv]÷(Vs÷Ve×500)÷T=0.643×ΔA

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,1×10^{-3} L;

Vs: Supernate volume, 0.1 mL;

Ve: Extract solution volume, 1 mL;

Cpr: 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 ΔA is greater than 0.6, it is recommended to dilute the sample with distilled water for determination. If ΔA 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 ΔA = A1-A2 = 1.23-1.067=0.163. Calculate the enzyme activity according to the sample mass:
   GOGAT activity (U/g weight) = 321.5 × ΔA÷W = 321.5×0.163÷0.1=524 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 ΔA=A1-A2= 1.416-1.404=0.012. Calculate the enzyme activity according to the sample mass:
   GOGAT activity (U/g weight) = 321.5×ΔA÷W= 321.5×0.012÷0.1=38.58 U/g mass.

Recent Product Citations:

[1] Hu Y, Li J, Lin H, Liu P, Zhang F, Lin X, Liang J, Tao Y, Jiang Y, Chen B. Ultrasonic treatment decreases Lyophyllum decastes fruiting body browning and affects energy metabolism. Ultrason Sonochem. 2022 Sep; 89:106111. doi: 10.1016/j.ultsonch.2022.106111. Epub 2022 Jul 30. PMID: 35998484; PMCID: PMC9421313

[2] Ahmad S, Wang GY, Muhammad I, Zeeshan M, Zhou XB. Melatonin and KNO3 Application Improves Growth, Physiological and Biochemical Characteristics of Maize Seedlings under Waterlogging Stress Conditions. Biology(Basel). 2022 Jan 9;11(1):99. doi: 10.3390/biology11010099. PMID: 35053096; PMCID: PMC8773118

[3] Iqbal A, He L, Ali I, Ullah S, Khan A, Akhtar K, Wei S, Fahad S, Khan R, Jiang L. Co-incorporation of manure and inorganic fertilizer improves leaf physiological traits, rice production and soil functionality in a paddy field. Sci Rep. 2021 May 11;11(1):10048. doi: 10.1038/s41598-021-89246-9. Erratum in: Sci Rep. 2021 Aug 17;11(1):17031. PMID: 33976273; PMCID: PMC8113589.

[4] 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.

[5] 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.

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:

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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

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