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Glutamate Synthase(GOGAT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: AK0599

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Store at 4℃ .

Reagent I: Liquid 20 mL×1. Store at 4℃ .

Reagent II: Powder×2. Store at 4℃ .

Reagent III: Powder×2. Store at 4℃ .

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, 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 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 ×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 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 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). ΔA=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 generation of 1 nmol of NADH in the reaction system per minute every milligram protein.

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

3. Bacteria or cultured 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.

GOGAT (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,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);

500: 5 million cells or bacteria;

10^9 : 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 hole 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>0.6), 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 (U/g mass) = 321.5 × ΔA÷W = 321.5 × 0.212÷0. 1 = 681.58 U/g mass.
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 a micro quartz cuvette.
GOGAT (U/g mass) = 321.5×ΔA÷W= 321.5× 0.0093÷0. 1 = 29.9 U/g mass.

Recent Product Citations：

[1] Fei Ding,Qiannan Hu,Meiling Wang,et al. Knockout of SlSBPASE 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, 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.

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

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AK0430/AK0429 Nitrite Content In Food Assay Kit